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SAR Studies on Tetrahydroisoquinoline Derivatives: The Role of Flexibility and Bioisosterism To Raise Potency and Selectivity toward P-glycoprotein

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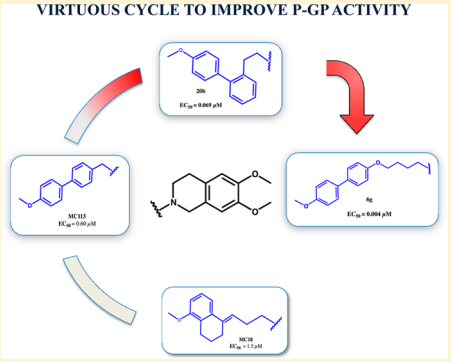
ABSTRACT: The development of P-glycoprotein (P-gp) ligands remains of considerable interest, mostly for investigating the protein's structure and transport mechanism. In recent years, many different generations of ligands have been tested for their ability to modulate P-gp activity. The aim of the present work is to perform SAR studies on tetrahydroisoquinoline derivatives in order to design potent and selective P-gp ligands. For this purpose, the effect of bioisosteric replacement and the role of flexibility have been investigated, and four series of tetrahydroisoquinoline ligands have been developed: (a) 2-aryloxazole bioisosteres, (b) elongated analogues, (c) 2*H*-chromene, and (d) 2-biphenyl derivatives. The results showed that both 2-biphenyl derivative **20b** and elongated derivative **6g** behaved as strong P-gp substrates. In conclusion, important aspects for developing potent and selective P-gp ligands have been highlighted, providing a solid starting point for further optimization.



INTRODUCTION

P-glycoprotein (P-gp) is a transmembrane transporter belonging to the ATP-binding cassette (ABC) family that requires the hydrolysis of ATP to run its transport mechanism.[1] This pump is localized at the apical side of the cell membrane in tissues that are usually involved in adsorption and excretion, such as the colon, kidney, adrenal gland, liver, and pancreas, as well as at the blood−brain barrier (BBB).[2] P-gp exerts a physiologically protective role by using ATP hydrolysis as the energy source for the translocation of several structurally unrelated molecules. The protein's activity prevents accumulation of endogenous (steroid hormones, cytokines) and exogenous (anticancer drugs, antiepileptics, anti-HIV drugs, antidepressants) substances in the brain, and its overexpression is the main cause of resistance to antineoplastic drugs in tumors.[3,4] Moreover changes in P-gp expression and function are involved in several neurological disorders, such as epilepsy and Parkinson's and Alzheimer's diseases.[5]

The ATP binding and hydrolysis that drive the conformational changes in P-gp associated with substrate transport have not yet been fully characterized, and the absence of a high-resolution structure of crystallized human P-glycoprotein is, to date, a strong limitation toward this goal.[6] The development of P-gp modulators remains of considerable interest to tackle this efflux protein and to investigate the protein's structure and transport mechanism. In recent years, many different generations of ligands have been tested for their ability to modulate P-gp activity.[7,8] Several P-gp ligands have also been used, after radiolabeling with ^{11}C or ^{18}F, as tools to monitor P-gp function and expression in vivo by positron emission tomography (PET).[9]

The third-generation modulator Tariquidar, subjected to clinical trials (Figure 1), showed high P-gp potency but poor selectivity because of its interaction with other ABC transporters such as breast cancer resistance protein (BCRP) and multidrug resistance associated protein 1 (MRP1).[2] In particular, Tariquidar was found to be a dual P-gp/BCRP substrate, resulting in the inability to visualize P-gp density in human brain by PET.[10,11] These findings suggest that compounds with subnanomolar P-gp binding affinities are needed to develop successful ligands.

Small molecules, namely, **MC18** and **MC266** (Figure 1), bearing a tetrahydroisoquinoline fragment displayed potent inhibitory and substrate profiles, respectively (activity data are reported in Table 1).[12−15] Starting from these two lead compounds, we constrained the spacer into a biphenyl fragment, obtaining a new series of P-gp ligands in which **MC70** and **MC113** (Figure 2; activity data are reported in Table 1) displayed high P-gp-inhibiting activity in the submicromolar range.[16,17] Moreover, [^{11}C]**MC113** has been studied in vivo for its ability to target P-gp, both at the BBB and in breast cancer, in specific mouse models. However, [^{11}C]**MC113** demonstrated a

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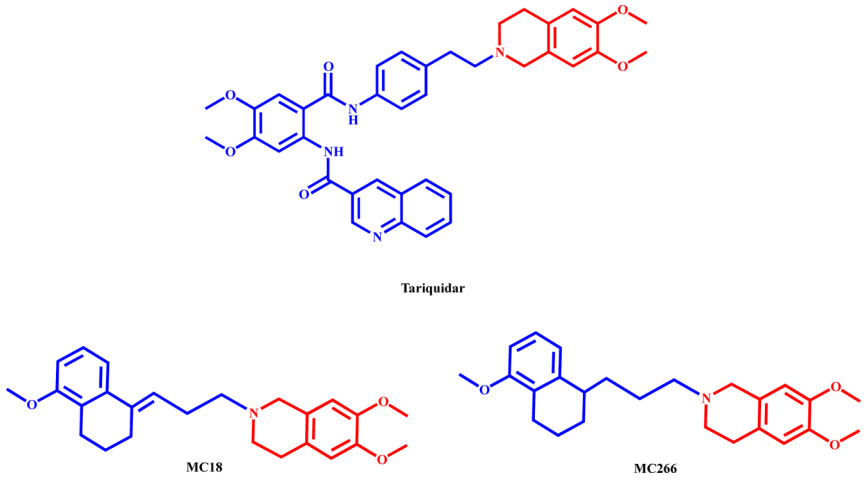

Figure 1. P-gp ligands.

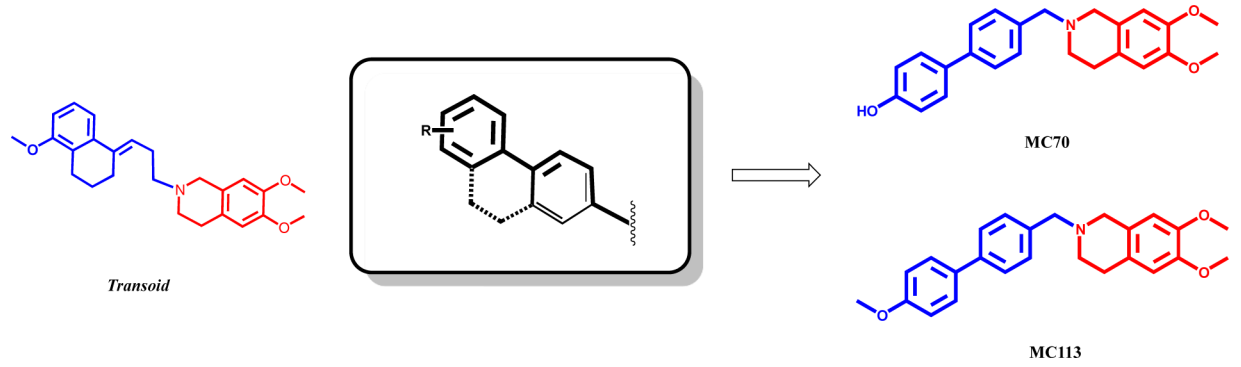

Figure 2. Transoid configuration of **MC18** and semirigid congeners.

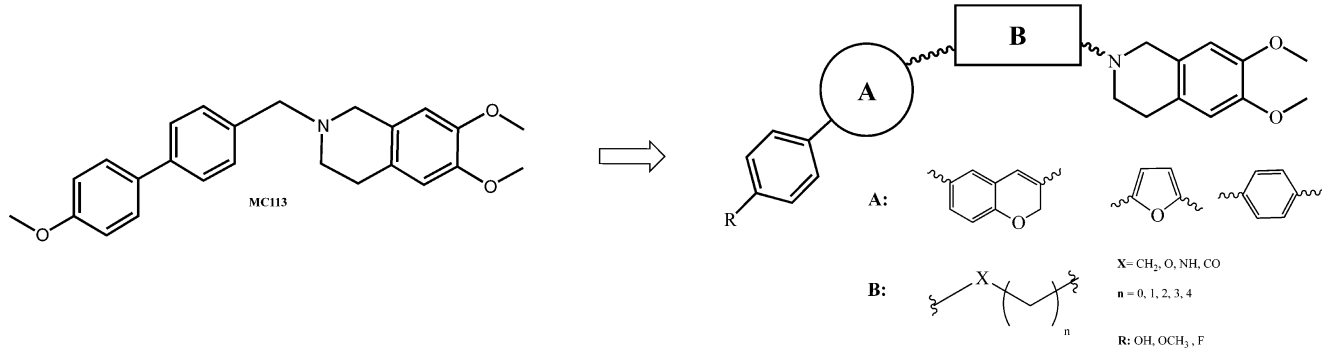

Figure 3. Structural modifications starting from **MC113**.

low P-gp-specific binding signal in vivo, probably due to its poor selectivity.[18,19]

Despite these results, starting from **MC113**, new P-gp ligands, with higher activity and selectivity with respect to that of the lead compound, have been synthesized. In particular, a series of molecular modifications were planned, and new P-gp modulators have been developed (Figure 3).

The first set of compounds has been designed as bioisosters of **MC113** in which the biphenyl fragment has been replaced with a 2-arylfuran moiety (Figure 4) that is variously substituted. In a previous work, 2-aryloxazole and 2-arylthiazole derivatives were synthesized as bioisosters of a 4-biphenyl series.[20]

Conversely, a second set of molecules was designed by introducing, between the biphenyl fragment and the basic moiety, an elongated spacer with the presence of O, CO, and NH

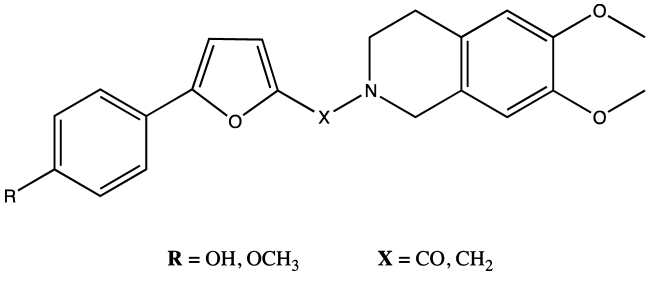

Figure 4. 2-Arylfuran derivatives.

(Figure 5). The aim of this approach was to evaluate the effect on activity and selectivity resulting from an increase in conformational flexibility in order to establish if a greater distance between

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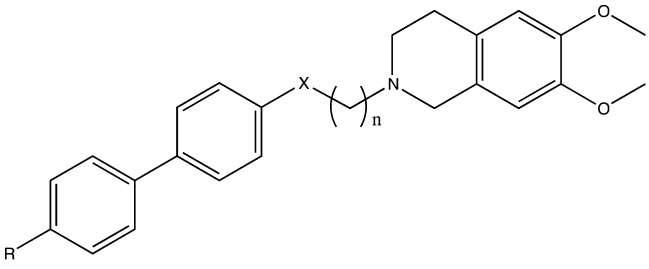

R = OH, OCH_3, F **X** = CH_2, O, NH **n** = 0, 1, 2, 3, 4

Figure 5. Elongated analogues of **MC113**.

basic and nonbasic moieties could be useful for targeting both substrate binding sites present in P-gp structure (Figure 6).[6,21]

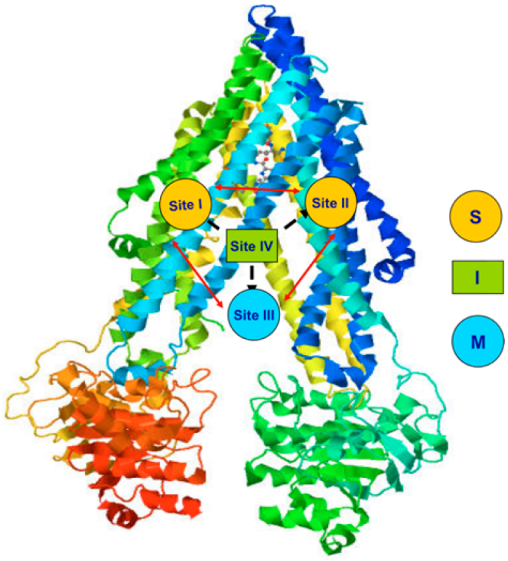

Figure 6. P-gp (PDB ID: 3G61) binding sites: S (substrate), I (inhibitor), and M (modulator).

Moreover the introduction of O, CO, and NH could give more information on the most suitable linkage between the tetrahydroisoquinoline and the aromatic fragment, which were confirmed to be molecular determinants for P-gp targeting.

To complete the investigation of elongated analogues of **MC113** and starting from the evidence that compounds with an O-alkyl spacer presented higher affinity toward the protein, conformationally constrained analogues, in which the O-alkyl spacer is blocked in a 2*H*-chromene nucleus (Figure 7), were prepared and evaluated in vitro.

Finally, a further attempt to better understand the active transoid configuration of **MC18** was to promote the cisoid configuration by introducing a 2-biphenyl fragment in place of the 4-biphenyl portion (Figure 8).

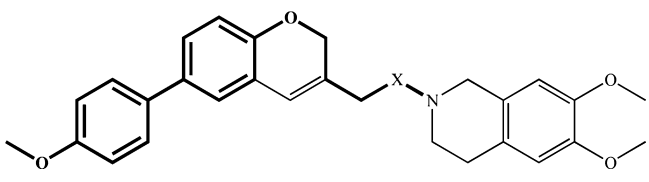

Figure 7. 2*H*-Chromene derivatives (X = CO, CH_2).

In all of the designed series, the choice of substituents was evaluated for the additional purpose of generating useful tools with easy radiolabeling points, such as a methoxy group or fluorine, for in vivo PET (^{11}C or ^{18}F) analysis to monitor P-gp activity and expression.[22−24]

RESULTS AND DISCUSSION

Chemistry. The syntheses of final compounds are depicted in Schemes 1−6. All synthesized molecules were characterized by ^1H NMR and mass spectrometry as well as, optionally, by elemental analysis.

Syntheses of Biphenyl Derivatives 6a−c,e−g (Scheme 1). The synthesis of **6a−c** started from the preparation of carboxylic acids **3a−c** by a Suzuki−Miyaura[26] coupling reaction followed by their condensation with 6,7-dimethoxytetrahydroisoquinoline to obtain amides **5a−c**, which underwent $LiAlH_4$ reduction to afford final amines **6a−c**. Derivative **6e** has been prepared starting from the Suzuki−Miyaura coupling reaction between 4-bromoanisole **1d** and 4-nitrophenylboronic acid **2d** to obtain nitro derivative **3d**,which was reduced by catalytic hydrogenation over palladium to derivative **4e**. Chloro derivative **7e**, obtained by nucleophilic substitution of 1-bromo-3-chloropropane, was condensed with 6,7-dimethoxytetrahydroisoquinoline to obtain final amine **6e**. The remaining amines, **6f** and **6g**, were prepared starting from commercially available hydroxyl derivatives **4f** and **4g** that were O-alkylated by means of 1-bromo-4-chlorobutane to give chloro derivatives **7f** and **7g**, which were condensed with 6,7-dimethoxytetrahydroisoquinoline to give amines **6f** and **6g**.

Synthesis of Biphenyl Derivative 11 (Scheme 2). Final amine **11** was prepared by N-alkylation of 6,7-dimethoxytetrahydroisoquinoline with 3-bromopropan-1-ol, obtaining alcohol **9**, which was converted to the corresponding chloride derivative **10**. Condensation of compound **10** with 6,7-dimethoxytetrahydroisoquinoline in the presence of NaH and 4′-hydroxy-4-methoxybiphenyl led to amine **11**.

Synthesis of Biphenyl Derivative 15 (Scheme 3). Hydroxyl derivative **4g** was subjected to O-alkylation using 2-chloroacetonitrile in the presence of sodium hydride (NaH) to obtain nitrile **12**, which was hydrolyzed to carboxylic acid **13**. Amide **14** was prepared by condensing acid **13** and 6,7-dimethoxytetrahydroisoquinoline. The crude was subsequently reduced to amine **15**.

Synthesis of 2-Biphenyl Derivatives 20a,b (Scheme 4). Carboxylic acids **18a,b** were prepared by a Suzuki−Miyaura coupling reaction, and they were condensed with 6,7-dimethoxytetrahydroisoquinoline to afford amides **19a,b**, which underwent $LiAlH_4$ reduction to final amines **20a,b**.

Synthesis of 2-Arylfuryl Derivatives 25a,b (Scheme 5). The starting step for the synthesis of final compounds **25a,b** consisted of a Suzuki−Miyaura coupling reaction between boronic acids **21a,b** and carboxylic acid **22** to prepare carboxylic acids **23a,b**, which were condensed with 6,7 dimethoxytetrahydroisoquinoline to obtain amides **24a,b** and subsequently reduced to corresponding amines **25a,b**.

Synthesis of 2*H*-Chromene Derivative 31 (Scheme 6). The preparation of amine **31** started from the synthesis of a 2*H*-chromene nucleus by a cyclization that involved aldehyde **26** and allyl cyanide. Nitrile **27** was hydrolyzed to the corresponding carboxylic acid **28**, which was condensed with 6,7-dimethoxytetrahydroisoquinoline to obtain amide **29**. Subsequently, a Suzuki reaction between amide **29** and 4-methoxybenzeneboronic acid

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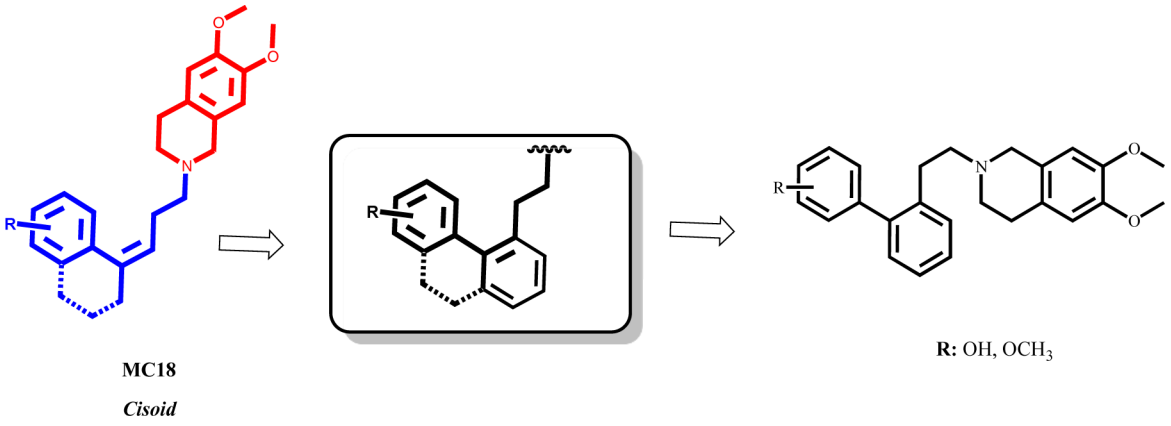
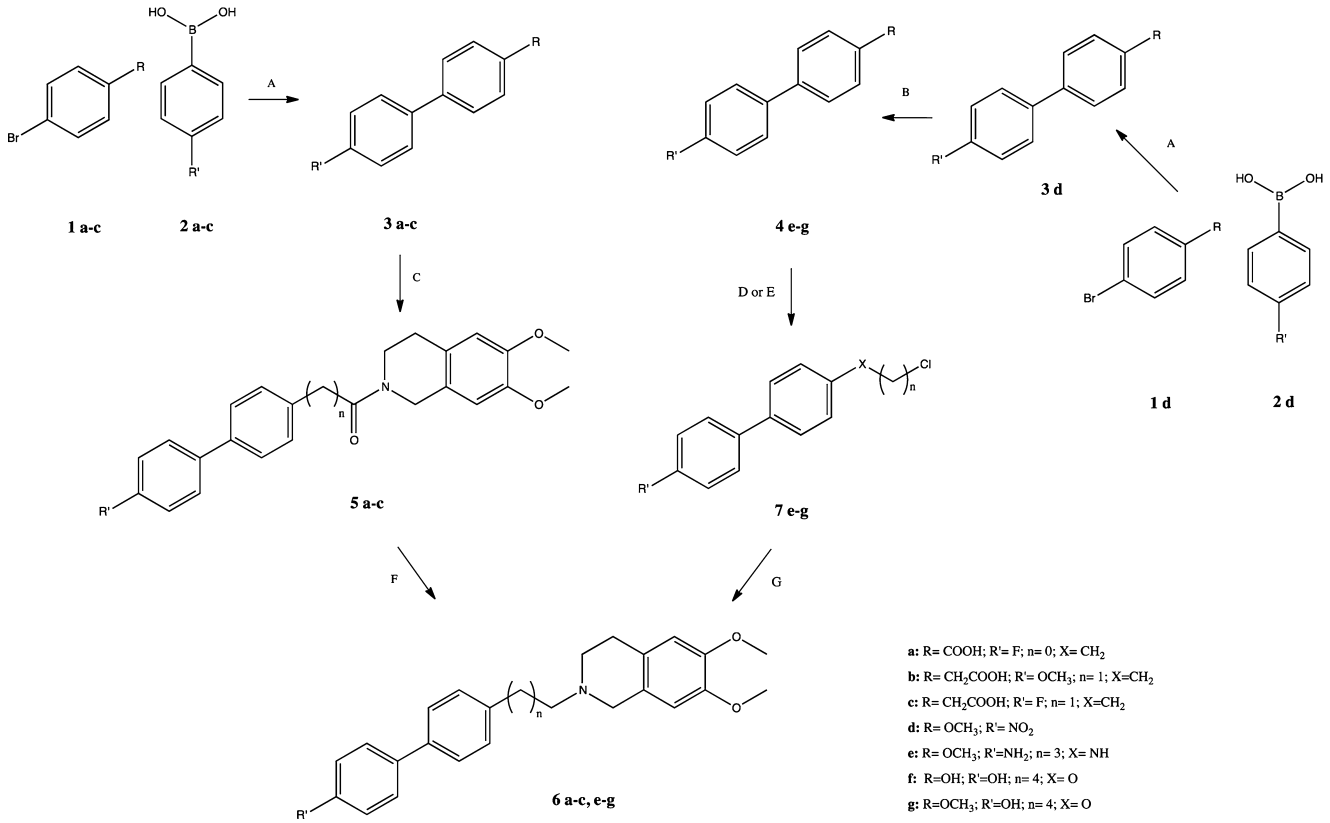


Figure 8. Promotion of the cisoid configuration of **MC18** using a 2-biphenyl fragment.

Scheme 1[a]



[a]Reagents: (A) $Pd(PPh_3)_4$, Cs_2CO_3, toluene; (B) H_2, Pd/C, EtOH; **4f** and **4g** are commercially available; (C) CDI, 6,7-dimethoxy,1,2,3,4-tetrahydroisoquinoline, THF; (D) 1-bromo-4-chlorobutane, NaH, toluene for **7f** and **7g**; (E) 1-bromo-3-chloropropane, K_2CO_3, CH_3CN for **7e**; (F) $LiAlH_4$, THF; (G) 6,7-dimethoxy,1,2,3,4-tetrahydroisoquinoline, Na_2CO_3, DMF.

obtained amide **31**, which was subjected to reduction to give amine **31**.

Biology. In order to establish the ability of the final compounds to modulate the activity of P-gp and MRP1 as well as their interacting mechanisms, each prepared ligand was studied in vitro using the following assays: (a) Calcein-AM assay to determine P-gp potency and selectivity and (b) the apparent permeability assay to investigate the interacting mechanism of the studied compound.

In particular, the effect of the tested molecules on the activities of P-gp and MRP1 were determined by evaluating Calcein accumulation in MDCK-MDR1 and MDCK-MRP1 cells overexpressing the P-gp and MRP1 transporters, respectively. Calcein-AM is a lipophilic pro-fluorescent probe that is a P-gp and MRP1 substrate. In the presence of a P-gp/MRP1 inhibitor, Calcein-AM is able to permeate the cell membrane, and it is hydrolyzed by cytosolic esterases to fluorescent Calcein. Because Calcein is hydrophilic and is not a P-gp/MRP1 substrate, it cannot cross the cell membrane, and a rapid increase in fluorescence can be measured. In Table 1, EC_{50} values are reported for each tested compound. EC_{50} values were determined by fitting the percent fluorescence increase versus log[dose].[23] Moreover, the activity of all synthesized ligands toward BCRP were studied in a Hoechst 33342 experiment[29] in

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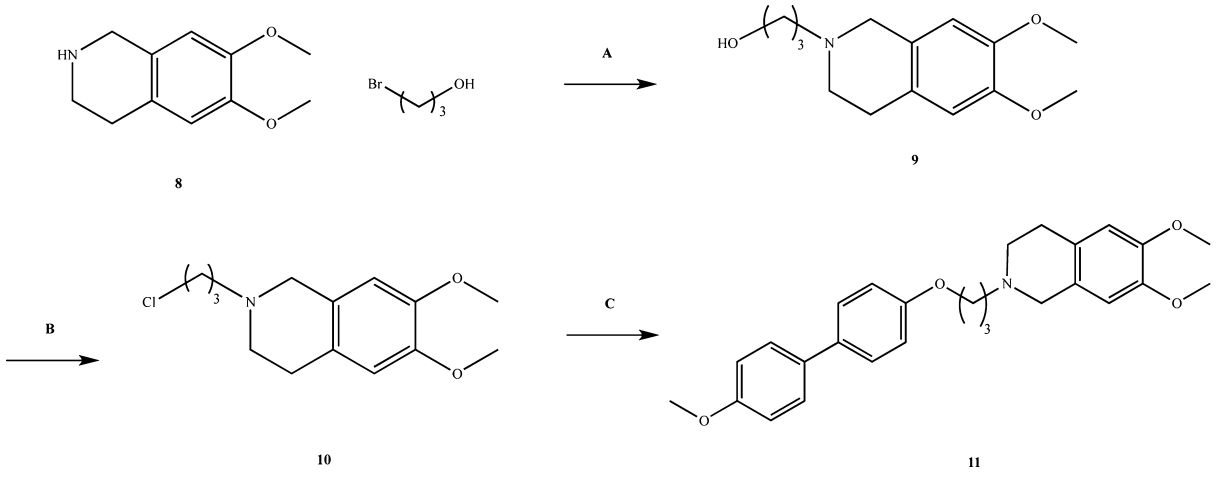
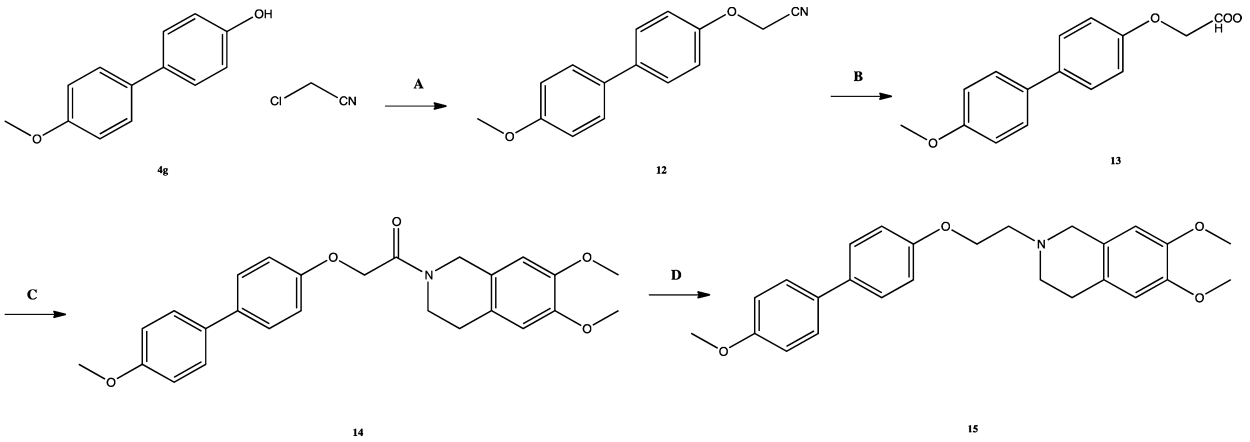
Scheme 2[a]



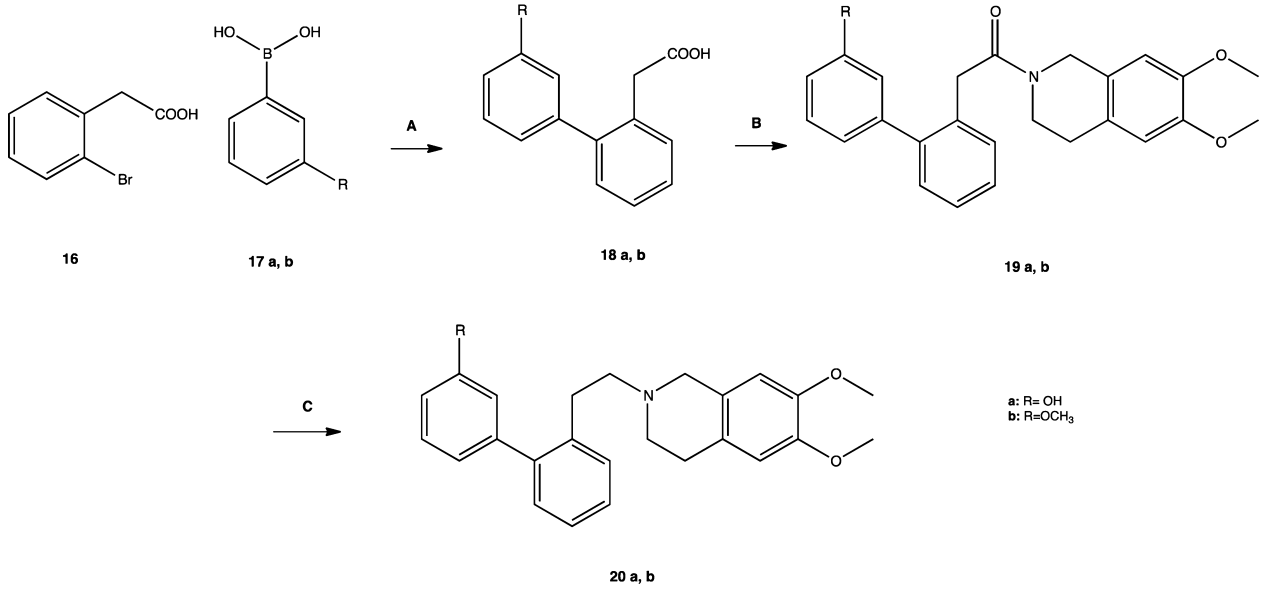
[a]Reagents: (A) K_2CO_3, CH_3CN; (B) $SOCl_2$, Et_3N; (C) NaH, DMF, 4′-hydroxy-4-methoxybiphenyl.

Scheme 3[a]



[a]Reagents: (A) NaH, toluene; (B) 2 N NaOH; (C) CDI, 6,7-dimethoxy,1,2,3,4-tetrahydroisoquinoline, THF; (D) $LiAlH_4$, THF.

Scheme 4[a]



[a]Reagents: (A) K_2CO_3, $Pd(OAc)_2$, DMGE/H_2O (3:1); (B) CDI, 6,7-dimethoxy,1,2,3,4-tetrahydroisoquinoline, THF; (C) $LiAlH_4$, THF.

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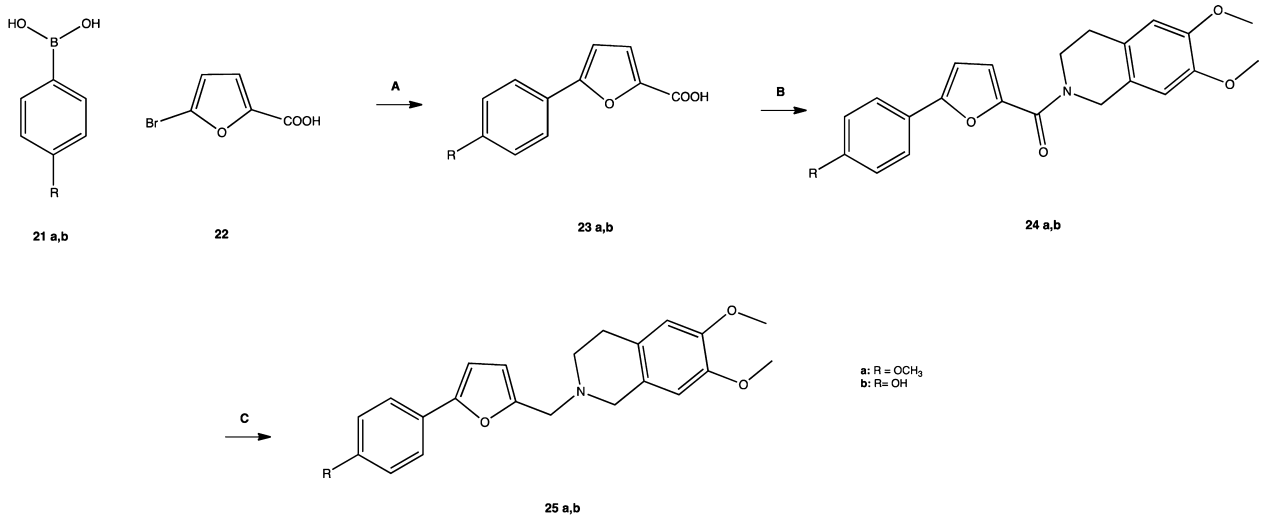
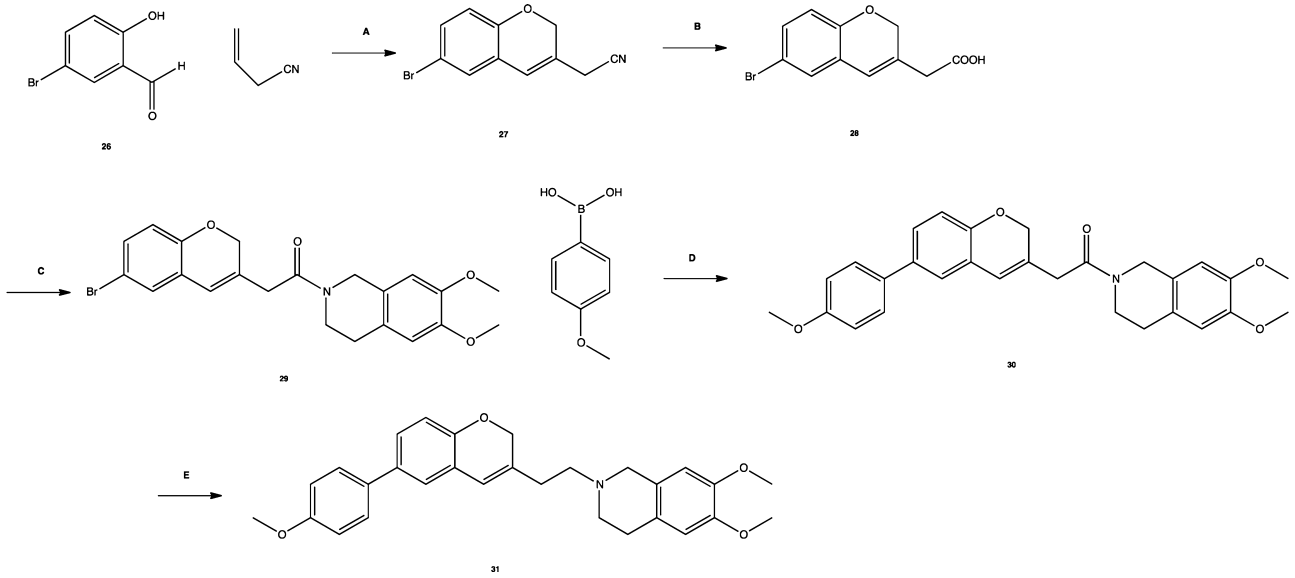
Scheme 5[a]



[a]Reagents: (A) $Pd(PPh_3)_4$, $EtOH/K_2CO_3$ s.s.; (B) CDI, 6,7-dimethoxy,1,2,3,4-tetrahydroisoquinoline, THF; (C) $LiAlH_4$, THF.

Scheme 6[a]



[a]Reagents: (A) allyl cyanide, DABCO; (B) 5 N NaOH; (C) CDI, 6,7-dimethoxy,1,2,3,4-tetrahydroisoquinoline, THF; (D) 4-methoxybenzeneboronic acid, $Pd(PPh_3)_4$, K_2CO_3, dioxane; (E) $LiAlH_4$, THF.

cells overexpressing BCRP (MDCK-BCRP). In this case, the inhibition of the pump was measured by the accumulation of the fluorescent dye Hoescht 33342 (a BCRP substrate) in the cells.

The permeability assay allows two fluxes through the cell monolayer to be studied, from the basolateral to the apical (B → A) and from the apical to the basolateral (A → B) compartments, thus predicting the interacting mechanism of the tested compound with the pump. In particular, if a studied compound is a P-gp inhibitor, then it displayed BA/AB (P_{app}, apparent permeability) < 2, whereas a P-gp substrate showed BA/AB > 2.[24] P_{app} values for each tested compound are reported in Table 1.

2-Arylfuryl Derivatives. 2-Aryloxazole and 2-arylthiazole derivatives, reported in a previous work, were designed as **MC70** bioisosteres.[20] They displayed decreased P-gp activity, and, in particular, the 2-aryloxazole moiety was found to be a versatile scaffold to obtain BCRP or MRP1 inhibitors. To complete the bioisosteric investigation, furyl derivatives were developed (Figure 4). Both methoxy (**24a**, **25a**) and hydroxy (**24b**, **25b**) substituted furyl derivatives were studied, and the difference in potency between amines and amides was evaluated. All furyl derivatives displayed good potency toward P-gp, and amide derivative **24a** displayed the best activity value (EC_{50} = 0.91 μM). This compound displayed better activity than that of the corresponding amine **25a** (EC_{50} = 4.3 μM). The hydroxyl amino and amido derivatives (**24b**, **25b**, respectively) displayed similar potency toward the pump, and both compounds were less potent than that of the methoxy derivatives. All tested furyl compounds are not active toward MRP1, and the furyl scaffold could be considered a useful fragment to gain P-gp selectivity. Moreover, it seems that, in addition to good selectivity, this bioisosteric substitution resulted in molecules with P_{app} > 2 that behaved as P-gp substrates.

Elongated Derivatives. The second set included molecules with various elongated spacers, between the biphenyl fragment

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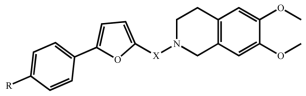
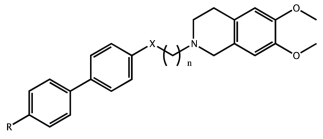
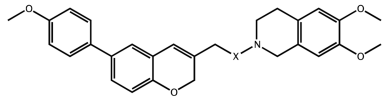
Table 1. Biological Data



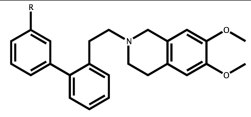
	R	X	MDR1 EC_{50} μM[a]± SEM	MRP1 EC_{50} μM[a]± SEM	P_{app}
24a[b]	OCH_3	C=O	0.91 ± 0.11	NA	5.2
24b[b]	OH	C=O	6.4 ± 0.20	NA	4.5
25a[b]	OCH_3	CH_2	4.3± 0.12	NA	3.7
25b[b]	OH	CH_2	8.1± 0.21	NA	5.7



	R	X	n	MDR1 EC_{50} μM[a]± SEM	MRP1 EC_{50} μM[a]± SEM	P_{app}
5b[b]	OCH_3	CH_2CO	0	0.80 ± 0.12	10	3.9
6b[b]	OCH_3	CH_2	1	0.57 ± 0.01	NA	38
6c[b]	F	CH_2	1	2.0 ± 0.13	NA	3.8
6a[b]	F	CH_2	0	4.9 ± 0.11	NA	3.8
11[b]	OCH_3	O	3	1.3 ± 0.30	NA	7.9
15[b]	OCH_3	O	2	0.93 ± 0.09	NA	47
6e[b]	OCH_3	NH	3	1.8 ± 0.12	NA	2.2
6f[b]	OH	O	4	NA	NA	1.5
6g[b]	OCH_3	O	4	0.004 ± 0.0001	NA	6.6



	R	X	MDR1 EC_{50} μM[a]± SEM	MRP1 EC_{50} μM[a]± SEM	P_{app}
31[b]	OCH_3	CH_2	1.2 ± 0.11	NA	4.9
30[b]	OCH_3	CO	5.8 ± 0.21	NA	3.2



	R	MDR1 EC_{50} μM[a]± SEM	MRP1 EC_{50} μM[a]± SEM	P_{app}
20a[b]	OH	0.23 ± 0.01	NA	3.2
20b[b]	OCH_3	0.069 ± 0.002	23	4.1

	MDR1 EC_{50} μM[a]± SEM	MRP1 EC_{50} μM[a]± SEM	P_{app}
MC18	1.5 ± 0.12	2.80	1.6
MC266	6.35 ± 0.11	NA	4.4
MC70	9.30 ± 0.01	9.30±0.22	1.3
MC113	0.60 ± 0.01	NA	4.4

[a]Data are the mean of three-independent determinations of duplicate samples. [b]Inactive toward BCRP.

and the basic moiety, optionally interrupted by O, CO, and NH, as reported in Figure 5. The first planned compound, in order to evaluate the effect of a progressive elongation of the alkyl spacer, was **6b**, derived from the insertion of one more methylene unit with respect to that in **MC113**. This compound displayed good activity (EC_{50} = 0.57 μM) and good selectivity toward P-gp (not active toward MRP1). P_{app} was greater than 2, and this value is in accordance with that of **MC113** (P_{app} = 4.4). The data found for **6b** was also compared with the activity and permeability values obtained for the corresponding amide derivative **5b**. The transition from the amine to the amide resulted in a loss of selectivity, but no relevant changes in the activity and interacting mechanism were observed. The second modification consisted of the insertion of a fluorine substituent in place of the methoxy group, both in elongated (n = 1, **6c**) or non-elongated (n = 0, **6a**) derivatives. Compounds **6a** and **6c** displayed less activity toward P-gp (EC_{50} = 4.9 and 2 μM, respectively) with respect to that of **MC113** and **6b**. A severe loss of selectivity was also observed in fluorine derivatives with respect to their corresponding methoxy-substituted compounds. Conversely, the permeability maintained a value greater than 2.

Derivatives **15**, **11**, and **6g** were designed by inserting an O-alkyl spacer, which has two (**15**), three (**11**), and four (**6g**) methylene units. The effect of this modification was an increase in activity for all compounds, whereas the selectivity was unchanged with respect to that of lead compound **MC113**. In particular, **6g** (O-alkyl with four methylene units) displayed the best P-gp activity, displaying activity in the nanomolar range (EC_{50} = 0.4 nM). Derivatives **16** and **11**, with shorter spacers (n = 2 and 3), displayed good potency (EC_{50} = 0.93 and 1 μM, respectively) toward the protein. All compounds bearing an O-alkyl spacer were inactive toward MRP1, and they displayed P_{app} values greater than 2, similar to that of **MC113**. The greater distance between the determinant portions that characterized these molecules seemed to improve the activity toward the protein. Specifically, the observed increase in potency could be due to the presence of a higher degree of flexibility that led the molecules to match the substrate binding sites of the protein in the most optimal way. Compound **6g** had the highest degree of flexibility and thus a higher possibility of reaching a conformation that is more suitable for matching the substrate binding sites.

In this series, the effect of the presence of a hydroxyl group, as a substituent on the biphenyl moiety, was investigated. Compound **6f**, bearing a hydroxyl group on the 4-biphenyl fragment, was inactive toward the target protein, whereas its P_{app} value, less than 2, suggests an interaction with other efflux transporters.

In addition, the presence of a NH group was evaluated in order to determine how the nature of the spacer influences activity and

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selectivity toward P-gp. The NH group was inserted in the alkyl spacer of compound **6e**, which has three methylene units. The final effect of this replacement of O with NH did not lead to changes in potency or selectivity.

2*H*-Chromene Derivatives. Starting from the evidence that compound **6g**, with an O-alkyl spacer of four methylene units, displayed the best P-gp potency value and in order to investigate the role of flexibility, conformationally constrained analogues in which the O-alkyl spacer is blocked in a 2*H*-chromene nucleus (Figure 6) were developed. Both amine derivative **31** and amide **30** showed less potency toward P-gp with respect to that of **6g**. They are inactive toward MRP1 and showed $P_{app} > 2$. Therefore, the conformational restriction led to compounds that are less active, and it is evident that flexibility could be a requirement to improve P-gp activity.

2-Biphenyl Derivatives. All performed studies originated with the aim of optimizing lead compound **MC113**, derived from a conformational restriction of the transoid active configuration of the P-gp inhibitor **MC18**. Another effort was to investigate the cisoid configuration of **MC18** in order to establish if the transoid configuration is important to inhibit the pump or if it is an outmoded requirement. Both compounds **20a** and **20b** displayed good potency toward P-gp (EC_{50} = 0.23 and 0.069 μM, respectively), but **20a** displayed better selectivity with respect to that of **20b**. P_{app} was greater than 2 for both 2-biphenyl derivatives, so they can be considered P-gp substrates.

CONCLUSIONS

These SAR studies led to the investigation of whether a biphenyl fragment could be considered an essential moiety for obtaining potent and selective P-gp ligands. The obtained results led us to discover two potent P-gp substrates: 2-biphenyl derivative **20b** and 4-biphenyl long-chain derivative **6g**. Both compounds displayed P-gp activity in the nanomolar range (0.069 μM for **20b** and 0.004 μM for **6g**).

Moreover, all compounds, tested in MDCK-BCRP cells,[29] were found to be inactive at less than 100 μM. These results and the activity of the ligands toward MRP1 allowed us to conclude that, with the exception of compounds **5b** and **20b**, all of the tested modifications, such as bioisosterism, spacer elongation, and conformational restriction, led to P-gp-selective ligands with variable potency respect to reference compound, on the basis of no basic fragment linked to the tetrahydroisoquinoline nucleus.

EXPERIMENTAL SECTION

Chemistry. General Procedures. 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. ^{1}H NMR spectra were recorded in $CDCl_3$ or DMSO-d_6 at 300 MHz on a Varian Mercury-VX spectrometer. All spectra were recorded on the free bases. All chemical shift values are reported in ppm (δ). Recording of mass spectra was done on an HP6890-5973MSD gas chromatograph/mass spectrometer; only significant m/z peaks, with their percentage of relative intensity given in parentheses, are reported. All spectra were in accordance with the assigned structures. ESI-MS analyses were performed on an Agilent 1100LC/MSD trap system VL. Purity of final compounds was established by combustion analysis of the corresponding hydrochloride salts, confirming a purity ≥95%.

General Procedure for Synthesis of Biphenyls 3a–d, 18a, and 18b, Aryloxazoles 23a,b, and Phenylchromene 30. The appropriate halides (1 mmol) and organoboronic acid (2 mmol) were reacted by palladium-catalyzed Suzuki reaction. Compounds **3a–d** were reacted in the presence of Cs_2CO_3 (1.5 mmol) and $Pd(PPh_3)_4$ (0.03 mmol) in toluene (20 mL) at 95 °C for 4 h. Biphenyls **18a,b** were reacted with K_2CO_3 (0.06 mmol) and $Pd(OAc)_2$ in DMGE/H_2O (3:1) at refluxing temperature overnight. Compounds **23a,b** were refluxed in EtOH (20 mL) in the presence of $Pd(PPh_3)_4$ (0.1 mmol) and K_2CO_3 s.s. (1 mmol) for 4–6 h. Compound **30** was synthesized using K_2CO_3 (3 mmol) and $Pd(PPh_3)_4$ (0.03 mmol) in dioxane (30 mL). HCl 1 N (15 mL) and AcOEt (15 mL) were added to the reaction mixture. Then, the solid was filtered and washed with $NaHCO_3$ ss and NaCl ss. The organic phase was separated, dried over Na_2SO_4, and evaporated. The residue was purified by silica gel column chromatography.

4′-Fluoro-[1,1′-biphenyl]-4-carboxylic Acid (3a). White solid. Eluted with $CHCl_3$. Yield 54%. ^{1}H NMR ($CDCl_3$) δ 7.29–7.42 (m, 4H), 7.83–7.85 (m, 2H), 7.61–7.70 (m, 2H), 10.50 (br s, 1H, exchangeable with D_2O). ESI$^-$/MS m/z: 215 [M–H]$^-$; ESI$^-$/MS/MS m/z: 171 (100)

2-(4′-Methoxy-[1,1′-biphenyl]-4-yl)acetic Acid (3b). Yellow solid. Eluted with $CHCl_3$. Yield 52%. ^{1}H NMR ($CDCl_3$) δ 3.70 (s, 2H, CH_2), 3.83 (s, 3H, CH_3), 6.90–7.58 (m, 8H). ESI$^-$/MS m/z: 241 [M − H]$^-$; ESI$^-$/MS/MS m/z: 197 (100), 198 (18).

2-(4′-Fluoro-[1,1′-biphenyl]-4-yl)acetic Acid (3c). White solid. Eluted with $CHCl_3$/AcOEt (8:2). Quantitative yield. ^{1}H NMR ($CDCl_3$) δ 3.69 (s, 2H), 7.15–7.39 (m, 8H), 10.80 (br s, 1H, exchangeable with D_2O). ESI$^-$/MS m/z: 229 [M − H]$^-$; ESI$^-$/MS/MS m/z: 185 (100).

4-Methoxy-4′-nitro-1,1′-biphenyl (3d). Yellow solid. Eluted with $CHCl_3$/AcOEt (8:2). Quantitative Yield. ^{1}H NMR ($CDCl_3$) δ 3.83 (s, 3H), 7.06–7.11 (m, 2H), 7.37–7.39 (m, 2H), 7.79–7.81 (m, 2H), 8.22–8.24 (m, 2H), 10.60 (br s, 1H, exchangeable with D_2O). GC-MS m/z: 229 (M$^+$, 100), 199 (22), 168 (21), 139 (45).

(3′-Hydroxybiphenyl-2-yl)acetic Acid (18a). Yellow oil. Eluted with $CHCl_3$/AcOEt (4:6). Yield 35%. ^{1}H NMR ($CDCl_3$) δ 3.62 (s, 2H) 5.29, (bs, 1H), 6.76–6.78 (m, 1H), 7.08–7.10 (m, 1H), 7.31–7.34 (m, 5H), 7.66–7.68 (m, 1H), 10.62 (br s, 1H, exchangeable with D_2O). ESI$^-$/MS m/z: 227 [M − H]$^-$; ESI$^-$/MS/MS m/z: 182.

2-(3′-Methoxy-[1,1′-biphenyl]-2-yl)acetic Acid (18b). White solid. Eluted with $CHCl_3$. Yield 11%. ^{1}H NMR ($CDCl_3$) δ 3.63 (s, 2H), 3.79 (s, 3H), 6.86–6.88 (m, 1H), 7.09–7.10 (m, 1H), 7.32–7.34 (m, 5H), 7.67–7.68 (m, 1H), 10.50 (br s, 1H, exchangeable with D_2O). ESI$^-$/MS m/z: 241 [M − H]$^-$; ESI$^-$/MS/MS m/z: 197 (100).

5-(4-Methoxyphenyl)furan-2-carboxylic Acid (23a). White solid. Eluted with $CHCl_3$/AcOEt (1:1). Yield 40%. ^{1}H NMR ($CDCl_3$) δ 3.82 (s, 3H), 7.06–7.11 (m, 2H), 7.40–7.42 (m, 2H), 7.69–7.70 (m, 2H), (m, 2H), 11 (br s, 1H, exchangeable with D_2O). ESI$^-$/MS m/z: 217 [M − H]$^-$; ESI$^-$/MS/MS m/z: 173 (100), 159 (50).

5-(4-Hydroxyphenyl)furan-2-carboxylic Acid (23b). White solid. Eluted with $CHCl_3$/AcOEt (1:1). Yield 69%. ^{1}H NMR ($CDCl_3$) δ 3.82 (s, 3H), 5.36 (br, 1H, exchangeable with D_2O), 7.06–7.11 (m, 2H), 7.40–7.42 (m, 2H), 7.62–7.65 (m, 2H), (m, 2H), 11 (br s, 1H, exchangeable with D_2O). ESI$^-$/MS m/z: 203 [M − H]$^-$; ESI$^-$/MS/MS m/z: 159 (100).

1-(6,7-Dimethoxy-3,4-dihydroisoquinolin-2(1*H*)-yl)-2-(6-(4-methoxyphenyl)-2*H*-chromen-3-yl)ethanone (30). Yellow oil. Eluted with hexane/AcOEt 7:3. Yield 21%. ^{1}H NMR ($CDCl_3$) δ 2.76–2.85 (m, 6H), 3.74–3.89 (m, 2H), 3.84 (s, 6H), 3.86 (s, 3H), 4.61 (s, 2H), 7.32 (s, 1H), 6.57–7.82 (m, 9H). ESI$^+$/MS m/z: 494 [M + Na]$^+$; ESI$^+$/MS/MS m/z: 304 (100).

General Procedure for Synthesis of Amides 5a–c, 14, 19a,b, 24a,b, and 29. A mixture of the appropriate carboxylic acid (1 mmol) and 1,1′-carbonyldiimidazole (1.1 mmol) in dry THF was stirred at room temperature overnight. A solution of 6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline (1 mmol) in dry THF was dropped into the reaction complex. The mixture was stirred at room temperature for 4 h. Fifty milliliters of H_2O was added to the reaction mixture, the aqueous phase was re-extracted with AcOEt (3 × 50 mL), and the combined solution was dried over Na_2SO_4 and evaporated. The residue was purified by silica gel column chromatography.

(6,7-Dimethoxy-3,4-dihydroisoquinolin-2(1*H*)-yl)(4′-fluoro-[1,1′-biphenyl]-4-yl)methanone (5a). Yellow oil. Eluted with $CHCl_3$/AcOEt 9:1. Yield 36%. ^{1}H NMR ($CDCl_3$) δ 2.74–2.95 (m, 4H), 3.80 (s, 2H), 3.83 (s, 3H), 3.86 (s, 3H), 6.59–6.61 (m, 2H), 7.12–7.17 (m, 1H), 7.31–7.34 (m, 1H), 7.53–7.61 (m, 6H). ESI$^+$/MS m/z: 414 [M + Na]$^+$; ESI$^+$/MS/MS m/z: 265 (8).

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1-(6,7-Dimethoxy-3,4-dihydroisoquinolin-2(1*H*)-yl)-2-(4′-methoxy-[1,1′-biphenyl]-4-yl)ethanone (5b). Yellow solid. Eluted with $CHCl_3$. Yield 36%. ^{1}H NMR ($CDCl_3$) δ 2.63 (m, 1H), 2.83 (m, 1H), 3.64 (m, 2H), 3.91−4.22 (m, 11 H), 4.53 (s, 1H), 4.73 (s, 1H), 6.55 (s, 1 H), 6.62 (s, 1H), 6.95 (d, 2H, J = 8 Hz), 7.25−7.30 (m, 2H), 7.48−7.53 (m, 4H). ESI$^+$/MS m/z: 418 $[M + H]^+$; ESI$^+$/MS/MS m/z: 194 (100), 165 (54). Anal. C, H, N [$C_{26}H_{27}NO_4$]. mp 144−146 °C.

1-(6,7-Dimethoxy-3,4-dihydroisoquinolin-2(1*H*)-yl)-2-(4′-fluoro-[1,1′-biphenyl]-4-yl)ethanone (5c). Yellow oil. Eluted with $CHCl_3$/AcOEt 1:1. Yield 40%. ^{1}H NMR ($CDCl_3$) δ 3.11−3.13 (m, 2H) 3.82−3.86 (m, 12H), 6.54 (s, 1H), 6.61 (s, 1H), 7.29−7.33 (m, 8H). GC-MS m/z: 405 (M^+, 100), 220 (62), 185 (54).

1-(6,7-Dimethoxy-3,4-dihydroisoquinolin-2(1*H*)-yl)-2-((4′-methoxy-[1,1′-biphenyl]-4-yl)oxy)ethanone (14). Yellow solid. Eluted with $CHCl_3$/AcOEt 1:1. Yield 31%. ^{1}H NMR ($CDCl_3$) δ 2.66−2.88 (m, 2H), 3.77−3.86 (m, 2H), 3.83 (s, 3H), 3.86 (s, 3H), 4.68 (s, 2H), 4.79 (s, 2H), 6.83 (s, 1H), 6.85 (s, 1H), 7.02−7.14 (m, 4H) 7.37−7.58 (m, 4H). ESI$^+$/MS m/z: 456 $[M + H]^+$; ESI$^+$/MS/MS m/z: 335 (100), 216 (29).

1-(6,7-Dimethoxy-3,4-dihydroisoquinolin-2(1*H*)-yl)-2-(3′-hydroxy-[1,1′-biphenyl]-2-yl)ethanone (19a). White oil. Eluted with $CHCl_3$/AcOEt 1:1. Yield 40%. ^{1}H NMR ($CDCl_3$) δ 2.45 (m, 1H) 2.70 (m, 1H), 3.10−3−12 (m, 2H), 3.60−3.87 (m, 8H), 4.20 (s, 1H), 4.63 (s, 1H), 5.69 (br s, 1H, exchangeable with D_2O), 6.37 (s, 1H), 6.39 (s, 1H), 6.92 (m, 1H), 7.29−7.39 (m, 6H), 7.60−7.62 (m, 1H). ESI$^-$/MS m/z: 403 $[M - H]^-$; ESI$^-$/MS/MS m/z: 387 (100), 209 (23).

1-(6,7-Dimethoxy-3,4-dihydroisoquinolin-2(1H)-yl)-2-(3′-methoxy-[1,1′-biphenyl]-2-yl)ethanone (19b). Yellow oil. Eluted with CH_2Cl_2/AcOEt (95:5). Quantitative yield. ^{1}H NMR ($CDCl_3$) δ 2.40−2.44 (m, 1H), 2.68−2.72 (m, 1H), 3.30−3.34 (m, 1H), 3.47−3.59 (m, 1H), 3.74−3.91 (m, 11H), 4.12 (s, 1H), 4.12 (s, 1H), 6.52 (s, 1H), 6.59 (s, 1H), 6.82−6.92 (m, 2H), 7.24−7.34 (m, 5H), 7.40−7.42 (m, 1H). ESI$^+$/MS m/z: 418 $[M + H]^+$; ESI$^+$/MS/MS m/z: 213 (18), 194 (100).

(6,7-Dimethoxy-3,4-dihydro-1*H*-isoquinolin-2-yl)-[5-(4-methoxyphenyl)-2-furyl]methanone (24a). White solid. Eluted with $CHCl_3$. Yield 93%. ^{1}H NMR ($CDCl_3$) δ 2.92−2.99 (m, 2H), 3.84 (s, 3H), 3.86 (s, 3H), 3.87 (s, 3H), 3.91−4.11 (m, 2H), 4.78−4.92 (m, 2H), 6.65 (s, 1H), 6.66 (s, 1H), 7.07−7.12 (m, 3H), 7.41 (d, 1H, J = 3.3 Hz), 7.67 (d, 2H, J = 8 Hz). ESI$^+$/MS m/z: 416 $[M + Na]^+$; ESI$^+$/MS/MS m/z: 302 (100), 171 (34). Anal. [$C_{23}H_{23}NO_5$] C, H, N. mp 175 °C. Recrystallized from $CHCl_3$/hexane.

(6,7-Dimethoxy-3,4-dihydroisoquinolin-2(1*H*)-yl)(5-(4-hydroxyphenyl)furan-2-yl)methanone (24b). White solid. Eluted with $CHCl_3$. Yield 33%. ^{1}H NMR (DMSO) δ 2.82−2.88 (m, 2H), 3.71 (s, 3H), 3.72 (s, 3H), 3.85−3.92 (m, 2H), 4.63−4.81 (m, 2H), 6.75 (s, 1H), 6.76 (s, 1H), 6.86 (d, 2H, J= 8 Hz), 7.13 (d, 1H, J = 3.3 Hz), 7.35 (d, 1H, J = 3.3 Hz), 7.81 (d, 2H), 9.77 (br s, 1H, OH exchangeable with D_2O). ESI$^-$/MS m/z: 378 $[M - H]^-$; ESI$^-$/MS/MS m/z: 363 (100), 187 (38). ESI$^+$/MS m/z: 402 $[M + Na]^+$; ESI$^+$/MS/MS m/z: 355 (35), 301 (100), 168 (58). Anal. [$C_{22}H_{21}O_5$] C, H, N. mp 110−112 °C. Recrystallized from $CHCl_3$/hexane.

2-(6-Bromo-2*H*-chromen-3-yl)-1-(6,7-dimethoxy-3,4-dihydroisoquinolin-2(1*H*)-yl)ethanone (29). Brown oil. Eluted with CH_2Cl_2/MeOH 98:2. Yield 48%. ^{1}H NMR ($CDCl_3$) δ 2.76−2.86 (m, 6H), 3.84 (s, 3H), 3.86 (s, 3H), (s, 2H), 4.46 (s, 2H), 6.84 (s, 1H), 6.86−6.88 (m, 2H), 7.32−7.35 (m, 2H), 8.07 (d, 1H, J = 2 Hz). ESI$^+$/MS m/z: 466 $[M + Na]^+$; ESI$^+$/MS/MS m/z: 275 (62), 214 (100).

4′-Methoxybiphenyl-4-amine (4e). A solution of biphenyl **3d** (1 mmol) in EOH (40 mL) and Pd/C 10% (1 mol) was purged with H_2. The reaction mixture was stirred under 1 atm H_2(g) at room temperature for 8 h. The resulting mixture was filtered, and the filtrate was concentrated to provide an oil that was purified on a silica gel column. Pink solid. Eluted with $CHCl_3$. Yield 52%. ^{1}H NMR ($CDCl_3$) δ 3.52 (s, 2H), 3.75 (s, 3H, OCH_3), 6.94 (d, 2H, J = 8 Hz), 7.03 (d, 2H, J = 8 Hz), 7.38 (d, 2H, J = 8 Hz), 7.52 (d, 2H, J = 8 Hz). GC-MS m/z: 199 (M^+, 100), 184 (86), 156 (28). mp 238−240 °C.

***N*-(3-Chloropropyl)-4′-methoxybiphenyl-4-amine (7e).** A solution of biphenyl **4e** (1 mmol), 1-bromo-3-chloropropane (0.6 mmol), and K_2CO_3 (0.7 mmol) in ACN (50 mL) was stirred for 4 h. The solvent was evaporated, and H_2O (40 mL) and $CHCl_3$ (40 mL) were added. The organic phase was separated, the aqueous phase was extracted with $CHCl_3$ (3 × 50 mL), and the combined solution was dried over Na_2SO_4 and evaporated. The residue was purified by silica gel column chromatography. Brown oil. Eluted with $CHCl_3$/MeOH (9:1). Yield 20%. ^{1}H NMR ($CDCl_3$) δ 2.14−2−16 (m, 2H), 3.35−3.37 (m, 2H), 3.68−3.71 (m, 2H), 6.71 (d, 2H, J = 8 Hz), 7.10 (d, 2H, J = 8 Hz), 7.57 (d, 2H, J = 8 Hz), 7.68 (d, 2H, J = 8 Hz). GC-MS m/z: 275 (M^+, 52), 212 (100).

General Procedure for Synthesis of 7f,g and 12. A solution of biphenyl **4f** or **4g** (commercially available) or **11** (1 mmol) in dry toluene (10 mL) was added to a suspension of NaH (0.5 mmol) in dry toluene (15 mL) and was stirred for 1 h. Chloroalkylderivative (2 mmol) in dry toluene (5 mL) was added, and the reaction mixture was refluxed overnight. H_2O was added until effervescence ceased. The aqueous phase was extracted with $CHCl_3$ (3 × 50 mL), and the combined solution was dried over Na_2SO_4 and evaporated. The residue was purified by silica gel column chromatography.

4′-(4-Chlorobutoxy)biphenyl-4-ol (7f). White solid. Eluted with $CHCl_3$/ MeOH (95:5). Yield 22%. ^{1}H NMR ($CDCl_3$) δ 1.76−1.78 (m, 4H), 3.66−3.68 (m, 2H), 4.06 (m, 2H), 5.01 (br s, 1H, exchangeable with D_2O), 6.85 (d, 2H, J = 8 Hz), 7.06 (d, 2H, J = 8 Hz), 7.62 (d, 2H, J = 8 Hz), 7.69 (d, 2H, J = 8 Hz). GC-MS m/z: 276 (M^+, 39), 186 (100).

4-(4-Chlorobutoxy)-4′-methoxy-1,1′-biphenyl (7g). White solid. Eluted with $CHCl_3$. Yield 63%. ^{1}H NMR ($CDCl_3$) δ 1.77−1.80 (m, 4H), 3.66−3.68 (m, 2H), 3.86 (s, 3H), 4.03 (m, 2H), 6.83 (d, 2H, J = 8 Hz), 6.85 (d, 2H, J = 8 Hz), 7.62 (d, 2H, J = 8 Hz), 7.67 (d, 2H, J = 8 Hz). GC-MS m/z: 290 (M^+, 100), 200 (91), 185 (65).

2-((4′-Methoxy-[1,1′-biphenyl]-4-yl)oxy)acetonitrile (12). White solid. Eluted with $CHCl_3$. Yield 96%. ^{1}H NMR ($CDCl_3$) δ 3.85 (s, 3H), 4.79 (s, 2H), 6.94−7.03 (m, 4H), 7.37−7.55 (m, 4H). ESI$^+$/MS m/z: 262 $[M + Na]^+$; ESI$^+$/MS/MS m/z: 179 (100).

General Procedure for Synthesis of Amines 6a−c, 15, 20a,b, 25a,b, and 31. A solution of appropriate amide **5a−c** (1 mmol) was added to a suspension of $LiAlH_4$ (2 mmol) in dry THF and refluxed for 2 h. Water was added to the cooled reaction until effervescence ceased. The aqueous phase was extracted with Et_2O (3 × 50 mL), and the combined solution was dried over Na_2SO_4 and evaporated. The residue was purified by silica gel column chromatography.

2-((4′-Fluoro-[1,1′-biphenyl]-4-yl)methyl)-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline (6a). White solid. Eluted with $CHCl_3$/MeOH (9:1). Yield 40%. ^{1}H NMR ($CDCl_3$) δ 2.77−2.98 (m, 4H) 3.57 (s, 2H), 3.73 (s, 2H), 3.77 (s, 3H), 3.84 (s, 3H), 6.49 (s, 1H), 6.60 (s, 1H), 7.09−7.12 (m, 2H) 7.40−7.58 (m, 6H). Anal. C, H, N [$C_{24}H_{24}NO_2F{\cdot}2H_2O{\cdot}HCl$]. mp 198−201 °C.

6,7-Dimethoxy-2-(2-(4′-methoxy-[1,1′-biphenyl]-4-yl)ethyl)-1,2,3,4-tetrahydroisoquinoline (6b). White solid. Eluted with $CHCl_3$. Yield 26%. ^{1}H NMR ($CDCl_3$) δ 2.67−3.10 (m, 8H), 3.67 (s, 2H), 3.85 (s, 9H), 6.56 (s, 1 H), 6.68 (s, 1H), 7.01 (d, 2H, J = 8 Hz) 7.26−7.30 (m, 2H), 7.47−7.55 (m, 4H). ESI$^+$/MS m/z: 404 $[M + H]^+$; ESI$^+$/MS/MS m/z: 240 (36), 211 (100), 179 (78). Anal. C, H, N [$C_{26}H_{29}NO_3{\cdot}2H_2O_2{\cdot}HCl$]. mp 214−216 °C.

2-(2-(4′-Fluoro-[1,1′-biphenyl]-4-yl)ethyl)-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline (6c). White solid. Eluted with $CHCl_3$. Yield 58%. ^{1}H NMR ($CDCl_3$) δ 2.77−2.98 (m, 8H) 3.64 (s, 2H), 3.85 (s, 6H), 6.54 (s, 1H), 6.60 (s, 1H), 7.09−7.12 (m, 2H) 7.29−7.32 (d, 2H, J = 8 Hz), 7.46−7.59 (m, 4H). ESI$^+$/MS m/z: 414 $[M + Na]^+$; ESI$^+$/MS/MS m/z: 240 (36), 211 (100), 179 (78). Anal. C, H, N [$C_{26}H_{29}NO_2F{\cdot}HCl$]. mp 202−203 °C.

6,7-Dimethoxy-2-(2-((4′-methoxy-[1,1′-biphenyl]-4-yl)oxy)-ethyl)-1,2,3,4-tetrahydroisoquinoline (15). Yellow solid. Eluted with $CHCl_3$/AcOEt 1:1. Yield 76%. ^{1}H NMR ($CDCl_3$) δ 2.86 (m, 4H), 2.95−3.00 (m, 2H), 3.76 (s, 2H), 3.83 (s, 6H), 3.86 (s, 3H), 4.25 (m, 2H), 6.52 (s, 1H), 6.59 (s, 1H), 6.94−6.99 (m, 4H), 7.35 (d, 2H, J = 8 Hz), 7.55 (d, 2H, J = 8 Hz). ESI$^+$/MS m/z: 420 $[M + H]^+$; ESI$^+$/MS/MS m/z: 218 (100), 179 (61).

2′-(2-(6,7-Dimethoxy-3,4-dihydroisoquinolin-2(1H)-yl)-ethyl)-[1,1′-biphenyl]-3-ol (20a). White solid. Eluted with $CHCl_3$. Yield 26%. ^{1}H NMR ($CDCl_3$) δ 2.66−2.69 (m, 4H), 2.79−2.95 (m, 4H), 3.49 (s, 2H), 3.75 (s, 3H), 3.79 (s, 3H), 5.40 (br s, 1H, exchangeable with D_2O), 6.38 (s, 1H), 6.51 (s, 1H), 6.53−6.60 (m, 1H),

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6.70−6.80 (m, 1H), 7.12−7.29 (m, 2H), 7.33−7.35 (m, 3H), 7.8 (m, 1H). ESI⁻/MS *m/z*: 388 [M − H]⁻; ESI⁻/MS/MS *m/z*: 373 (100). Anal. C, H, N [$C_{25}H_{27}NO_3$ (HCl)]. mp > 250 °C.

6,7-Dimethoxy-2-(2-(3′-methoxy-[1,1′-biphenyl]-2-yl)ethyl)-1,2,3,4-tetrahydroisoquinoline (20b). White solid. Eluted with $CHCl_3$. Yield 64%. ^{1}H NMR ($CDCl_3$) δ 2.66−2.68 (m, 4H), 2.71−2.79 (m, 2H), 2.81−2.92 (m, 2H), 3.42 (s, 2H), 3.82 (s, 9H), 6.43 (s, 1H), 6.54 (s, 1H), 6.86−6.92 (m, 2H), 7.21−7.35 (m, 6H). ESI⁺/MS *m/z*: 426 [M + Na]⁺; ESI⁺/MS/MS *m/z*: 365 (100). Anal. C, H, N [$C_{26}H_{29}NO_3 \times$ (HCl)]. mp 183−186.

6,7-Dimethoxy-2-[[5-(4-methoxyphenyl)-2-furyl]methyl]-3,4-dihydro-1*H*-isoquinoline (25a). White solid. Eluted with $CHCl_3$. Yield 17%. ^{1}H NMR ($CDCl_3$) δ 2.81−2.86 (m, 4H), 3.64 (s, 2H), 3.78 (s, 2H), 3.81 (s, 3H), 3.82 (s, 3H), 3.83 (s, 3H), 6.32 (d, 1H, J = 3.3 Hz), 6.46 (d, 1H, J = 3.3 Hz), 6.51 (s, 1H), 6.58 (s, 1H), 6.88−6.93 (m, 2H), 7.58−7.63 (m, 2H). ESI⁺/MS *m/z*: 402 [M + Na]⁺. ESI⁺/MS/MS *m/z*: 387 (49), 349 (93), 210 (100), 135 (61). Anal. [$C_{23}H_{25}NO_4 \times H_2O \times$ HCl] C, H, N. mp 209−211 °C. Recrystallized from MeOH/hexane.

4-(5-((6,7-Dimethoxy-3,4-dihydroisoquinolin-2(1*H*)-yl)methyl)furan-2-yl)phenol (25b). Green solid. Eluted with $CHCl_3$/AcOEt 9:1. Yield 44%. ^{1}H NMR (DMSO) δ 2.65−2.73 (m, 4H), 3.22−3.38 (m, 4H), 3.64 (s, 3H), 3.66 (s, 3H), 6.36 (d, 1H, J = 3.3 Hz), 6.85−6.96 (m, 4H), 7.12 (d, 1H, *J* = 3.3 Hz), 7.78 (d, 2H, *J* = 8 Hz), 9.64 (br s, 1H, OH exchangeable with D_2O). ESI⁻/MS *m/z*: 364 [M − H]⁻. ESI⁻/MS/MS *m/z*: 349 (30); 172 (100). ESI⁺/MS *m/z*: 388 [M + Na]⁺. ESI⁺/MS/MS *m/z*: 296 (9); 183 (7). Anal. [$C_{22}H_{23}O_4N$·HCl] C, H, N. mp 234−237 °C.

6,7-Dimethoxy-2-(2-(6-(4-methoxyphenyl)-2*H*-chromen-3-yl)ethyl)-1,2,3,4-tetrahydroisoquinoline (31). White solid. Eluted with $CHCl_3$. Yield 62%. ^{1}H NMR ($CDCl_3$) δ 2.65−2.83 (m, 4H), 3.09 (d, 1H, *J* = 7 Hz), 3.34 (d, 1H, *J* = 7 Hz), 3.51 (d, 1H, *J* = 7 Hz), 3.55 (d, 1H, *J* = 7 Hz), 3.82 (s, 2H), 3.83 (s, 3H), 3.84 (s, 3H), 3.85 (s, 3H) 4.46 (s, 2H), 6.51 (s, 1H), 6.53 (s, 1H), 7.02−7.08 (m, 3H), 7.18 (s, 1H), 7.48−7.65 (m, 3H), 7.82 (d, 1H, *J* = 1 Hz). ESI⁺/MS *m/z*: 480 [M + Na]⁺; ESI⁺/MS/MS *m/z*: 465 (23), 216(100). Anal. [$C_{29}H_{31}O_4N$·HCl] C, H, N. mp 155−156 °C. Recrystallized from MeOH/Et_2O.

General Procedure for Synthesis of Amines 6e−g. Amine was synthesized by alkylation of 6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline (1.2 mmol) by acyl chloride **3b** (1 mmol) in DMF (20 mL) using Na_2CO_3 as base (2 mmol). The mixture was refluxed overnight. The DMF was evaporated, and the residue was partitioned between H_2O (20 mL) and $CHCl_3$ (20 mL). The organic phase was separated, the aqueous phase was extracted with $CHCl_3$ (3 × 50 mL), and the collected solutions were dried over Na_2SO_4 and evaporated. The residue was purified by silica gel column chromatography.

***N*-(3-(6,7-Dimethoxy-3,4-dihydroisoquinolin-2(1*H*)-yl)propyl)-4′-methoxy-[1,1′-biphenyl]-4-amine (6e).** Yellow solid. Eluted with $CHCl_3$/MeOH (97:3). Quantitative yield. ^{1}H NMR ($CDCl_3$) δ 1.67−1.69 (m, 2H), 2.45−2.47 (m, 2H), 2.78−2.82 (m, 4H), 3.65−3.67 (m, 2H), 3.72 (s, 2H), 3.84 (s, 9H), 4.21 (br s, 1H, NH exchangeable with D_2O), 6.51−6.52 (m, 2H), 6.85 (s, 1H), 6.87 (s, 1H), 7.10 (d, 2H, *J* = 8 Hz), 7.56 (d, 2H, *J* = 8 Hz), 7.68 (d, 2H, *J* = 8 Hz). ESI⁺/MS *m/z*: 455 [M+ Na]⁺, ESI⁺/MS/MS *m/z*: 451 (100), 425 (45), 258 (17), 192 (23). Anal. [$C_{27}H_{32}N_2O_3$·HCl] C, H, N.

4′-(4-(6,7-Dimethoxy-3,4-dihydroisoquinolin-2(1*H*)-yl)-butoxy)-[1,1′-biphenyl]-4-ol (6f). White solid. Eluted with CH_2Cl_2/MeOH (9:1). Yield 16%. ^{1}H NMR ($CDCl_3$) δ 1.85 (s, 4H), 2.62−2.65 (m, 2H), 2.78−2.85 (m, 4H), 3.62 (s, 2H), 3.83 (s, 6H), 4.01−4.10 (m, 2H), 5.35 (br s, 1H, OH exchangeable with D_2O), 6.52 (s, 1H), 6.59 (s, 1H) 6.83−6.88 (m, 4H), 7.39−7.42 (m, 4H). ESI⁺/MS *m/z*: 456 [M + Na]⁺; ESI⁺/MS/MS *m/z*: 248 (100), 179 (12). Anal. [$C_{27}H_{31}NO_4$·HCl] C, H, N. mp 178−180 °C.

6,7-Dimethoxy-2-(4-((4′-methoxy-[1,1′-biphenyl]-4-yl)oxy)-butyl)-1,2,3,4-tetrahydroisoquinoline (6g). White solid. Eluted with CH_2Cl_2/MeOH (98:2). Yield 29%. ^{1}H NMR ($CDCl_3$) δ 1.79−1.90 (m, 4H), 2.55−2.60 (m, 2H), 2.70−2.80 (m, 2H), 2.82−2.84 (m, 2H), 3.57 (s, 2H), 3.83 (s, 9H,), 4.01−4.05 (m, 2H), 6.52(s, 1H), 6.59 (s, 1H), 6.93−6.96 (m, 4 H), 7.44−7.48 (m, 4H). ESI⁺/MS *m/z*: 448 [M + H]⁺; ESI⁺/MS/MS *m/z*: 284 (47), 255 (61), 231 (89), 179 (100). Anal. [$C_{28}H_{33}NO_4$·HCl·H_2O] C, H, N. mp 222−223 °C.

3-(6,7-Dimethoxy-1,2,3,4-tetrahydroisoquinoline-2-yl)-propan-1-ol (9). A mixture of 6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline (**8**) (1.5 mmol), the appropriate 1-bromo alkylalcohol (1 mmol), and K_2CO_3 (1.5 mmol) in acetonitrile (20 mL) was refluxed overnight. The solvent was evaporated, and the residue was partitioned between H_2O (20 mL) and $CHCl_3$ (20 mL). The organic phase was separated, the aqueous phase was extracted with $CHCl_3$ (3 × 50 mL), and the collected organic fractions were dried over Na_2SO_4 and evaporated. Eluted with $CHCl_3$/MeOH (9:1). Yellow oil. Yield 95%. ^{1}H NMR ($CDCl_3$) δ 1.60−1.62 (m, 2H), 2.45−2.47 (m, 2H), 2.78−2.95 (m, 4H), 3.50−3.52 (m, 2H), 3.65 (br s, 1H, OH exchangeable with D_2O), 3.70 (s, 2H), 3.84 (s, 6H), 6.83 (s, 1H), 6.85 (s, 1H). GC-MS *m/z*: 251 (M^+, 17), 206 (100), 164 (53).

2-(3-Chloropropyl)-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline (10). Compound **9** was refluxed for 4 h with thionyl chloride (2 mL) and Et_3N (0.5 mL). The solvent was evaporated, and the residue was treated with Na_2CO_3 s.s. to pH 10. The aqueous phase was extracted with $CHCl_3$ (3 × 50 mL), and the collected organic fractions were dried over Na_2SO_4 and evaporated. Eluted with $CHCl_3$/MeOH (9:1). Brown oil. Yield 37%. ^{1}H NMR ($CDCl_3$) δ 2.01−2.03 (m, 2H), 2.46−2.47 (m, 2H), 2.77−2.96 (m, 4H), 3.69−3.71 (m, 4H), 3.84 (s, 6H), 6.77 (s, 1H), 6.81 (s, 1H). GC-MS *m/z:* 271 (M^+ + 2, 1), 269 (M^+, 25), 206 (100), 164 (67).

6,7-Dimethoxy-2-(3-((4′-methoxy-[1,1′-biphenyl]-4-yl)oxy)-propyl)-1,2,3,4-tetrahydroisoquinoline (11). One millimole of 4′-hydroxy-4-methoxy-biphenyl dissolved in dry DMF (1 mL) was dropped into a suspension of NaH (2 mmol) in dry DMF (4 mL). The mixture was stirred at room temperature for 1 h. A solution of compound **10** (2 mmol) in dry DMF (3 mL) was added to the mixture reaction, which was then heated to reflux overnight. Water was added until effervescence ceased. The solvent was evaporated, and the residue was partitioned between H_2O (20 mL) and $CHCl_3$ (20 mL). The organic phase was separated, the aqueous phase was extracted with $CHCl_3$ (3 × 50 mL), and the collected organic fractions were dried over Na_2SO_4 and evaporated. Eluted with $CHCl_3$. The product was transformed in the hydrochloric salt and recrystallized from MeOH. Yield 90%. ^{1}H NMR ($CDCl_3$) δ 1.82−1.84 (m, 2H), 2.45−2.47 (m, 2H), 2.74−2.93 (m, 4H), 3.68 (s, 2H), 3.84 (s, 9H), 4.12−4.14 (m, 2H), 6.81 (s, 1H), 6.83 (s, 1H), 7.10−7.15 (m, 4H), 7.65−7.67 (m, 4H). ESI⁺/ MS *m/z:* 434 [M + H]⁺; ESI⁺/MS/MS *m/z:* 208 (45), 179 (100).

2-((4′-Methoxy-[1,1′-biphenyl]-4-yl)oxy)acetic Acid (13). A solution of compound **6** (1.26 mmol) dissolved in 4 N NaOH (50 mL) was refluxed for 4 h. After cooling, the solution was acidified with 6 N HCl, the aqueous phase was extracted with AcOEt (3 × 50 mL), and the collected organic fractions were dried over Na_2SO_4 and evaporated. The residue was purified by silica gel column chromatography. Eluted with $CHCl_3$. White solid. Yield 92%. ^{1}H NMR ($CDCl_3$) δ 3.85 (s, 3H), 4.56 (s, 2H), 7.03−7.09 (m, 4H), 7.58−7.62 (m, 4H), 10.69 (br s, 1H, exchangeable with D_2O). ESI⁻/MS *m/z*: 257 [M − H]⁻; ESI⁻/MS/MS *m/z*: 199 (12).

2-(6-Bromo-2*H*-chromen-3-yl)acetonitrile (27). A mixture of 5-bromosalicylaldehyde (1.0 mmol) (**26**) and acrylonitrile (5.0 mmol) in DABCO (1.0 mmol) was refluxed for 24 h. The solvent was evaporated, and the residue was partitioned between H_2O (20 mL) and AcOEt (20 mL). The organic phase was separated, the aqueous phase was extracted with $CHCl_3$ (3 × 50 mL), and the collected organic fractions were dried over Na_2SO_4 and evaporated. The residue was purified on silica gel column chromatography. Yellow solid. Eluted with CH_2Cl_2. Yield 37%. ^{1}H NMR ($CDCl_3$) δ 3.08 (s, 2H), 4.60 (s, 2H), 7.05 (s, 1H), 6.87−7.37 (m, 3H). GC-MS *m/z:* 251 (M^+ + 2, 29), 249 (M^+, 30), 236 (100).

2-(6-Bromo-2*H*-chromen-3-yl)acetic Acid (28). Nitrile **27** was refluxed overnight in the presence of 5 M NaOH. The aqueous mixture was acidified with 3 N HCl and extracted with AcOEt (3 × 15 mL). The collected organic fractions were dried over Na_2SO_4 and evaporated. Yellow solid. Eluted with $CHCl_3$. Yield 28%. ^{1}H NMR ($CDCl_3$) δ 2.90 (s, 2H), 4.61 (s, 2H), 7.05 (s, 1H), 6.87−7.37 (m, 3H), 10.90 (br s, 1H, exchangeable with D_2O). ESI⁻/MS *m/z*: 267 [M − H]⁻; ESI⁻/MS/MS *m/z*: 223 (79), 225 (100).

Biology. Calcein-AM Experiment. These experiments were carried out as described by Feng et al.[25] with minor modifications.

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Each cell line (50 000 cells per well) was seeded into black 96-well plates with 100 μL of medium and allowed to become confluent overnight. Test compounds were solubilized in 100 μL of 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 of phosphate-buffered saline (PBS) to yield a final concentration of 2.5 μM, and the plate was incubated for another 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 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 from the fluorescence of untreated cells. In treated wells, the increase in fluorescence was measured relative to that of the basal level. EC_{50} values were determined by fitting the percent fluorescence increase versus log[dose].[27]

Permeability Experiment. *Preparation of Caco-2 Monolayer.* Caco-2 cells were harvested with trypsin−EDTA and seeded onto a MultiScreen Caco-2 assay system at a density of 10 000 cells/well. The culture medium was replaced every 48 h for the first 6 days and every 24 h thereafter, and after 21 days in culture, the Caco-2 monolayer was utilized for the permeability experiments. The transepithelial electrical resistance (TEER) of the monolayers was measured daily before and after the experiment using an epithelial voltohmmeter (Millicell-ERS; Millipore, Billerica, MA). Generally, TEER values obtained are greater than 1000 Ω for a 21 day culture.[28]

Drug Transport Experiment. Apical to basolateral (P_{app} A → B) and basolateral to apical (P_{app} B → A) permeability of drugs was measured at 120 min at the concentration of 100 μM. Drugs were dissolved in Hank's balanced salt solution (HBSS, pH 7.4) and sterile filtered. After 21 days of cell growth, the medium was removed from filter wells and from the receiver plate. The filter wells were filled with 75 μL of fresh HBSS buffer, and the receiver plate, with 250 μL per well of the same buffer. This procedure was repeated twice, and the plates were incubated at 37 °C for 30 min. After incubation, the HBSS buffer was removed, and, in some wells, drug solutions were added to the filter well (75 μL); HBSS without drug was added to the corresponding receiver plate (250 μL). For other wells, the drug solutions were added to the basolateral side (250 μL), and HBSS without drug was added to the corresponding filter wells.

The plates were incubated at 37 °C for 120 min. After incubation, samples were removed both from the apical (filter well) and basolateral (receiver plate) sides of the monolayer and then were stored in a freezer (−20 °C) pending analysis. The concentration of compounds was analyzed using UV−vis spectroscopy. The apparent permeability (P_{app}), in units of nm/s, was calculated using the following equation

$$P_{app} = [VA/(\text{area} \times \text{time})] \times ([\text{drug}]_{acceptor}/[\text{drug}]_{initial})$$

where VA is the volume (in mL) in the acceptor well, area is the surface area of the membrane (0.11 cm^2 of the well), time is the total transport time in seconds (7200 s), $[\text{drug}]_{acceptor}$ is the concentration of the drug measured by UV spectroscopy, and $[\text{drug}]_{initial}$ is the initial drug concentration (1×10^{-4} M) in the apical or basolateral well.[28]

Analytical Methods. Samples from in vitro permeability studies were analyzed using a Shimadzu spectrophotometer, UV-1800. For each compound, a calibration curve was constructed at the appropriate wavelength, reported in Table 2.

Hoechst 33342 Experiment. These experiments were carried out as described by Bauer et al. with modifications.[29] Each cell line (30 000 cells per well) was seeded into black 96-well plates with 100 μL of medium and allowed to become confluent overnight. One-hundred microliters of test compounds was solubilized in culture medium and added to the monolayers. The 96-well plate was incubated at 37 °C for 30 min. Hoechst 33342 was added in 100 μL of PBS to yield a final concentration of 8 μM, and the 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 three 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. Under these experimental conditions, Hoechst 33342 accumulation in the absence and presence of tested compounds was evaluated, and the basal level of fluorescence was estimated from untreated cells. In treated wells, the increase in fluorescence with respect to that of the basal level was measured. EC_{50} values were determined by fitting the fluorescence increase percentage versus log[dose].

Table 2. Wavelength of Absorption (λ) and Molar Extinction Coefficient (ε) of Each Tested Compound

	λ (nm)	ε^a
24a	314	13 440
24b	315	12 810
25a	285	20 720
25b	285	11 970
5b	227	33 210
6b	262	9810
6c	228	2004
6a	227	32 630
11	232	9920
15	287	10 250
6e	232	10 132
6f	267	9905
6g	260	848
31	278	19 061
30	228	36 682
20a	232	21 440
20b	232	18 390

[a]Molar extinction coefficient measures of how strongly a substance absorbs light at a particular wavelength and is represented in units of M^{-1} cm^{-1}.

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Notes

The authors declare no competing financial interest.

ABBREVIATIONS USED

EDTA, ethylenediaminetetraacetic acid; SAR, structure−activity relationship; PDB, Protein Data Bank; CDI, 1′-carbonyldiimidazole; ACN, acetonitrile; DABCO, 1,4-diazabicyclo[2.2.2]-octane; Calcein-AM, calcein acetoxymethyl ester; EC_{50}, half maximal effective concentration; PFA, paraformaldehyde solution

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