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(Article begins on next page)
New tetrahydroisoquinoline based P-glycoprotein modulators: decoration of the biphenyl core gives selective ligands.


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Abstract: P-glycoprotein (P-gp, MDR1) is a membrane transporter expressed in several districts of our body. It exerts a crucial defense role as it effluxes hundreds of potentially toxic substances. However, P-gp is one of the main causes of the failure in cancer chemotherapy, as a number of chemotherapeutic agents are P-gp substrates. Another interesting implication regards the correlation between P-gp expression impairment and the onset of several central nervous system pathologies such as Alzheimer’s and Parkinson’s diseases. In view of these considerations, in the present study a new series of P-gp modulators has been designed, synthesized and evaluated for their activity towards P-gp and other two sister proteins (BCRP and MRP1). The compounds, structurally correlated to the potent but non-selective P-gp inhibitor MC70 [4’-(6,7-dimethoxy-3,4-dihydro-1H-isooquinolin-2-ylmethyl)biphenyl-4-0l], proved fairly selective towards P-gp, with a potency in the low micromolar range. Compounds 5a, 5d and 12d proved capable to restore doxorubicin toxicity in resistant cancer cells.

Introduction
P-glycoprotein (P-gp, also known as MDR1 or ABCB1) is a membrane protein belonging to ATP-binding cassettes (ABC) superfamily; it is a complex molecular machinery able to recognize and efflux hundreds of structurally uncorrelated substances exploiting energy derived by ATP hydrolysis. It has a strategic localization in a number of organs and
tissues, thus exerting a crucial defence role against toxic substances, both of endogenous and exogenous origin, and constituting an essential component of several biological barriers.\[1\] P-gp has raised great attention since several years ago, owing to its involvement in multidrug resistance (MDR), a phenomenon that represents one of the main causes of cancer chemotherapy failure. The transporter is overexpressed in cancer cells and is responsible of the efflux of several chemotherapeutic agents.\[2\] The wide expression of P-gp on the surface of endothelial cells at the blood-brain barrier makes it a gatekeeper for central nervous system (CNS), as it prevents potentially harmful substances from entering, but also hampers many structurally and functionally uncorrelated drugs, compromising the success of pharmacological treatment of different CNS disorders and tumours. More recently, an interesting connection has emerged between P-gp and the onset of Alzheimer’s Disease (AD), Parkinson’s Disease (PD), epilepsy and other CNS diseases: an impairment in the expression level of the protein has been observed in the early stages of these neurological disorders.\[3,4\] Starting from this background, medicinal chemistry efforts to target P-gp have been undertaken, mainly in the aim of obtaining inhibitors which should be co-administered with chemotherapeutic agents subjected to P-gp mediated efflux, thus restoring therapy efficacy. According to this approach a number of MDR reversal agents have been reported, usually classified in three generations, but most of them suffer of poor in vivo pharmacological profile. In addition the potential application in the early diagnosis through imaging techniques such as Positron Emission Tomography (PET) of several neurological disorders has been proposed for P-gp ligands, in view of the involvement of the transporter in the onset of these CNS pathologies: in particular radiolabeled substrates are useful to measure in vivo function of the transporter at the blood brain barrier (BBB).\[5\] In the ongoing effort to obtain selective and potent P-gp ligands, in the present study the authors have carried out several modifications on MC70 (Figure 1), a previously studied P-gp inhibitor.\[6,7\] MC70 shows a good P-gp inhibiting potency (EC$_{50}$ = 0.69 $\mu$M), on the other hand displaying a non-selective profile towards P-gp.

![Structure and biological activity profile of MC70.](image)

The authors have previously explored structure activity relationships of MC70 derivatives: in particular they focused on functionalization of phenolic group with alkyl and oxyalkyl chains,\[8\] and with more complex moieties containing variously substituted furazan (1,2,5-oxadiazole) ring.\[9\] Reported herein is a series of derivatives where the biphenyl moiety was modulated: in particular the aim of the study was to evaluate, in terms of inhibition potency and selectivity on P-gp, the effect of substituents able to modify electronic
properties and endowed with hydrogen bond donor or acceptor properties. The compounds have been synthesized through an inexpensive and straightforward route; compared to MC70, potency was slightly decreased, EC50 values being in the low micromolar range for most compounds, but all the ligands display high selectivity towards P-gp, being essentially inactive against MRP1 and BCRP. Three of them have been further evaluated in co-administration with doxorubicin and proved able to restore intracellular concentration of the anthracycline to different extent. A sensible insight of the binding pose of the most potent compound has been gained through a molecular docking study.

Results and discussion

Synthesis of target compounds

The target compounds were synthesized according to the methods reported in Scheme 1 and 2. In brief, the key step is a Suzuki coupling reaction,10 carried out with palladium on activated charcoal in a ligand free fashion in aqueous environment, starting from the 4-bromobenzylalcohols 1a-f (Scheme 1), or the 4-bromophenols 8a-e (Scheme 2). The substrates were coupled with 4-hydroxyphenylboronic acid or 4-hydroxymethylphenylboronic acid respectively. The coupling products were then reacted with an excess of p-toluensulfonyl chloride in presence of triethylamine, to give the di-tosylate derivatives 3a-f (Scheme 1) or 10a-e (Scheme 2). The latter was used without purification to obtain benzylation of 6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline. Finally the removal of the phenyl tosylate group was achieved through hydrolysis with NaOH in THF/CH3OH mixture.

The two benzylic alcohols 1c and 1d were synthesized starting from the corresponding benzoic acids through reduction with BH3-THF complex (Scheme 1).
Scheme 1. Synthesis of target compounds 5a-f. Reagents and conditions: a) 4-hydroxybenzeneboronic acid, KOH, Pd/C cat., H₂O, 130 °C, 3 hours; b) p-toluenesulfonyl chloride, Et₃N, DMAP cat., CH₂Cl₂, room temperature, 6 hours; c) 6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline hydrochloride, DBU, CH₃CN, 70 °C, 18 hours; d) NaOH, THF/CH₃OH 2/1, 65 °C, 1 hour; e) BH₃–THF complex, THF, N₂, 18 hours, room temperature.

As for compound 16, the procedure reported above for coupling reaction starting from 1-bromo-4-methoxy-2-nitrobenzene 13 did not afford the desired intermediate, probably due to the poor solubility of 13. For this reason the Suzuki coupling reaction was carried out in a more “traditional” manner (Scheme 2) using palladium tetrakis triphenylphosphine as catalyst in 1,4-dioxane/water mixture; the intermediate 14 was subdued to hydrolysis of methoxy group with BBr₃, obtaining the concomitant bromination of benzylic alcohol. The bromo derivative 15 was then reacted with 6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline, yielding compound 16.

Finally the two amino substituted compounds 17a and 17b were obtained through catalytic hydrogenation of the nitro derivatives 12c and 16 (Scheme 2).

Scheme 2. Synthesis of target compounds 12a-e, 16, 17a,b. Reagents and conditions: a) 4-hydroxymethylbenzeneboronic acid, KOH, Pd/C cat., H₂O, 130 °C, 3 hours; b) p-
toluenesulfonyl chloride, Et$_3$N, DMAP cat., CH$_2$Cl$_2$, room temperature, 6h; c) 6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline hydrochloride, DBU, CH$_3$CN, 70 °C, 18 hours; d) NaOH, THF/CH$_3$OH 2/1, 65 °C, 1 hour; e) 4-hydroxymethylbenzeneboronic acid, K$_2$CO$_3$, Pd[(C$_6$H$_5$)$_3$P]$_4$, 1,4-dioxane/water 2/1, N$_2$, 90 °C, 18 hours; f) BBr$_3$, CH$_2$Cl$_2$, 18 hours, room temperature; g) H$_2$, Pd/C cat., CH$_3$OH, room temperature, 3 hours.

**Biological evaluation**

All the new synthesized compounds have been tested to establish their P-gp interacting mechanism as substrate or inhibitor by three assays: 1) the inhibition of the transport of a fluorescent or a pro-fluorescent substrate of the transporter;[11] 2) the determination of the apparent permeability value (P$_{app}$);[11] 3) the detection of the ATP cell level depletion;[11] the results are reported in Table 1.

**Table 1. Biological characterization of target compounds.**

<table>
<thead>
<tr>
<th>Compound</th>
<th>Structure</th>
<th>MDR1 EC$_{50}$ (μM) [a]</th>
<th>MRP1 EC$_{50}$ (μM) [a]</th>
<th>BCRP EC$_{50}$ (μM) [a]</th>
<th>ATP consumption</th>
<th>P$_{app}$ [b]</th>
</tr>
</thead>
<tbody>
<tr>
<td>5a</td>
<td><img src="image" alt="Structure 5a" /></td>
<td>1.60 ± 0.27</td>
<td>na [c]</td>
<td>na</td>
<td>0</td>
<td>4.5</td>
</tr>
<tr>
<td>5b</td>
<td><img src="image" alt="Structure 5b" /></td>
<td>4.73 ± 0.90</td>
<td>na</td>
<td>na</td>
<td>0</td>
<td>4.1</td>
</tr>
<tr>
<td>5c</td>
<td><img src="image" alt="Structure 5c" /></td>
<td>8.4 ± 1.58</td>
<td>na</td>
<td>na</td>
<td>0</td>
<td>5.8</td>
</tr>
<tr>
<td>5d</td>
<td><img src="image" alt="Structure 5d" /></td>
<td>1.51 ± 0.30</td>
<td>38 ± 7.6</td>
<td>na</td>
<td>0</td>
<td>4.4</td>
</tr>
<tr>
<td>5e</td>
<td><img src="image" alt="Structure 5e" /></td>
<td>6.2 ± 1.24</td>
<td>na</td>
<td>na</td>
<td>0</td>
<td>3.7</td>
</tr>
<tr>
<td>5f</td>
<td><img src="image" alt="Structure 5f" /></td>
<td>15.2 ± 3.00</td>
<td>na</td>
<td>na</td>
<td>0</td>
<td>2.9</td>
</tr>
<tr>
<td>12a</td>
<td><img src="image" alt="Structure 12a" /></td>
<td>2.0 ± 0.40</td>
<td>na</td>
<td>na</td>
<td>0</td>
<td>3.9</td>
</tr>
</tbody>
</table>
The first assay is performed in a cell line overexpressing P-gp (MDCK-MDR1 cells) and it detects the ability of the ligands to compete with the efflux of the pro-fluorescent P-gp substrate Calcein-AM towards P-gp; it is the measure of the potency of each compound towards the target. The second assay evaluates a ratio basolateral-apical vs apical-basolateral (BA/AB) representative of two contributions, the passive diffusion (BA) and active transport (AB) in a system mimicking BBB, such as Caco2 cells; if this ratio is < 2, the compound can be considered a P-gp inhibitor, otherwise ($P_{app}$ > 2) it behaves as a substrate. The third assay measures the consumption of ATP in MDCK-MDR1 cells. Generally, a substrate, since transported by the pump, induces an ATP consumption while an inhibitor, inhibiting the binding of ATP on its site on P-gp, is not transported and thus it does not induce a decrease in ATP cell level. The selectivity of all compounds towards the sister proteins BCRP and MRP1 has been also tested by the measure of the ability of the compounds to interfere with the transport BCRP- or MRP1-mediated of the fluorescent BCRP substrate Hoechst33342 or the pro-fluorescent MRP1 substrate Calcein-AM.

As depicted in Table 1, all of the compounds were found less active than the lead compound MC70 ($EC_{50} = 0.69 \mu M$) displaying $EC_{50}$ values ranging from 1.51 to 15.2 $\mu M$, and among them 5a ($EC_{50} = 1.60 \mu M$), 5d ($EC_{50} = 1.51 \mu M$), and 12d ($EC_{50} = 1.86 \mu M$), displayed the best activity values. However, all compounds were more selective towards

<table>
<thead>
<tr>
<th>Compound</th>
<th>Structure</th>
<th>$EC_{50}$ (na)</th>
<th>$P_{app}$</th>
<th>Selectivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>12b</td>
<td><img src="image1.png" alt="Structure" /></td>
<td>2.8 ± 0.56</td>
<td>na</td>
<td>na</td>
</tr>
<tr>
<td>12c</td>
<td><img src="image2.png" alt="Structure" /></td>
<td>12.7 ± 2.50</td>
<td>28.9</td>
<td>na</td>
</tr>
<tr>
<td>17a</td>
<td><img src="image3.png" alt="Structure" /></td>
<td>6.9 ± 1.32</td>
<td>na</td>
<td>0</td>
</tr>
<tr>
<td>12d</td>
<td><img src="image4.png" alt="Structure" /></td>
<td>1.86 ± 0.37</td>
<td>62</td>
<td>0</td>
</tr>
<tr>
<td>12e</td>
<td><img src="image5.png" alt="Structure" /></td>
<td>2.17 ± 0.43</td>
<td>na</td>
<td>na</td>
</tr>
<tr>
<td>16</td>
<td><img src="image6.png" alt="Structure" /></td>
<td>2.54 ± 0.48</td>
<td>na</td>
<td>na</td>
</tr>
<tr>
<td>17b</td>
<td><img src="image7.png" alt="Structure" /></td>
<td>5.4 ± 1.00</td>
<td>na</td>
<td>0</td>
</tr>
</tbody>
</table>

[a] The value is the mean of three independent experiments, sample in duplicate. [b] Apparent permeability (the value is from two independent experiments). [c] Not active at 100 $\mu M$. 

The value is the mean of three independent experiments, sample in duplicate. [b] Apparent permeability (the value is from two independent experiments). [c] Not active at 100 $\mu M$. 

The table shows the $EC_{50}$ values and $P_{app}$ values for the compounds tested. The compounds are ordered by their $EC_{50}$ values, with the lead compound MC70 having the lowest $EC_{50}$ value. The selectivity is indicated by the $P_{app}$ values, with $P_{app}$ values greater than 2 indicating selectivity.
P-gp, proving inactive towards BCRP and MRP1. Only compound 5d and 12c showed a moderate MRP1 activity (EC\(_{50}\) = 38 \(\mu\)M and 28.9 \(\mu\)M, respectively). All the ligands, having a \(P_{\text{app}}\) values > 2 and not inducing an ATP cell depletion, behaved as substrates of the Category IIIB\[6\]. As a whole only slight differences can be observed along the series, but noteworthy methoxy substituent on the benzylic ring of the biphenyl fragment led to the most active compounds (EC\(_{50}\) = 1.60 \(\mu\)M for 5a, EC\(_{50}\) = 1.51 \(\mu\)M for 5d); the presence of nitro substituent on both rings of the biphenyl fragment proved detrimental for P-gp activity: in particular, 5f (EC\(_{50}\) = 15.2 \(\mu\)M) and 12c (EC\(_{50}\) = 12.7 \(\mu\)M) were about 10-fold less active than the best compound of the series (5d, EC\(_{50}\) = 1.51 \(\mu\)M) and about 20-fold than the lead compound MC70 (EC\(_{50}\) = 0.69 \(\mu\)M).

**Molecular docking study**

A structure based study was then carried to furnish fresh insights into the binding mode of the new compounds and to support explication to the EC\(_{50}\) data. To fulfill this topic our previously published P-gp receptor model\[^9\] served as valuable tool to explain ligand pose of the most active compound (5d). In our past study we have postulated that the “inward-outward facing” of the P-gp scaffold should facilitate the “pulling out” of substrates as soon as they pass the cell bilayer, while active inhibitors would hamper the P-gp flipping depending on molecular shape and pharmacophoric features of the molecules; in line with this view, molecular docking further supports this evidence.

As it might be perceived from Figure 2 reporting the binding mode of 5d, the ligand scaffold properly fits the binding site space delimitated by the two six helices-transmembrane domains (TMDs), and deeply locks into the crevice comprising the intracellular moiety of the same domains, with a kind of “reversed wedge” pose that might be responsible for the hampering of P-gp flipping upon ligand binding.
Figure 2. Binding mode of 5d into the MDR1 binding site. Water molecules are represented as red spheres, and the extracellular and intracellular sides are at top and bottom of the scene respectively. The free energy of binding calculated with hydration force field of AutoDock is -8.62 kcal/mol, while the contact surface area measures 440 Å².

Interestingly the tetrahydroisoquinoline ring is placed close to one of the TMDs, and it occupies a mainly hydrophobic receptor slot, surrounded by Tyr310, Phe336, Phe728 and Phe983 generating extensive favourable contacts and \( \pi-\pi \) stacking, as well as a stable binding through two water molecules enlacing hydrogen bonding bridges with Tyr310 and Ser979. The rest of the aromatic moiety points to the opposite TMD with the phenolic group making polar contacts with the backbone of Met949 and the methoxy group interacting with Tyr953 throughout a water molecule coordinating a hydrogen bond, and as long as this is concerned, the same methoxy substituent should oblige, to some extent, the biphenyl motif in a non-planar conformation. Indeed, this steric hindrance, in combination with an hydrogen bond formation, might clarify, at least within this series of derivatives, the better EC\(_{50}\) value of 5d.

Co-administration of doxorubicin with 5a, 5d and 12d

Compounds 5a, 5d, and 12d have been evaluated in co-administration with doxorubicin, in order to study their efficacy as doxorubicin-rehabilitating agents in the treatment of resistant tumours (Figure 3). Preliminary data demonstrated that the three compounds were not cytotoxic at 48h and 72h (data not shown) and doxorubicin at 10 µM is not able to induce cell death as effluxed by P-gp in the resistant tumours cell model overexpressing P-gp (MDCK-MDR1 cells). When MC70, 5a, 5d, and 12d were co-administrated with doxorubicin, they restore its antineoplastic cytotoxicity. In fact, while the lead compound MC70 and 5a, 5d displayed a moderate ability to restore doxorubicin effect (20% of cytotoxicity increase for MC70 and 15% for 5a, 5d), 12d was able to produce an increase a 60% of doxorubicin citotoxicity, demonstrating its ability to almost completely rehabilitate the access of the antineoplastic drug in tumour cells.

![Graph](image-url)  
**Figure 3.** Co-administration of doxorubicin (10 µM) with compounds MC70, 5a, 5d, and 12d (10 µM). Antiproliferative effect of MC70, 5a, 5d, and 12d (10 µM) alone (gray bars)
and co-administered with doxorubicin (10 μM) (black bars) at 48h in MDCK-MDR1 cell line. Ctr bar represents the administration of 10 μM doxorubicin alone.

Immunoblotting experiments on MC70, 5a, 5d and 12d
The same compounds (MC70, 5a, 5d and 12d) which were evaluated in co-administration with doxorubicin were further studied in immunoblotting experiments to verify a possible interference with P-gp expression, in the same experimental condition of cell viability tests. As it can be seen in Figure 4, no differences in P-gp expression were observed in treated cells compared to the control, thus supporting the hypothesis of a direct effect of the compounds on the transporter.

Figure 4. Immunoblotting experiment with compounds MC70, 5a, 5d, and 12d (10 μM). Proteins were extracted from MDCK-MDR1 cells after 48 hours of incubation. Actin levels were used as protein loading control. CTRL bands refer to untreated cells. The figure is representative of 1 out of 3 experiments with similar results.

Conclusion
A new series of P-gp modulators has been developed through the “decoration” of the biphenyl moiety of MC70 with substituents endowed with different electronic and hydrogen bond donor/acceptor properties. The compounds were synthesized exploiting a straightforward and inexpensive route. They displayed slightly lower potency than the parent compound but proved highly selective, thus representing suitable candidates to be used in imaging techniques for measurement of P-gp function at BBB.

A molecular docking simulation carried out for the most potent compound (5d, EC50 = 1.51 μM) highlighted an additional anchoring for the biphenyl moiety through the methoxy substituent, which engages a hydrogen bond mediated by a water molecule. Besides the biochemical characterization aimed to clarify the mechanism of interaction of the ligands with P-gp, the most potent compounds (5a, 5d, and 12d) and MC70 were evaluated in a co-administration assay with doxorubicin, and proved efficient to restore drug toxicity against doxorubicin resistant MDCK-MDR1 cells. A possible effect on P-gp expression was ruled out by performing immunoblotting experiment on the same selected compounds. The present study thus represents an additional investigation of a previously started structure activity relationship study on tetrahydroisoquinoline derivatives, and furnishes additional information about the mode of binding of this class of compounds which can be
useful for future development of potent P-gp modulators. Moreover, compound 12d displayed a better profile than the lead compound MC70, as co-administrating agent to restore the antineoplastic therapy in resistant tumours.

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Conflict of interest
The authors declare no conflict of interest.

Contributions
S. Guglielmo and N. Colabufo desigend the study and wrote the manuscript.
S. Guglielmo planned the synthetic strategy.
M. Contino planned and performed the biological assays to define the P-gp interacting mechanism and participated in the editing of the manuscript.
M.G. Perrone performed the permeability assays.
R. Giampietro performed the co-administration assay.
A. Carriere and D. Zaccaria performed the molecular docking study.
K. Chegaev and V. Borio carried out the synthesis of compounds.
B. Rolando carried out the structural characterization and purity assessment.
C. Riganti and K. Zabielska-Koczywas acrried out the immunoblotting experiments.
R. Fruttero carried out literature research and contributed to write the manuscript.

References:
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Supporting Information

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Section 1. Chemistry

Materials and instrumentation. $^1$H and $^{13}$C-NMR spectra were recorded on a Bruker Avance 300 at 300 and 75 MHz respectively, using SiMe$_4$ as the internal reference. Chemical shifts (δ) are given in parts per million (ppm. The following abbreviations are used to designate the multiplicities: s = singlet, d = doublet, dd = doublet of doublets, t = triplet, q = quartet, m = multiplet. Low resolution mass spectra were recorded on a Micromass Quattro micro™ API (Waters Corporation, Milford, MA, USA) with electrospray ionization. Melting points(mp) were determined with a capillary apparatus (Büchi 540). Flash column chromatography was performed on silica gel (Merck Kieselgel 60, 230-400 mesh ASTM). The progress of the reactions was followed by thin layer chromatography (TLC) on 5×20 cm plates with a layer thickness of 0.2 mm. The purity of target compounds was assessed by RP-HPLC. Analyses were performed on a HP1100 chromatograph system (Agilent Technologies, Palo Alto, CA, USA). The analytical column was a LiChrosphere® C18 5µM (Merck KGaA, 64271 Darmstadt, Germany). UV signals were recorded at 210, 226 and 254 nm. All compounds were dissolved in eluent and injected through a 20 µL loop. Compounds $^{1}$d$^{[1]}$ and $^{1}$e$^{[2]}$ were prepared according to reported procedure.

General procedure for the synthesis of 2a-f. The appropriate benzyl alcohol, 4-hydroxybenzeneboronic acid (1.5 eq), potassium hydroxide (4 eq) and 10% dispersion of palladium on charcoal (0.02 eq) were added to 15 mL of water in a sealed vessel. The mixture was stirred at 130 °C for 3 hours, then cooled to room temperature, acidified to pH 3 with 6M HCl and extracted with ethyl acetate. The organic phase was washed with brine, dried and evaporated under reduced pressure. The crude product was purified through flash chromatography on silica gel.

4'-(Hydroxymethyl)-3'-methoxybiphenyl-4-ol (2a). Eluent: petroleum ether/ethyl acetate 70/30. Yield: 78%. Mp (iPrOH) = 153.4 - 154.1 °C. MS ESI-: 229 [M-1]. $^1$H-NMR (DMSO-d$_6$) δ 9.54 (br. s. 1H, C$_6$H$_4$OH), 7.51 (m, 2H), 7.38 (d, J = 7.7 Hz, 1H), 7.12 (m, 2H), 6.85 (m, 2H), 5.01 (br. s. 1H, CH$_2$OH), 4.51 (br. s. 2H, CH$_2$OH), 3.84 (s, 3H, OCH$_3$). $^{13}$C-NMR (DMSO-d$_6$) δ 157.88, 157.22, 140.87, 132.02, 129.26, 128.62, 128.32, 118.54, 116.46, 108.79, 58.65, 56.01.
3'-Fluoro-4’-(hydroxymethyl)biphenyl-4-ol (2b). Eluent: petroleum ether/ethyl acetate 60/40. Yield: 75%. Mp (iPrOH) = 164 – 165 °C. MS ESI-: 217 [M-1]. 1H-NMR (DMSO-d6) δ 9.63 (s, 1H, C6H4OH), 7.42 (m, 5H), 6.85 (d, J = 8.2 Hz, 2H), 5.26 (t, J = 5.8 Hz, 1H, CH2OH), 4.56 (d, J = 5.5 Hz, 2H, CH2OH). 13C-NMR (DMSO-d6) δ 160.14 (d, J = 242 Hz), 157.53, 141.10 (d, J = 8.25 Hz), 129.58 (d, J = 5.2 Hz), 129.48 (d, J = 2.25 Hz), 127.81, 126.92 (d, J = 15.8 Hz), 115.79, 112.10 (d, J = 22.5 Hz), 56.64 (d, J = 3.8 Hz).

4’-(Hydroxymethyl)-3’-nitrobiphenyl-4-ol (2c). Eluent: petroleum ether/ethyl acetate 70/30. Yield: 30%. Mp (EtOH) = 181 – 183 °C (dec.). MS ESI-: 244 [M-1]. 1H-NMR (DMSO-d6) δ 9.73 (s, 1H, C6H4OH), 8.17 (s, 1H), 7.61 (d, J = 1.6 Hz, 1H), 7.59 (d, J = 8.8 Hz, 2H), 6.88 (d, J = 8.8 Hz, 2H), 5.55 (t, J = 5.5 Hz, 1H, CH2OH), 4.82 (d, J = 5.5 Hz, 2H, CH2OH). 13C-NMR (DMSO-d6) δ 157.97, 147.52, 139.84, 135.77, 130.69, 129.05, 128.28, 127.99, 121.05, 115.98, 59.77.

4’-(Hydroxymethyl)-2’-methoxybiphenyl-4-ol (2d). Eluent: petroleum ether/ethyl acetate 70/30. Yield: 88%. Mp (EtOH) = 176 – 177 °C. MS ESI-: 229 [M-1]. 1H-NMR (DMSO-d6) δ 9.44 (s, 1H, C6H4OH), 7.10 (m, 4H), 6.82 (m, 3H), 5.13 (t, J = 5.5 Hz,. 1H, CH2OH), 4.38 (d, J = 4.9 Hz, 2H, CH2OH), 3.77 (s, 3H, OCH3). 13C-NMR (DMSO-d6) δ 158.20, 156.27, 140.73, 132.47, 130.77, 130.42, 130.12, 114.92, 112.87, 112.14, 60.92, 55.03.

2’-Fluoro-4’-(hydroxymethyl)biphenyl-4-ol (2e). Eluent: petroleum ether/ethyl acetate 70/30. Yield: 76%. Sticky solid. MS ESI-: 217 [M-1]. 1H-NMR (DMSO-d6) δ 9.62 (s, 1H, C6H4OH), 7.38 (m, 3H), 7.18 (d, J = 9.9 Hz, 2H), 6.86 (d, J = 8.2 Hz, 2H), 5.33 (t, J = 5.8 Hz, 1H, CH2OH), 4.53 (d, J = 5.5 Hz, 2H, CH2OH). 13C-NMR (DMSO-d6) δ 158.98 (d, J = 244 Hz), 157.14, 143.83 (d, J = 7.5 Hz), 129.97 (d, J = 3.75 Hz), 129.88 (d, J = 3.0 Hz), 126.41 (d, J = 12.75 Hz), 125.72, 122.52 (d, J = 3.0 Hz), 115.44, 113.69 (d, J = 22.5 Hz), 62.04 (d, J = 1.5 Hz).

4’-(Hydroxymethyl)-2’-nitrobiphenyl-4-ol (2f). Eluent: petroleum ether/ethyl acetate 70/30. Yield: 28%. Sticky solid. MS ESI-: 244 [M-1]. 1H-NMR (DMSO-d6) δ 9.67 (br. s, 1H, C6H4OH), 7.79 (s, 1H), 7.61 (m, 1H), 7.44 (m, 1H), 7.11 (m, 2H), 6.82 (m, 2H), 4.60 (s, 2H, CH2OH). 13C-NMR (DMSO-d6) δ 157.56, 148.85, 143.11, 133.21, 131.39, 130.14, 128.95, 127.18, 121.29, 115.61, 61.61.

General procedure for the synthesis of 1c, f. The appropriate benzoic acid was dissolved in anhydrous tetrahydrofuran under nitrogen atmosphere in an ice – water bath. Borane – tetrahydrofuran complex (1M, 3 eq) was added dropwise over a period of 1 hour.
The mixture was stirred at room temperature for 18 hours and then cooled in an ice-water bath and treated dropwise with water. The solvent was removed under reduced pressure, and the residue was partitioned between ethyl acetate and water. The aqueous layer was extracted with ethyl acetate and the organic layers were combined, washed with brine, dried, filtered, and concentrated to give the title product.

(4-Bromo-2-nitrophenyl)methanol (1c). The product was used without further purification. Yield 94%. MS ESI+: 233 [M-H]+. 1H-NMR (CDCl₃) δ 7.81 (d, J = 8.2 Hz, 1H), 7.68 (d, J = 8.8 Hz 1H), 7.58 (m, 1H), 5.34 (s, 1H, CH₂OH), 4.97 (s, 2H, CH₂OH). 13C-NMR (CDCl₃) δ 137.03, 136.88, 131.06, 129.47, 127.84, 121.47, 61.95.

(4-Bromo-3-nitrophenyl)methanol (1f). The crude product was purified through flash chromatography. Eluent petroleum ether/ethyl acetate 65/35. Yield 60%. MS ESI+: 233 [M-H]+.

1H-NMR (CDCl₃) δ 7.83 (m, 1H), 7.69 (d, J = 8.2 Hz, 1H), 7.40 (m, 1H), 4.73 (s, 2H, CH₂OH).

13C-NMR (CDCl₃) δ 142.05, 134.96, 131.07, 128.56, 123.39, 112.82, 63.14.

General procedure for the synthesis of 9a-e. The appropriate phenol, 4-hydroxymethylbenzeneboronic acid (1.5 eq), potassium hydroxide (4 eq) and 10% dispersion of palladium on charcoal (0.02 eq) were added to 15 mL of water in a sealed vessel. The mixture was stirred at 130 °C for 3 hours, then cooled to room temperature, acidified to pH 3 with 6M HCl and extracted with ethyl acetate. The organic phase was washed with brine, dried and evaporated under reduced pressure. The crude product was reacted as such or purified through flash chromatography on silica gel.

4'-{(Hydroxymethyl)-3-methoxybiphenyl-4-ol (9a). Sticky solid. Yield 76%. MS ESI+: 213 [M - 17]+ 254 [M-H]+. 1H-NMR (DMSO-d⁶) δ 9.09 (br. s., 1H, C₆H₃O), 7.57 (d, J = 8.2 Hz, 2H), 7.33 (m, 2H), 7.18 (d, J = 1.6 Hz, 1H), 7.07 (dd, J = 8.0, 1.9 Hz, 1H), 6.85 (d, J = 8.2 Hz, 1H), 5.20 (br. s., 1H, CH₂OH), 4.52 (s, 2H, CH₂OH), 3.85 (s, 3H, OCH₃). 13C-NMR (DMSO-d⁶) δ 147.95, 146.25, 140.77, 138.88, 131.48, 126.96, 125.89, 119.02, 115.89, 110.65, 62.71, 55.68.

3-Fluoro-4'-{(hydroxymethyl)biphenyl-4-ol (9b). Yield 83%. Mp (EtOH) = 151 – 152 °C. MS ESI+: 217 [M - 1]. 1H-NMR (DMSO-d⁶) δ 9.97 (br. s., 1H, C₆H₃O), 7.57 (d, J = 8.2 Hz, 2H), 7.46 (d, J = 13.2 Hz, 1H), 7.34 (m, 3H), 7.03 (m, 1H), 5.22 (t, J = 5.0 Hz 1H, CH₂OH), 4.52 (d, J = 5.5 Hz, 2H, CH₂OH). 13C-NMR (DMSO-d⁶) δ 151.36 (d, J = 239 Hz), 144.35 (d, J = 12 Hz), 137.40, 134.0 (d, J = 4.5 Hz), 131.72 (d, J = 6.8 HZ), 127.03, 125.84, 122.54, 118.14 (d, J = 3.8 Hz), 114.10 (d, J = 18 Hz), 62.64.

4'-{(Hydroxymethyl)-3-nitrobiphenyl-4-ol (9c). Yield 65%. Mp (iPrOH) = 111 – 112 °C. MS ESI+: 244 [M - 1]. 1H-NMR (DMSO-d⁶) δ 11.09 (br. s., 1H, C₆H₃O), 8.12 (d, J = 2.2
Hz, 1H), 7.85 (dd, J = 8.8, 2.2 Hz, 1H), 7.62 (d, J = 8.2 Hz, 2H), 7.40 (d, J = 8.2 Hz, 2H), 7.21 (d, J = 8.8 Hz, 1H), 5.24 (br. s., 1H, CH$_2$OH), 4.54 (s, 2H, CH$_2$OH). $^{13}$C-NMR (DMSO-d$_6$) δ 151.29, 142.02, 137.31, 136.24, 133.09, 131.34, 127.15, 126.01, 122.47, 119.67, 662.59.

4'-{Hydroxymethyl}-2-methoxybiphenyl-4-ol (9d). Yield 74%. Mp (n-Hexane / ethyl acetate) = 147 – 148 °C. MS ESI−: 229 [M - 1]. $^1$H-NMR (DMSO-d$_6$) δ 9.58 (s, 1H, C$_6$H$_3$OH), 7.32 (m, 4H), 7.06 (d, J = 8.2 Hz, 1H), 6.5 (s, 1H), 6.44 (d, J = 8.2 Hz, 1H), 5.24 (t, J = 5.5 Hz, 1H, CH$_2$OH), 4.50 (d, J = 4.9 Hz, 2H, CH$_2$OH), 3.69 (s, 3H, OCH$_3$).

$^{13}$C-NMR (DMSO-d$_6$) δ 158.18, 157.12, 140.22, 136.88, 130.83, 128.11, 126.16, 120.69, 107.46, 99.46, 62.84, 55.27.

4'-{Hydroxymethyl}-2-fluorobiphenyl-4-ol (9e). Yield 67%. Mp (MeOH) = 132.5 – 133.2 °C. MS ESI−: 217 [M - 1]. $^1$H-NMR (DMSO-d$_6$) δ 159.89 (d, J = 214.5 Hz), 158.19 (d, J = 16.5 Hz), 114.32, 133.87, 131.08 (d, J = 6 Hz), 128.20 (d, J = 6 Hz), 126.67, 118.85 (d, J = 13.5 Hz), 127.15 (d, J = 3 Hz), 102.08 (d, J = 24.8 Hz), 62.70.

(4'-{Methoxy-2'-nitrophenyl-4-yl})methanol (14). 1-bromo-4-methoxy-2-nitrobenzene, 4-hydroxymethylbenzenboronic acid (1.5 eq), potassium carbonate (2 eq), and tetrakis(triphenylphosphine)palladium (0.05 eq) were added to a mixture of 1,4-dioxane/water 2/1 under nitrogen atmosphere. The reaction mixture was stirred at 90 °C for 18 hours, then cooled to room temperature, concentrated under reduced pressure to remove volatile and extracted with ethyl acetate. The organic extracts were washed with brine, dried and evaporated under reduced pressure. The crude product was purified through flash chromatography on silica gel eluting with petroleum ether/ethyl acetate 65/35, to yield the title product as a pale yellow solid. Yield 46%. Mp (iPrOH) = 134.5 – 135.7 °C. MS ESI+: 282 [M + Na$^+$]. $^1$H-NMR (DMSO-d$_6$) δ 7.53 (d, J = 2.7 Hz, 1H), 7.45 (d, J = 8.8 Hz, 1H), 7.37 (m, 2H), 7.31 (dd, J = 8.2, 2.7 Hz, 1H), 7.24 (m, 2H), 5.27 (t, J = 5.8 Hz, 1H, CH$_2$OH), 4.54 (d, J = 6.0 Hz, 2H, CH$_2$OH), 3.87 (s, 3H, OCH$_3$). $^{13}$C-NMR (DMSO-d$_6$) δ 158.81, 149.59, 142.30, 135.00, 132.66, 127.57, 126.98, 126.80, 118.77, 109.02, 62.57, 56.09.

4'-{Bromomethyl}-2-nitrobenzyl-4-ol (15). (4'-methoxy-2'-nitrophenyl-4-yl)methanol was dissolved in dry dichloromethane; the solution was cooled in ice – water bath and boron tribromide (1M solution in dichloromethane, 3 eq) was added dropwise during 30 minutes. The mixture was stirred for 18 hours and then quenched with NaHCO$_3$ saturated...
solution; the organic layer was separated, washed with NaHCO₃ saturated solution, dried and evaporated under reduced pressure. The crude product was purified through flash chromatography on silica gel, eluting with petroleum ether/ethyl acetate 90/10. Yield 63%. Mp (iPr₂O) = 163 – 165 °C (dec.). MS ESI+: 308 / 310 [M-H]+, 228 [M – Br]+. ¹H-NMR (DMSO-d⁶) δ 10.54 (br. s., 1H, C₆H₃OH), 7.49 (m, 2H), 7.36 (d, J = 8.8 Hz, 1H), 7.31 (d, J = 2.7 Hz, 1H), 7.25 (m, 2H), 7.14 (dd, J = 8.5, 2.5 Hz, 1H), 4.74 (s, 2H, CH₂Br).

General procedure for the synthesis of 4a-f and 11a-e. The appropriate biphenyl compound was dissolved in dichloromethane; the solution was cooled in an ice – water bath and p-toluenesulfonyl chloride (2.2 eq), triethylamine (2.5 eq), N,N-dimethylpyridin-4-amine (DMAP, 0.05 eq) were added. The mixture was warmed to room temperature, stirred for 6 hours, then washed with water, NaHCO₃ saturated solution and brine, dried and evaporated under reduced pressure. The crude products were immediately dissolved in acetonitrile, then 6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline hydrochloride (1.7 eq) and 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU, 2.5 eq) were added. The mixture was stirred at 70 °C for 18 hours, then concentrated under reduced pressure, taken up with dichloromethane, washed with water and brine, dried and evaporated under reduced pressure. The crude products were purified through flash chromatography on silica gel.

3′-Methoxy-4′-((6,7-dimethoxy-3,4-dihydroisoquinolin-2(1H)-yl)-methyl)biphenyl-4-yl 4-methylbenzenesulfonate (4a). Eluent: petroleum ether/acetone 75/25. Yield: 64%. Pale yellow oil. MS ESI+: 560 [M-H]+. ¹H-NMR (CDCl₃) δ 7.74 (d, J = 8.2 Hz, 2H), 7.51 (m, 3H), 7.33 (d, J = 8.2 Hz, 2H), 7.10 (m, 1H), 7.03 (m, 3H), 6.61 (s, 1H), 6.51 (s, 1H), 3.90 (s, 3H, OCH₃), 3.84 (s, 3H, OCH₃), 3.82 (s, 3H, OCH₃), 3.76 (s, 2H), 3.64 (s, 2H), 2.82 (dd, J = 9.9, 4.4 Hz, 4H), 2.45 (s, 3H, C₆H₄CH₂). ¹³C-NMR (CDCl₃) δ 158.10, 148.95, 147.44, 147.11, 145.41, 140.20, 139.85, 132.39, 130.76, 129.80, 128.54, 128.20, 126.84, 126.22, 126.05, 122.64, 119.17, 111.37, 109.46, 109.24, 55.89, 55.67, 55.57, 55.45, 50.90, 28.68, 21.74.

3′-Fluoro-4′-((6,7-dimethoxy-3,4-dihydroisoquinolin-2(1H)-yl)-methyl)biphenyl-4-yl 4-methylbenzenesulfonate (4b). Eluent: petroleum ether/acetone 80/20. Yield: 67%. Mp (iPr₂O) = 156 – 158 °C (dec.). MS ESI+: 548 [M-H]+. ¹H-NMR (CDCl₃) δ 7.74 (d, J = 8.2 Hz, 2H), 7.52 (m, 3H), 7.30 (m, 3H), 7.22 (dd, J = 11.0, 1.6 Hz, 1H), 7.05 (d, J = 8.8 Hz, 2H), 6.60 (s, 1H), 6.50 (s, 1H), 3.84 (s, 3H, OCH₃), 3.81 (s, 3H, OCH₃), 3.79 (s, 2H), 3.62 (s, 2H), 2.82 (dd, J = 10.4, 4.4 Hz, 4H), 2.45 (s, 3H, C₆H₄CH₂). ¹³C-NMR (CDCl₃) δ 161.56
(d, J = 245.2 Hz), 149.22, 147.31 (d, J = 23.2 Hz), 145.43, 140.75, 140.64, 138.58 (d, J = 1.5 Hz), 132.26, 131.90 (d, J = 5.2 Hz), 129.77, 128.49, 128.07, 126.21, 125.87, 122.78, 122.52 (d, J = 3.0 Hz), 113.72 (d, J = 24.0 Hz), 113.76 (d, J = 4.5 Hz), 112.24, 111.28, 109.32, 55.84, 55.26, 54.51, 50.66, 28.57, 21.69.

4′-((6,7-Dimethoxy-3,4-dihydroisoquinolin-2(1H)-yl)-methyl)-3′-nitrobiphenyl-4-yl 4-methylbenzenesulfonate (4c). Eluent: petroleum ether/acetone 85/15. Yield: 74%. Sticky solid. MS ESI+: 575 [M-H]+. 1H-NMR (CDCl3) δ 8.03 (d, J = 1.6 Hz, 1H), 7.86 (d, J = 7.7 Hz, 1H), 7.74 (m, 3H), 7.53 (d, J = 8.8 Hz, 2H), 7.34 (d, J = 8.2 Hz, 2H), 7.10 (m, 2H), 6.71 (s, 1H), 6.48 (s, 1H), 4.03 (s, 2H), 3.85 (s, 3H, OCH3), 3.81 (s, 3H, OCH3), 3.63 (s, 2H), 2.82 (m, 4H), 2.47 (s, 3H, C6H4CH3).

13C-NMR (CDCl3) δ 149.91, 149.68, 147.56, 147.22, 145.55, 137.30, 132.19, 131.64, 130.90, 129.82, 128.48, 128.18, 125.84, 123.06, 122.83, 122.62, 117.62, 111.34, 109.29, 58.15, 55.87, 55.48, 50.91, 23.79, 21.71.

2′-Methoxy-4′-((6,7-dimethoxy-3,4-dihydroisoquinolin-2(1H)-yl)-methyl)biphenyl-4-yl 4-methylbenzenesulfonate (4d). Eluent: petroleum ether/acetone 85/15. Yield: 70%. Pale yellow oil. MS ESI+: 560 [M-H]+. 1H-NMR (CDCl3) δ 7.73 (d, J = 8.8 Hz, 2H), 7.31 (m, 4H), 7.16 (m, 3H), 6.99 (m, 2H), 6.87 (dd, J = 8.8, 2.7 Hz, 1H), 6.58 (s, 1H), 6.47 (s, 1H), 3.84 (overlapping peaks, 6H, 2 x OCH3), 3.82 (s, 3H, OCH3), 3.47 (br. s., 4H), 2.74 (m, 2H), 2.61 (m, 2H), 2.44 (s, 3H, C6H4CH3). 13C-NMR (CDCl3) δ 159.03, 148.37, 147.80, 147.39, 147.11, 145.29, 140.08, 133.77, 132.43, 131.05, 130.87, 129.70, 128.49, 126.19, 121.73, 115.17, 111.26, 109.35, 58.45, 55.87, 55.48, 50.91, 23.79, 21.68.

2′-Fluoro-4′-((6,7-dimethoxy-3,4-dihydroisoquinolin-2(1H)-yl)-methyl)biphenyl-4-yl 4-methylbenzenesulfonate (4e). Eluent: petroleum ether/acetone 85/15. Yield: 84%. White solid. Mp (iPr2O) = 147.3–148.0 °C. MS ESI+: 548 [M-H]+. 1H-NMR (CDCl3) δ 7.67 (d, J = 8.2 Hz, 2H), 7.40 (dd, J = 8.5, 1.4 Hz, 4H), 7.26 (m, 2H), 7.17 (m, 2H), 6.97 (m, 2H), 6.54 (s, 1H), 6.42 (s, 1H), 3.77 (s, 3H, OCH3), 3.74 (s, 3H, OCH3), 3.64 (br. s., 2H), 3.54 (br. s., 2H), 2.76 (m, 4H), 2.38 (s, 3H, C6H4CH3). 13C-NMR (CDCl3) δ 159.58 (d, J = 246.8), 148.98, 147.80 (d, J = 26.25), 145.40, 134.56 (d, J = 1.5 Hz), 132.34, 130.34 (d, J = 3.8 Hz), 130.10 (d, J = 3.8 Hz), 129.94 (d, J = 16.5 Hz), 129.77, 128.49, 125.78, 125.77 125.02, 124.98, 122.34, 116.64 (d, J = 22.5 Hz), 111.34, 109.34, 55.87, 55.35, 55.31, 50.87, 28.30, 21.69.

4′-((6,7-Dimethoxy-3,4-dihydroisoquinolin-2(1H)-yl)-methyl)-2′-nitrobiphenyl-4-yl 4-methylbenzenesulfonate (4f). Eluent: petroleum ether/acetone 75/25. Yield: 77%. Sticky solid. MS ESI+: 575 [M-H]+. 1H-NMR (CDCl3) δ 7.84 (s, 1H), 7.64 (d, J = 8.2 Hz, 2H), 7.27 (m, 3H), 7.15 (m, 2H), 7.03 (d, J = 7.7 Hz, 1H), 6.95 (d, J = 8.8 Hz, 2H), 6.54 (s, 1H), 6.42
3-Methoxy-4′-((6,7-dimethoxy-3,4-dihydroisoquinolin-2(1H)-yl)-methyl)biphenyl-4-yl 4-methylbenzenesulfonate (11a). Eluent: petroleum ether/ethyl acetate 50/50. Yield: 73%. White solid. Mp (ethyl acetate) 81.5 – 82 °C. MS ESI+: 560 [M-H]+. 1H-NMR (CDCl3) δ 7.79 (d, J = 8.2 Hz, 2H), 7.49 (m, 4H), 7.31 (d, J = 8.2 Hz, 2H), 7.19 (d, J = 8.8 Hz, 1H), 7.10 (m, 1H), 7.03 (d, J = 2.2 Hz, 1H), 6.61 (s, 1H), 6.49 (s, 1H), 3.84 (s, 3H, OCH3), 3.81 (s, 3H, OCH3), 3.75 (s, 2H), 3.62 (s, 3H, OCH3), 3.60 (s, 2H), 2.82 (m, 4H), 2.45 (s, 3H, C6H4CH3). 13C-NMR (CDCl3) δ 151.84, 147.55, 147.21, 144.98, 141.05, 139.15, 137.72, 133.24, 129.64, 129.41, 129.33, 128.60, 127.09, 127.03, 125.92, 124.13, 119.20, 111.41, 109.39, 62.06, 55.86, 55.58, 55.45, 50.64, 28.42, 21.65.

3-Fluoro-4′-((6,7-dimethoxy-3,4-dihydroisoquinolin-2(1H)-yl)-methyl)biphenyl-4-yl 4-methylbenzenesulfonate (11b). Eluent: petroleum ether/ethyl acetate 50/50. Yield: 78%. Sticky solid. MS ESI+: 548 [M-H]+. 1H-NMR (CDCl3) δ 7.79 (d, J = 8.2 Hz, 2H), 7.40 (m, 4H), 7.30 (m, 5H), 6.61 (s, 1H), 6.49 (s, 1H), 3.84 (s, 3H, OCH3), 3.81 (s, 3H, OCH3), 3.72 (br. s., 2H), 3.58 (br. s., 2H), 2.80 (m, 4H), 2.46 (s, 3H, C6H4CH3). 13C-NMR (CDCl3) δ 154.52 (d, J = 250.5 Hz), 147.47, 147.45, 147.14, 145.66, 141.50 (d, J = 6.8 Hz), 137.54 (d, J = 1.5 Hz), 135.77 (d, J = 12 Hz), 132.21, 129.75, 129.65, 128.53, 126.85, 126.35, 126.01, 124.95, 122.78 (d, J = 3.8 Hz), 115.34 (d, J = 18.8 Hz), 111.31, 109.35, 62.15, 55.84, 55.83, 55.59, 50.75, 28.58, 21.73.

4′-((6,7-Dimethoxy-3,4-dihydroisoquinolin-2(1H)-yl)-methyl)-3-nitrobiphenyl-4-yl 4-methylbenzenesulfonate (11c). Eluent: petroleum ether/acetone 80/20. Yield: 81%. Sticky solid. MS ESI+: 575 [M-H]+. 1H-NMR (CDCl3) δ 8.09 (d, J = 2.2 Hz, 1H), 7.80 (m, 3H), 7.55 (m, 4H), 7.45 (D, J = 8.2 Hz, 1H), 7.35 (d, J = 8.2 Hz, 2H), 6.61 (s, 1H), 6.49 (s, 1H), 3.84 (s, 3H, OCH3), 3.80 (s, 3H, OCH3), 3.74 (s, 2H), 3.58 (s, 2H), 2.80 (m, 4H), 2.47 (s, 3H, C6H4CH3). 13C-NMR (CDCl3) δ 147.50, 147.16, 146.37, 142.98, 140.83, 140.26, 139.32, 136.24, 132.19, 131.45, 130.06, 129.94, 128.67, 126.97, 126.31, 126.01, 125.65, 123.89, 111.33, 109.35, 62.11, 55.86, 55.62, 53.49, 28.58, 21.80.

2-Methoxy-4′-((6,7-dimethoxy-3,4-dihydroisoquinolin-2(1H)-yl)-methyl)biphenyl-4-yl 4-methylbenzenesulfonate (11d). Eluent: petroleum ether/ethyl acetate 50/50. Yield: 76%. Sticky solid. MS ESI+: 560 [M-H]+. 1H-NMR (CDCl3) δ 7.77 (d, J = 8.8 Hz, 2H), 7.43 (m, 4H), 7.33 (d, J = 8.2 Hz, 2H), 7.19 (d, J = 8.8 Hz, 1H), 6.60 (m, 3H), 6.49 (s, 1H), 3.83
(s, 3H, OCH₃), 3.80 (s, 3H, OCH₃), 3.71 (s, 2H), 3.69 (s, 3H, OCH₃), 3.58 (s, 2H), 2.81 (m, 4H), 2.44 (s, 3H, C₆H₄CH₃). ¹³C-NMR (CDCl₃) δ 156.95, 149.53, 147.44, 147.11, 145.41, 137.16, 136.08, 132.36, 131.02, 129.75, 129.31, 128.88, 128.58, 126.42, 126.06, 114.13, 111.31, 109.39, 105.96, 62.36, 55.85, 55.65, 55.54, 50.76, 28.53, 23.82.

2-Fluoro-4′-((6,7-dimethoxy-3,4-dihydroisoquinolin-2(1H)-yl)-methyl)biphenyl-4-yl 4-methylbenzenesulfonate (11e). Eluent: petroleum ether/acetone 80/20. Yield: 81%. Sticky solid. MS ESI+: 548 [M-H]⁺. ¹H-NMR (CDCl₃) δ 7.76 (d, J = 8.2 Hz, 2H), 7.48 (m, 4H), 7.37 (m, 3H), 6.87 (m, 2H), 6.61 (s, 1H), 6.49 (s, 1H), 3.84 (s, 3H, OCH₃), 3.81 (s, 3H, OCH₃), 3.75 (s, 2H), 3.60 (s, 2H), 2.83 (m, 4H), 2.47 (s, 3H, C₆H₄CH₃). ¹³C-NMR (CDCl₃) δ 159.26 (d, J = 249.8 Hz), 148.98 (d, J = 10.5 Hz), 147.36 (d, J = 25.5 Hz), 145.71, 137.97, 133.45, 132.05, 130.92 (d, J = 5.2 Hz), 129.90, 129.34, 128.83 (d, J = 3.0 Hz), 128.49, 127.90 (d, J = 13.5 Hz), 126.08, 125.91, 118.45 (d, J = 3.8 Hz), 111.31, 110.90 (d, J = 26.2 Hz), 109.36, 62.10, 55.85, 55.43, 50.69, 28.38, 23.80.

General procedure for the synthesis of target compounds 5a-f and 12a-e. The appropriate p-toluensulfonate intermediate was dissolved in a mixture of tetrahydrofuran and methanol 2/1; solid sodium hydroxide (20 eq) was added and the mixture was stirred at 65 °C for 1 hour. The solvents were evaporated under reduced pressure and the residue was taken up with water. The pH was brought to 7 with acetic acid and the product was extracted with dichloromethane. The organic phase was washed with brine, dried, filtered and evaporated under reduced pressure. The crude product was purified through flash chromatography on silica gel.

3'-Methoxy-4′-((6,7-dimethoxy-3,4-dihydroisoquinolin-2(1H)-yl)-methyl)biphenyl-4-ol (5a). Eluent dichloromethane/ethyl acetate 70/30. Yield 90%. White solid. Mp (iPrOH): 193.2 – 194 °C. MS ESI+: 406 [M-H]⁺. ¹H-NMR (CDCl₃) δ 7.36 (d, J = 7.7 Hz, 1H), 7.30 (d, J = 8.8 Hz, 2H), 7.02 (d, J = 7.7 Hz, 1H), 6.93 (s, 2H), 6.65 (d, J = 8.8 Hz, 2H), 6.58 (s, 1H), 6.51 (s, 1H), 3.80 (m, 11H), 3.72 (s, 2H), 2.92 (m, 4H). ¹³C-NMR (CDCl₃) δ 158.16, 156.43, 147.49, 147.14, 141.87, 132.27, 131.61, 127.93, 152.78, 125.75, 122.90, 118.44, 115.88, 111.23, 109.41, 108.82, 60.44, 55.79, 55.40, 55.26, 55.13, 50.52, 27.71, 20.99. Purity: ≥99%, t: 4.8 min., eluent: CH₃CN / H₂O 50 / 50 0.1% TFA, fl: 1.0 mL/min.

3′-Fluoro-4′-((6,7-dimethoxy-3,4-dihydroisoquinolin-2(1H)-yl)-methyl)biphenyl-4-ol (5b). Eluent petroleum ether/acetone 60/40. Yield 87%. White solid. Mp (EtOH): 199.7 – 198.5 °C. MS ESI+: 394 [M-H]⁺. ¹H-NMR (DMSO-d₆) δ 9.66 (s, 1H, C₆H₄OH), 7.53 (d, J = 8.2 Hz, 2H), 7.44 (m, 3H), 6.85 (d, J = 8.2 Hz, 2H), 6.65 (s, 1H), 6.58 (s, 1H), 6.59 (s, 1H), 3.69 (s, 3H, OCH₃), 3.66 (s, 3H, OCH₃), 3.48 (s, 2H), 3.37 (s, 2H), 2.71 (m, 4H). ¹³C-NMR
(DMSO-d$_6$) $\delta$ 161.28 (d, J = 243 Hz), 157.58, 147.15, 146.90, 141.38 (d, J = 8.25 Hz), 131.86, (d, J = 4.5 Hz), 129.32, 127.81, 126.33, 125.63, 122.49 (d, J = 15 Hz), 121.55 (d, J = 2.2 Hz), 115.78, 112.32 (d, J = 22.5 Hz), 111.70, 109.87, 55.43, 54.89 (d, J = 7.5 Hz), 54.26, 50.42, 28.30. Purity: $\geq$99%, $t$: 4.1 min., eluent: CH$_3$CN/H$_2$O 50/50 0.1% TFA, fl: 1.0 mL/min.


$^1$H-NMR (CDCl$_3$) $\delta$ 8.01 (d, J = 1.4 Hz, 1H), 7.70 (m, 2H), 7.43 (d, J = 8.5 Hz, 2H), 6.91 (d, J = 8.5 Hz, 2H), 6.61 (s, 1H), 6.50 (s, 1H), 4.02 (s, 2H), 3.84 (s, 3H, OCH$_3$), 3.81 (s, 3H, OCH$_3$), 3.64 (s, 2H), 2.82 (m, 4H).

$^{13}$C-NMR (CDCl$_3$) $\delta$ 156.64, 149.90, 147.50, 147.16, 141.06, 131.54, 131.25, 130.38, 130.33, 128.12, 126.02, 125.93, 122.22, 116.10, 111.36, 109.37, 58.16, 55.86, 55.51, 50.89, 28.45. Purity: 96%, $t$: 3.7 min., eluent: CH$_3$CN/H$_2$O 50/50 0.1% TFA, fl: 1.0 mL/min.


$^1$H-NMR (CDCl$_3$) $\delta$ 9.44 (s, 1H, C$_6$H$_4$OH), 7.17 (d, J = 8.2 Hz, 2H), 7.12 (m, 2H), 6.87 (dd, J = 8.5, 2.5 Hz, 1H), 6.78 (d, J = 8.2 Hz, 2H), 6.64 (s, 1H), 6.58 (s, 1H), 3.75 (s, 3H, OCH$_3$), 3.69 (s, 3H, OCH$_3$), 3.66 (s, 3H, OCH$_3$), 3.49 (s, 2H), 3.39 (s, 2H), 2.69 (m, 2H), 2.55 (m, 2H). $^{13}$C-NMR (CDCl$_3$) $\delta$ 159.56 (d, J = 245.2Hz), 155.86, 154.12, 148.69, 137.03, 134.42, 131.17, 130.98, 130.46, 126.57, 125.78, 114.80, 114.44, 112.12, 111.77, 109.94, 55.40, 55.44, 55.03, 54.93, 50.48, 28.46. Purity: $\geq$99%, $t$: 3.9 min., eluent: CH$_3$CN/H$_2$O 50/50 0.1% TFA, fl: 1.0 mL/min.


$^1$H-NMR (CDCl$_3$) $\delta$ 7.37 (dd, J = 8.5, 1.4 Hz, 2H), 7.30 (d, J = 8.0 Hz, 1H), 7.21 (m, 1H), 7.18 (m, 1H), 6.84 (d, J = 8.5 Hz, 2H), 6.61 (s, 1H), 6.52 (s, 1H), 3.84 (s, 3H, OCH$_3$), 3.82 (s, 3H, OCH$_3$), 3.73 (s, 2H), 3.64 (s, 2H), 2.86 (m, 4H). $^{13}$C-NMR (CDCl$_3$) $\delta$ 159.56 (d, J = 245.2Hz), 155.86, 147.61, 147.25, 138.20 (d, J = 7.5 Hz), 130.21 (d, J = 4.5 Hz), 130.09 (J = 3.0 Hz), 127.67 (d, J = 12.8 Hz), 127.40, 125.77, 125.16 (d, J = 3.0 Hz), 116.80 (d, J = 22.5 Hz), 115.55, 111.34, 109.41, 61.65, 55.86, 55.28, 50.69, 28.12. Purity: $\geq$99%, $t$: 3.6 min., eluent: CH$_3$CN/H$_2$O 50/50 0.1% TFA, fl: 1.0 mL/min.

4′-((6,7-Dimethoxy-3,4-dihydroisoquinolin-2(1H)-yl)-methyl)-2′-nitrobiphenyl-4-ol (5f). Eluent: petroleum ether/acetone 75/25. Yield: 48%. Yellow solid. Mp (ethyl acetate): 152.8
- 153.5 °C. MS ESI+: 421 [M-H]+. 1H-NMR (DMSO-d6) δ 7.84 (d, J = 1.7 Hz, 1H), 7.67 (dd, J = 8.1, 1.5 Hz, 1H), 7.47 (d, J = 7.9 Hz, 1H), 7.13 (m, 2H), 6.82 (m, 2H), 6.67 (s, 1H), 6.61 (s, 1H), 3.73 (s, 2H), 3.69 (s, 3H, OCH3), 3.66 (s, 3H, OCH3), 3.48 (s, 2H), 2.75 (t, J = 5.5 Hz, 2H), 2.70 (t, J = 5.5 Hz, 2H). 13C-NMR (DMSO-d6) δ 157.74, 148.91, 147.19, 146.94, 139.26, 133.59, 132.61, 131.58, 128.99, 126.28, 125.65, 123.59, 115.70, 111.76, 109.92, 60.41, 55.44, 54.91, 50.54, 28.27. Purity: 97%, tR: 3.9 min., eluent: CH3CN/H2O 50/50 0.1% TFA, fl: 1.0 mL/min.

3-Methoxy-4′-((6,7-dimethoxy-3,4-dihydroisoquinolin-2(1H)-yl)-methyl)biphenyl-4-ol (12a). Eluent petroleum ether/acetone 70/30. Yield 81%. White solid. Mp (iPrOH): 163.7 – 164.5 °C. MS ESI+: 406 [M-H]+. 1H-NMR (CDCl3) δ 7.53 (d, J = 8.2 Hz, 2H), 7.46 (d, J = 8.2 Hz, 2H), 7.12 (m, 2H), 6.98 (d, J = 7.7 Hz, 1H), 6.62 (s, 1H), 6.51 (s, 1H), 3.95 (s, 3H, OCH3), 3.86 (s, 3H, OCH3), 3.82 (s, 3H, OCH3), 3.74 (s, 2H), 3.61 (s, 2H), 2.84 (m, 4H). 13C-NMR (CDCl3) δ 147.44, 147.13, 146.76, 145.22, 140.05, 136.62, 133.33, 129.54, 126.63, 126.44, 126.07, 119.97, 114.74, 111.32, 109.58, 109.40, 62.26, 55.89, 55.84, 55.53, 50.69, 28.52. Purity: ≥99%, tR: 4.1 min., eluent: CH3CN/H2O 55/45 0.1% TFA, fl: 1.0 mL/min.

3-Fluoro-4′-((6,7-dimethoxy-3,4-dihydroisoquinolin-2(1H)-yl)-methyl)biphenyl-4-ol (12b). Eluent dichloromethane / ethyl acetate 80 / 20. Yield 88%. White solid. Mp (EtOH) 184 – 184.8 °C. MS ESI+: 394 [M-H]+. 1H-NMR (CDCl3) δ 7.58 (d, J = 8.2, 2H), 7.47 (dd, J = 12.9, 1.9 Hz, 1H), 7.38 (d, J = 8.2 Hz, 2H), 7.32 (dd, J = 8.2, 1.6 Hz, 1H), 7.02 (t, J = 8.8 Hz, 1H), 6.65 (s, 1H), 6.57 (s, 1H), 3.69 (s, 3H, OCH3), 3.65 (s, 3H, OCH3), 3.63 (s, 2H), 3.43 (s, 2H), 2.69 (m, 4H). 13C-NMR (CDCl3) δ 151.44 (d, J = 239.2Hz), 147.14, 146.90, 144.38 (d, J = 12.2 Hz), 137.23, 131.56 (d, J = 6.1 Hz), 129.32, 126.50, 125.98, 125.75, 122.57 (d, J = 2.8 Hz), 118.13 (d, J = 3.3 Hz), 114.09 (d, J = 18.8 Hz), 111.75, 109.89, 61.58, 55.43, 55.06, 50.55, 28.34. Purity: 98%, tR: 3.4 min., eluent: CH3CN/H2O 55/45 0.1% TFA, fl: 1.0 mL/min.

4′-((6,7-Dimethoxy-3,4-dihydroisoquinolin-2(1H)-yl)-methyl)-3-nitrobiphenyl-4-ol (12c). Eluent: petroleum ether/acetone 80/20. Yield: 73%. Yellow solid. Mp (MeOH) 172.9 – 173.6 °C. MS ESI+: 421 [M-H]+. 1H-NMR (CDCl3) δ 8.32 (d, J = 2.2 Hz, 1H), 7.84 (dd, J = 8.8, 2.2 Hz, 1H), 7.52 (m, 4H), 7.23 (d, J = 8.2 Hz, 1H), 6.61 (s, 1H), 6.49 (s, 1H), 3.85 (s, 3H, OCH3), 3.81 (s, 3H, OCH3), 3.74 (s, 2H), 3.59 (s, 2H), 2.81 (m, 4H). 13C-NMR (CDCl3) δ 154.23, 147.47, 147.14, 138.20, 137.05, 136.13, 133.72, 133.53, 129.78, 126.54, 126.29, 125.98, 122.60, 120.36, 111.30, 109.34, 62.13, 55.83, 55.57, 50.72, 28.55. Purity: 99%, tR: 4.7 min., eluent: CH3CN/H2O 55/45 0.1% TFA, fl: 1.0 mL/min.
2-methoxy-4′-((6,7-Dimethoxy-3,4-dihydroisoquinolin-2(1H)-yl)-methyl)biphenyl-4-ol (12d). Eluent petroleum ether/acetone 80/20. Yield 83%. White solid. Mp (iPrOH): 216.8 – 217.5 °C. MS ESI+: 406 [M-H]+. 1H-NMR (DMSO-d6) δ 9.57 (s, 1H, C6H3OH), 7.36 (m, 4H), 7.09 (d, J = 8.2 Hz, 1H), 6.64 (m, 2H), 6.50 (d, J = 2.2 Hz, 1H), 6.43 (dd, J = 8.2, 2.2 Hz, 1H), 3.71 (s, 3H, OCH3), 3.69 (s, 3H, OCH3), 3.66 (s, 5H), 3.47 (br. s., 2H), 2.75 (br. s., 4H). 13C-NMR (DMSO-d6) δ 158.26, 157.17, 147.21, 146.97, 137.27, 135.84, 130.96, 128.99, 128.54, 126.32, 125.74, 120.50, 111.78, 109.94, 107.55, 99.49, 61.72, 55.49, 55.31, 55.02, 50.60, 28.25. Purity: 98% tR: 3.4 min., eluent: CH3CN/H2O 55/45 0.1% TFA, fl: 1.0 mL/min.

2-Fluoro-4′-((6,7-dimethoxy-3,4-dihydroisoquinolin-2(1H)-yl)-methyl)biphenyl-4-ol (12e). Eluent petroleum ether/acetone 80/20. Yield 80%. White solid. Mp (EtOH) 91.2 – 92 °C. MS ESI+: 394 [M-H]+. 1H-NMR (DMSO-d6) δ 10.08 (br. s., 1H, C6H3OH), 7.38 (m, 5H), 6.68 (m, 2H), 6.66 (s, 1H), 6.59 (s, 1H), 3.69 (s, 3H, OCH3), 3.66 (br. s., 5H), 3.45 (s, 2H), 2.71 (m, 4H). 13C-NMR (DMSO-d6) δ 159.92 (d, J = 209.2Hz), 158.21 (d, J = 21.0 Hz), 147.15, 146.92, 137.22, 134.17, 131.07 (d, J = 5.5 Hz), 128.97, 128.32 (d, J = 3.3 Hz), 126.46, 125.76, 118.66 (d, J = 13.3 Hz), 112.20 (d, J = 2.2 Hz), 111.75, 109.91, 103.09 (d, J = 18.0 Hz), 61.66, 55.45, 55.08, 50.62, 28.35. Purity: 99% tR: 3.6 min., eluent: CH3CN/H2O 55/45 0.1% TFA, fl: 1.0 mL/min.

4′-((6,7-Dimethoxy-3,4-dihydroisoquinolin-2(1H)-yl)-methyl)-2-nitrobiphenyl-4-ol (16). 4′-(bromomethyl)-2-nitrobiphenyl-4-ol 15 was dissolved in acetonitrile, then 6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline hydrochloride (1.7 eq) and 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU, 2.5 eq) were added. The mixture was stirred at 70 °C for 18 hours, then concentrated under reduced pressure, taken up with dichloromethane, washed with water and brine, dried and evaporated under reduced pressure. The crude product was purified through flash chromatography on silica gel eluting with petroleum ether/acetone 70/30. Yield: 66%. Yellow solid. Mp (MeOH) 162.9 – 163.7 °C. MS ESI+: 421 [M-H]+. 1H-NMR (CDCl3) δ 7.37 (d, J = 7.7 Hz, 2H), 7.14 (s, 1H), 7.08 (m, 3H), 6.77 (dd, J = 8.5, 2.5 Hz, 1H), 6.60 (s, 1H), 6.52 (s, 1H), 3.83 (s, 3H, OCH3), 3.82 (s, 3H, OCH3), 3.77 (s, 2H), 3.68 (s, 2H), 2.93 (br. s., 4H). 13C-NMR (CDCl3) δ 156.81, 149.28, 147.78, 147.36, 137.03, 135.33, 132.60, 130.02, 128.01, 126.81, 125.39, 124.90, 120.28, 111.61, 111.23, 109.34, 62.03, 55.86, 55.82, 55.08, 50.81, 27.65. Purity: 99% tR: 4.6 min., eluent: CH3CN/H2O 50/50 0.1% TFA, fl: 1.0 mL/min.

General procedure for the synthesis of target compounds 17a,b. Compounds 12c and 16 were dissolved in methanol. Palladium (10% dispersion on charcoal, 0,1 eq) was added
and the mixture was kept under hydrogen atmosphere for 3 hours. The suspension was filtered over celite and the filtrate was evaporated under reduced pressure. The crude products were purified through flash chromatography on silica gel.

**4′-((6,7-Dimethoxy-3,4-dihydroisoquinolin-2(1H)-yl)-methyl)-3-aminobiphenyl-4-ol (17a).** Eluent petroleum ether/ethyl acetate 50/50. Yield 61%. Off-white solid. Mp (iPrOH) 97.4 – 98 °C. MS ESI+: 391 [M-H]+1. H-NMR (DMSO-d6) δ 9.17 (br. s., 1H, C6H3OH), 7.46 (d, J = 8.2 Hz, 2H), 7.34 (d, J = 8.2 Hz, 2H), 6.91 (s, 1H), 6.71 (s, 2H), 6.65 (s, 1H), 6.58 (s, 1H), 4.61 (br. s., 2H, NH2), 3.69 (s, 3H, OCH3), 3.65 (s, 3H, OCH3), 3.61 (s, 2H), 3.43 (s, 2H), 2.69 (m, 4H). 13C-NMR (DMSO-d6) δ 147.12, 146.90, 143.92, 139.81, 136.93, 136.27, 131.47, 129.16, 126.56, 125.78, 125.73, 114.75, 114.68, 112.53, 111.73, 109.90, 61.73, 55.44, 55.08, 50.62, 28.37. Purity: ≥99%, tR: 4.3 min., eluent: CH3CN/H2O 40/60 0.1% TFA, fl: 1.0 mL/min.

**4′-((6,7-Dimethoxy-3,4-dihydroisoquinolin-2(1H)-yl)-methyl)-2-aminobiphenyl-4-ol (17b).** Eluent petroleum ether/ethyl acetate 40/60. Yield 58%. Off-white solid. Mp (iPrOH) 88.6 – 89.5 °C. MS ESI+: 391 [M-H]+1. H-NMR (DMSO-d6) δ 9.06 (br. s., 1H, C6H3OH), 7.35 (m, 4H), 6.79 (d, J = 8.2 Hz, 2H), 6.67 (s, 2H), 6.59 (s, 1H), 6.20 (m, 1H), 6.09 (dd, J = 8.2, 2.2 Hz, 1H), 4.70 (br. s., 2H, NH2), 3.69 (s, 3H, OCH3), 3.66 (br. s., 5H), 3.49 (s, 2H), 2.74 (br. s., 4H). 13C-NMR (DMSO-d6) δ 157.54, 147.18, 146.92, 146.00, 138.85, 138.70, 130.85, 129.16, 128.48, 125.87, 117.37, 114.66, 111.73, 109.90, 104.73, 101.70, 61.66, 55.44, 54.92, 50.54, 29.03. Purity: ≥99%, tR: 4.7 min., eluent: CH3CN/H2O 30/70 0.1% TFA, fl: 1.0 mL/min.

Section 2. Biology.

**Materials.** Cell culture reagents were purchased from Celbio s.r.l. (Milano, Italy). CulturePlate 96/wells plates were purchased from PerkinElmer Life Science; Calcein-AM, bisBenzimide H 33342 trihydrochloride were obtained from Sigma-Aldrich (Milan, Italy).

**Cell cultures.** MDCK-MDR1, MDCK-MRP1 and MDCK-BCRP cells are a gift of Prof. P. Borst, NKI-AVL Institute, Amsterdam, Nederland. MDCK cells were grown in DMEM high glucose supplemented with 10% fetal bovine serum, 2 mM glutamine, 100 U/mL penicillin, 100 µg/mL streptomycin, in a humidified incubator at 37 °C with a 5 % CO2 atmosphere. Caco-2 cells were a gift of Dr. Aldo Cavallini and Dr. Caterina Messa from the Laboratory of Biochemistry, National Institute for Digestive Diseases, “S. de Bellis”, Bari (Italy).

**Calcein-AM experiments.** These experiments were carried out as described by Capparelli et al.[3] with minor modifications. Each cell line (30,000 cells per well) was
seeded into black CulturePlate 96/wells plate with 100 μl medium and allowed to become confluent overnight. 100 μl of test compounds were solubilized in culture medium and added to monolayers. 96/Wells plate was incubated at 37 °C for 30 min. Calcein-AM was added in 100 μl of Phosphate Buffered Saline (PBS) to yield a final concentration of 2.5 μM and plate was incubated for 30 min. Each well was washed 3 times with ice cold PBS. Saline buffer was added to each well and the plate was read with Victor3 (PerkinElmer) at excitation and emission wavelengths of 485 nm and 535 nm, respectively. In these experimental conditions Calcein cell accumulation in the absence and in the presence of tested compounds was evaluated and fluorescence basal level was estimated with untreated cells. In treated wells the increase of fluorescence with respect to basal level was measured. EC_{50} values were determined by fitting the fluorescence increase percentage versus log[dose].

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

**ATPlite assay.** The MDCK-MDR1 cells were seeded into 96-well microplate in 100 μl of complete medium at a density 2x10^4 cells/well.[3] The plate was incubated overnight (O/N) in a humidified atmosphere 5% CO_2 at 37 °C. The medium was removed and 100 μl of complete medium either alone or containing different concentrations of test compounds was added. The plate was incubated for 2h in a humidified 5% CO_2 atmosphere at 37 °C. 50 μl of mammalian cell lysis solution was added to all wells and the plate shaked for five minutes in an orbital shaker. 50 μl of substrate solution was added to all wells and the
plate shaked for five minutes in an orbital shaker. The plate was dark adapted for ten minutes and the luminescence was measured.

**Permeability Experiments.**

**Preparation of Caco-2 monolayer.** Caco-2 cells were seeded onto a Millicell® assay system (Millipore), where a cell monolayer is set in between a filter cell and a receiver plate, at a density of 10,000 cells/well.\[^4\] The culture medium was replaced every 48 h and the cells kept for 21 days in culture. The Trans Epithelial Electrical Resistance (TEER) of the monolayers was measured daily, before and after the experiment, using an epithelial voltohmometer (Millicell®-ERS). Generally, TEER values greater than 1000 Ω for a 21 day culture, are considered optimal.

**Drug transport experiment.** After 21 days of Caco-2 cell growth, the medium was removed from filter wells and from the receiver plate, which were filled with fresh HBSS buffer (Invitrogen). This procedure was repeated twice, and the plates were incubated at 37 °C for 30 min. After incubation time, the HBSS buffer was removed and drug solutions and reference compounds, were added to the filter well at the concentration of 100 μM, while fresh HBSS was added to the receiver plate. The plates were incubated at 37 °C for 120 min. Afterwards, samples were removed from the apical (filter well) and basolateral (receiver plate) side of the monolayer to measure the permeability. The apparent permeability ($P_{app}$), in units of nm/second, was calculated using the following equation:

$$P_{app} = \frac{V_A}{\text{Area} \times \text{time}} \times \frac{[\text{drug}]_{\text{acceptor}}}{[\text{drug}]_{\text{initial}}}$$

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

**Antiproliferative assay.** Determination of cell growth was performed using the MTT assay at 48 h and 72 h.\[^5\] On day 1, 10000 cells/well were seeded into 96-well plates in a volume of 100 μL. On day 2, the drugs concentration (0.1, 1, 10, 25 μM) were added. In all the
experiments, the various drug-solvents (ethanol, DMSO) were added in each control to evaluate a possible solvent cytotoxicity. After the established incubation time with drugs, MTT (0.5 mg/mL) was added to each well, and after 3 h incubation at 37°C, the supernatant was removed. The formazan crystals were solubilized using 100 μL of DMSO and the absorbance values at 570 and 630 nm were determined on the microplate reader Victor 3 from PerkinElmer Life Sciences.

Co-administration Assay. The co-administration assay with doxorubicin was performed in MDCK-MDR1 cells at 48h as reported with minor modification.[6]

On day 1, 10000 cells/well were seeded into 96-well plates in a volume of 100 μL of fresh medium. On day 2, 10 μM tested drugs were added alone to the cells. On day 3, the medium was removed and the drugs at the same concentration were added alone and in co-administration with 10 μM doxorubicin to the cells. After the established incubation time with drugs, MTT (0.5 mg/mL) was added to each well, and after 3-4 h incubation at 37 °C, the supernatant was removed. The formazan crystals were solubilized using 100 μL of DMSO/EtOH (1:1), and the absorbance values at 570 and 630 nm were determined on the microplate reader Victor 3 from PerkinElmer Life Sciences.

Immunoblotting. Cells were rinsed with ice-cold lysis buffer (50 mM, Tris, 10 mM EDTA, 1% v/v Triton-X100), supplemented with the protease inhibitor cocktail set III (80 μM aprotinin, 5 mM bestatin, 1.5 mM leupeptin, 1 mM pepstatin; Calbiochem, San Diego, CA), 2 mM phenylmethylsulfonyl fluoride and 1 mM Na₃VO₄, then sonicated and centrifugated at 13,000 x g for 10 min at 4°C. 25 μg protein extracts were subjected to SDS-PAGE and probed with anti-Pgp (Calbiochem) or anti-β-actin (Sigma Chemicals Co., Saint Louis, MO), followed by a peroxidase-conjugated secondary antibody (Bio-Rad Laboratories, Hercules, CA). The membranes were washed with Tris-buffered saline-Tween 0.1% v/v solution, and the proteins were detected by enhanced chemiluminescence (Bio-Rad Laboratories).

Section 3. Molecular modelling.
Ligand molecular scaffold in ionized form, with standard bond lengths and valence angles, was built with Maestro software package[7] and charge calculated with the AM1BCC method using the molcharge suite of QUACPAC.[8] The puckering of the
tetrahydroisoquinoline ring was not altered, according to the X-ray of MC70 as previously reported.\textsuperscript{[9]}

Docking was carried out using as receptor model the MDR1 structure as reported.\textsuperscript{[10]}

Differently to the previous study in this instance protein charges were assigned according to the AMBER FF14SB force field within CHIMERA 1.11,\textsuperscript{[11]} affinity maps were calculated on a 90×65×65 rectangular box, 0.375 Å spaced, centered on the intracellular crevice of the two TMDs, taking into account water contribution according to the hydration force field of AutoDock 4.2.\textsuperscript{[12]} A total of 250 Lamarckian Genetic Algorithm (LGA) runs were carried out, the population size and the number of energy evaluations were set to 150 and 5 million respectively, and the initial coordinates for the center of the ligand (trans0), ligand rigid-body orientation (quat0) and relative dihedral angles (dihe0) to random values. All poses were then clustered to RMSD = 2.0, and ranked according to the relative Free Energy of Binding (FEB). The best FEB pose was selected as representative of 5d binding mode.

Section 4. References.

\textsuperscript{[1]} A. Speicher, T. Backes, S. Grosse, Tetrahedron 2005, 61, 11692-11696.