Potent and selective tariquidar bioisosters as potential PET radiotracers for imaging P-gp

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ABSTRACT

Compounds 8a–d have been designed as bioisosters of tariquidar for imaging P-gp expression and density by PET. The results displayed that compounds 8b and 8d could be considered potential P-gp/BCRP ligands suitable as 11C and 18F radiotracers, respectively.

The Adenosine triphosphate (ATP) Binding Cassette (ABC) transporter P-glycoprotein (P-gp) is expressed in several tissues such as small intestine, Blood Brain Barrier (BBB), Blood Cerebro Spinal Fluid Barrier (BCSF), Blood Testis Barrier (BTTB), hepatocytes and kidneys. P-gp modulates both xenobiotics absorption and excretion in these barriers and organs. Furthermore, P-gp overexpression is one of the main cause of the failure of chemotherapeutic treatment as it modulated chemotherapeutic drugs efflux. In addition, since several drugs are P-gp substrates and the efflux pump saturation could induce an alteration in gastrointestinal tract absorption and in brain barrier permeation, there is a considerable interest in the quantification of P-gp expression and function by non-invasive imaging analysis such as PET. In this scenario, an important goal is the development of P-gp PET radiotracers displaying high affinity and selectivity towards the target.

Among P-gp radiotracers, [11C]tariquidar has been the mostly studied, as P-gp inhibitor (Fig. 1); on this radiotracer some PET studies have been performed and recent results demonstrated that it was dose-dependently transported by P-gp and BCRP pumps.

In our laboratory, MC18 and MC113 have been designed as P-gp ligands and their biological evaluation classified them as potent and selective P-gp inhibitors; thus, these ligands have been [11C]-radiolabelled and tested in vivo by microPET analysis (Fig. 2). [11C]MC18 displayed in vivo and in vitro interesting and consistent results while [11C]MC113 showed high brain uptake but it was unsuitable to visualize P-gp expression in murine tumor model.

In order to potentiate the activity and selectivity of our lead compounds (MC18, MC113), we developed bioisosters bearing arylthiazole moiety as depicted in Figure 3 and among them MC90 that displayed, with respect to our lead compounds, superimposed results. Comparing MC90 to tariquidar we hypothesized that the arylthiazole fragment could be considered as biosisosters of 3-carboxamidouquinoline (A) (Fig. 1). Since, tetrahydroisoxquinoline moiety is the same (C) and the N-phenylbenzamide fragment (B) could be considered the pivotal part of the molecule useful to increase P-gp activity in nanomolar range, we designed a bioequivalent fragment inserting it between A and C of MC90 structure to obtain a set of compounds belonging to general formula reported in Figure 4. The substituents on arylthiazole moiety have been selected taking into account the radiolabelling reaction for obtaining 11C- or 18F-PET probes. For this purpose, methoxy derivative (8b) and the corresponding hydroxy derivative (8a) as precursor for [11C]8b and fluorodervative (8d) and the corresponding nitroderivative (8c) as precursor for [18F]8d have been planned. Compounds 4 and 5 (amide and thioamide, respectively) have been prepared to demonstrate the role of arylthiazole fragment in these molecules since the basic moiety (C) was the same while central nucleus (B) was our hypothesis. All compounds have been tested in the three known biological assays for determining: (i) P-gp activity; (ii) ATP-ase activity; (iii) Apparent Permeability (Papp). All these results permit to establish the potency and the P-gp interacting mechanism (substrate or inhibitor or modulator). In particular, substrates activate ATP-ase whereas inhibitors are...
unable to stimulate this site. Furthermore, the Apparent Permeability \((P_{\text{app}})\) represents the contribution of two different fluxes: the first, from basolateral to apical is representative for passive transport, while the flux from apical to basolateral is representative for active transport. Both fluxes contributed to define the Apparent Permeability so that \(BA/AB\) ratio is \(\geq 2\) for substrates while it is \(\leq 2\) for P-gp inhibitors.

The synthesis of arylthiazole derivatives A, B, and C is depicted in Scheme 1. The carboxamide 3 was prepared with the commercially available 4-hydroxybenzaldehyde (1) and 6-chloronicotinamide (2) in DMF. Compound 4 was obtained by condensing compound 3 with 6,7-dimethoxy-1,2,3,4-tetrahydrioisoquinoline in dry \(\text{CH}_2\text{Cl}_2\) and the intermediate was reduced in the presence of \(\text{NaBH}_4\). The amide function was treated with Lawesson’s reagent in dry THF to give the corresponding thioamide (5). The crude 5 was condensed with the appropriate arylbromoketone 7A–D, synthesized starting from appropriate alkyketone 6A–D, leading to arylthiazoles A, B, and C.

The biological results were listed in Table 1 where reference compounds MC18, MC113, and MC90 are reported. All arylthiazoles

\[
\begin{align*}
\text{A} & \\
\text{B} & \\
\text{C} & \\
\end{align*}
\]

\[\text{Figure 1. } [^{11}\text{C}]\text{tariquidar.}\]

\[\text{Figure 2. } P\text{-gp radiotracers.}\]

\[\text{Figure 3. Arylthiazole derivative MC90.}\]

\[\text{Figure 4. General formula of arylthiazole derivatives A, B, and C.}\]

\[\text{Figure 5. MC90.}\]

\[\text{[}^{11}\text{C}]\text{MC18} \quad [^{11}\text{C}]\text{MC113}\]
8a–d displayed submicromolar P-gp activity ranging from 0.25 to 0.95 \text{M}. Moreover, all ligands were inactive towards MRP1 (EC_{50} >100 \mu \text{M}). Surprisingly, compound 8a was more active towards BCRP (EC_{50} = 0.018 \mu \text{M}) than towards P-gp (EC_{50} = 0.25 \mu \text{M}). Compound 8b displayed superimposed P-gp/BCRP activities whereas compounds 8c and 8d were moderately more potent towards P-gp than towards BCRP.

The derivatives 4 and 5, lacking arylthiazole nucleus, were about 100-fold less potent than thiazoles 8a–d confirming that this moiety plays a crucial role in P-gp activity. Apparent Permeability (P_{app}) and ATP-ase activity for each compound indicated that compounds 8a–d could be considered as P-gp transported substrates. The same results have been obtained for tariquidar.

With respect to lead compounds MC18 (P-gp inhibitor), MC113 recently studied as \textsuperscript{[11C]}MC113\textsuperscript{10} and MC90, thiazole derivatives 8a–d displayed comparable P-gp activity but were inactive towards MRP1 pump. However, other substituents are needed to better investigate their role in determining P-gp intrinsic activity. These preliminary results indicated that compound 8b could be considered P-gp/BCRP ligand tariquidar-like although less potent towards P-gp with respect to reference compound. Compound 8d could be considered potential P-gp/BCRP radiotracer tariquidar-like even if it was less potent than 8a and tariquidar.\textsuperscript{7} Since P-gp density at the human blood-brain barrier is in nanomolar range,\textsuperscript{14} it is need a PET radiotracer displaying inhibitory activity in the same range to visualize P-gp expression. By contrast, in order to measure P-gp activity are sufficient radiotracers, as verapamil,

\textbf{Scheme 1.} Reagents and conditions: K_{2}CO_{3}, DMF (A); 6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline, NaBH\textsubscript{4}, CH\textsubscript{2}CL\textsubscript{2} (B); Lawesson’s reagent THF (C); Br\textsubscript{2}/CHCl\textsubscript{3} (D); EtOH (E).
and desmethylloperamide, displaying P-gp activity in micromolar range. Moreover, these results demonstrated that the B fragment, present in derivatives 8a-d, increased P-gp activity 10-fold with respect to MC90, lacking this fragment. However, compounds 8a-d were an order of magnitude less active than tariquidar. In conclusion, derivatives 8a-d could be considered tariquidar bioiso- sters where arylthiazole moiety A mimicked the quinoline-3-carboxamide and the inserted B fragment, present in 8a-d, could be considered a bioequivalent moiety of the corresponding fragment belonging to tariquidar.

Supplementary data

Supplementary data (the synthesis and characterization of intermediates depicted in Scheme 1 are reported). Elemental analyses for compounds 4, 5, 8a-d are included. Moreover, the biological protocols and the corresponding references) associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2012.12.084.

References and notes

13. Experimental section: 3-(2-(4-(4-(3,4-Dihydro-6,7-dimethoxyisoquinolin-2(1H)-yl)methyl)pyridin-2-yl)thiazol-2-yl)phenyl]pyridin-2-yl]phenol (8a). Yellow oil, 60% yield from column chromatography (CHCl3). ESI/MS m/z 552 (M++1, 8) 549 (13), 350 (100). H NMR δ 2.78–2.84 (m, 4H, CH2CH2NC6H5), 3.70 (s, 2H, CH3CH2NCH3), 3.82 (s, 3H, OCH3), 3.84 (s, 3H, OCH3), 6.50–8.80 (m, 15H, aromatic and OH). Anal. (C32H29N3O4S2HCl) C, H, N (hydrochloride salt, white solid).

Table 1

<table>
<thead>
<tr>
<th>Compound</th>
<th>R</th>
<th>P-gp</th>
<th>BCRP</th>
<th>MRP1</th>
<th>Apparent Permeability BA/AB</th>
<th>ATP-ase</th>
</tr>
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<tbody>
<tr>
<td>8a</td>
<td>3-OH</td>
<td>0.25 ± 0.02</td>
<td>0.018 ± 0.005</td>
<td>&gt;100</td>
<td>0.9</td>
<td>Y (20%)p</td>
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<tr>
<td>8b</td>
<td>3-OCH3</td>
<td>0.95 ± 0.04</td>
<td>0.50 ± 0.02</td>
<td>&gt;100</td>
<td>2.5</td>
<td>Y (20%)p</td>
</tr>
<tr>
<td>8c</td>
<td>4-NO2</td>
<td>0.51 ± 0.03</td>
<td>10 ± 0.5</td>
<td>&gt;100</td>
<td>2.7</td>
<td>Y (16%)p</td>
</tr>
<tr>
<td>8d</td>
<td>4-F</td>
<td>0.41 ± 0.02</td>
<td>1.0 ± 0.2</td>
<td>&gt;100</td>
<td>4.5</td>
<td>Y (18%)p</td>
</tr>
<tr>
<td>MC18d</td>
<td></td>
<td>1.50</td>
<td>90</td>
<td>2.80</td>
<td>1.64</td>
<td></td>
</tr>
<tr>
<td>MC113</td>
<td></td>
<td>0.6 ± 0.03</td>
<td>15 ± 3</td>
<td>21 ± 2</td>
<td>2.6</td>
<td></td>
</tr>
<tr>
<td>MC90b</td>
<td></td>
<td>1.94</td>
<td>30</td>
<td>2.7</td>
<td>6.1</td>
<td></td>
</tr>
<tr>
<td>Tariquidar</td>
<td></td>
<td>0.044 ± 0.001</td>
<td>0.010 ± 0.005</td>
<td>Not tested</td>
<td>22</td>
<td>Y (30%)p</td>
</tr>
</tbody>
</table>

* The result is the mean of three independent experiments sample in duplicate.
* The percentage at 50 µM of the effect is in parenthesis.
* See Ref. 8 for MC18 and Ref. 11 for MC18 and MC90.
aromatic). Anal. (C$_{32}$H$_{28}$N$_4$O$_5$S/C$_1$$_2$HCl/C$_1$H$_2$O) C, H, N (hydrochloride salt, white solid).

2-(4-(5-(4-(4-Fluorophenyl)thiazol-2-yl)pyridin-2-yl oxy)benzyl)-1,2,3,4-tetrahydro-6,7-dimethoxyisoquinoline (8d). Yellow oil, 20% yield from column chromatography (eluent CHCl$_3$/MeOH 19:1). ESI+/MS $m/z$ 554 (M$^+1$, 8) 390 (39) 539 (100). 1H NMR $\delta$ 2.75–2.84 (m, 4H, NC$_6$H$_4$C$_2$H$_2$), 3.58 (s, 2H, OC$_6$H$_4$C$_2$H$_2$N), 3.70 (s, 2H, CH$_2$CH$_2$NC$_6$H$_4$), 3.82 (s, 3H, OCH$_3$), 3.84 (s, 3H, OCH$_3$). 6.51–8.80 (m, 14H, aromatic). Anal. (C$_{32}$H$_{28}$N$_4$O$_5$S/C$_1$$_2$HCl) C, H, N (hydrochloride salt, white solid).