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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 ^{11}C and ^{18}F radiotracers, respectively.

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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 (BTB), hepatocytes and kidneys.[1] P-gp modulates both xenobiotics absorption and excretion in these barriers and organs.[2] Furthermore, P-gp overexpression is one of the main cause of the failure of chemotherapeutic treatment as it modulated chemotherapeutic drugs efflux.[3] 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.[4] In this *scenario*, an important goal is the development of P-gp PET radiotracers displaying high affinity and selectivity towards the target.[5,6]

Among P-gp radiotracers, [^{11}C]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.[7]

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;[8,9] thus, these ligands have been [^{11}C]-radiolabelled and tested in vivo by microPET analysis (Fig. 2).[6,10] [^{11}**C**]**MC18** displayed in vivo and in vitro interesting and consistent results while [^{11}**C**]**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[11] 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 arylthizole fragment could be considered as biosisoster of 3-carboxamidequinoline (**A**) (Fig. 1). Since, tetrahydroisoquinoline 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 ^{11}C- or ^{18}F-PET probes. For this purpose, methoxy derivative (**8b**) and the corresponding hydroxy derivative (**8a**) as precursor for [^{11}**C**]**8b** and fluoroderivative (**8d**) and the corresponding nitroderivative (**8c**) as precursor for [^{18}**F**]**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 (P_{app}).[4] 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

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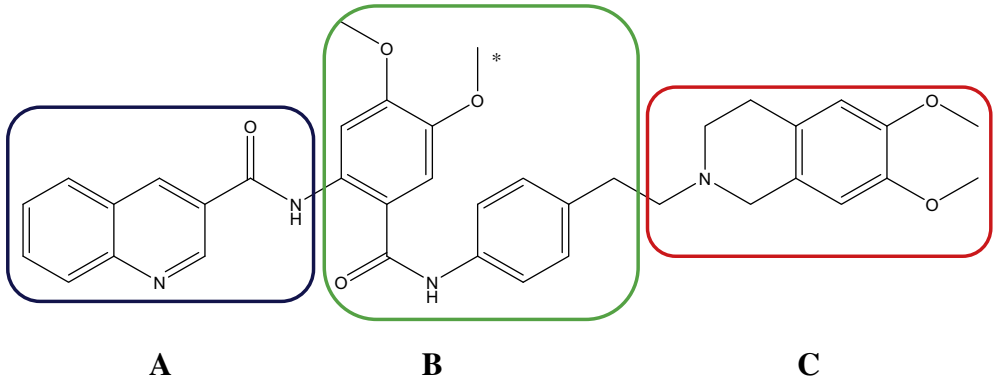


Figure 1. [^{11}C]tariquidar.

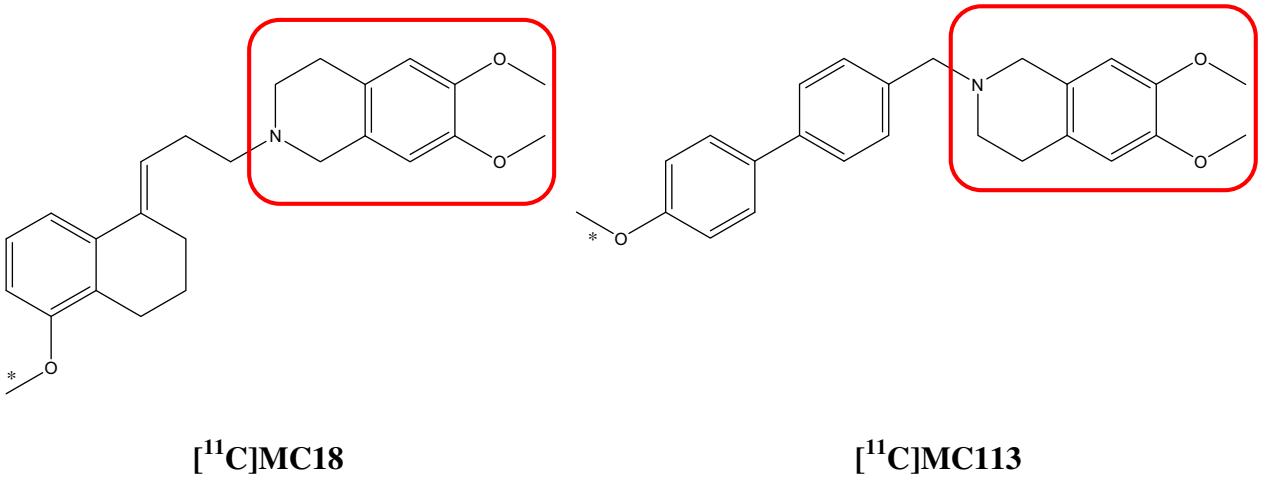


Figure 2. P-gp radiotracers.

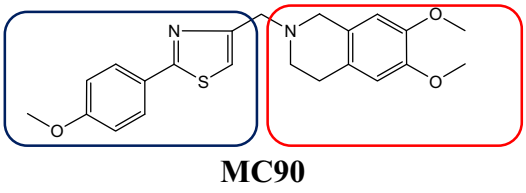


Figure 3. Arylthiazole derivative **MC90**.

unable to stimulate this site. Furthermore, the Apparent Permeability (P_{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 $\geqslant$2 for substrates while it is $\leqslant$2 for P-gp inhibitors.

The synthesis of arylthiazole derivatives **8a–d**, **4** and **5** 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**[12] with 6,7-dimethoxy-1,2,3,4-tetrahyidroisoquinoline in dry CH_2Cl_2 and the intermediate was reduced in the presence of $NaBH_4$. 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 **8a–d**. [13]

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

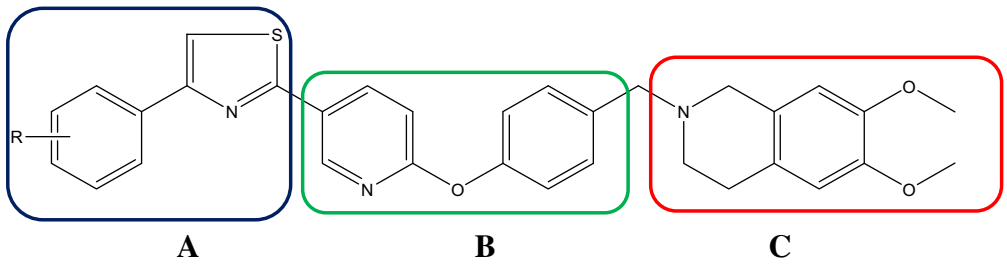
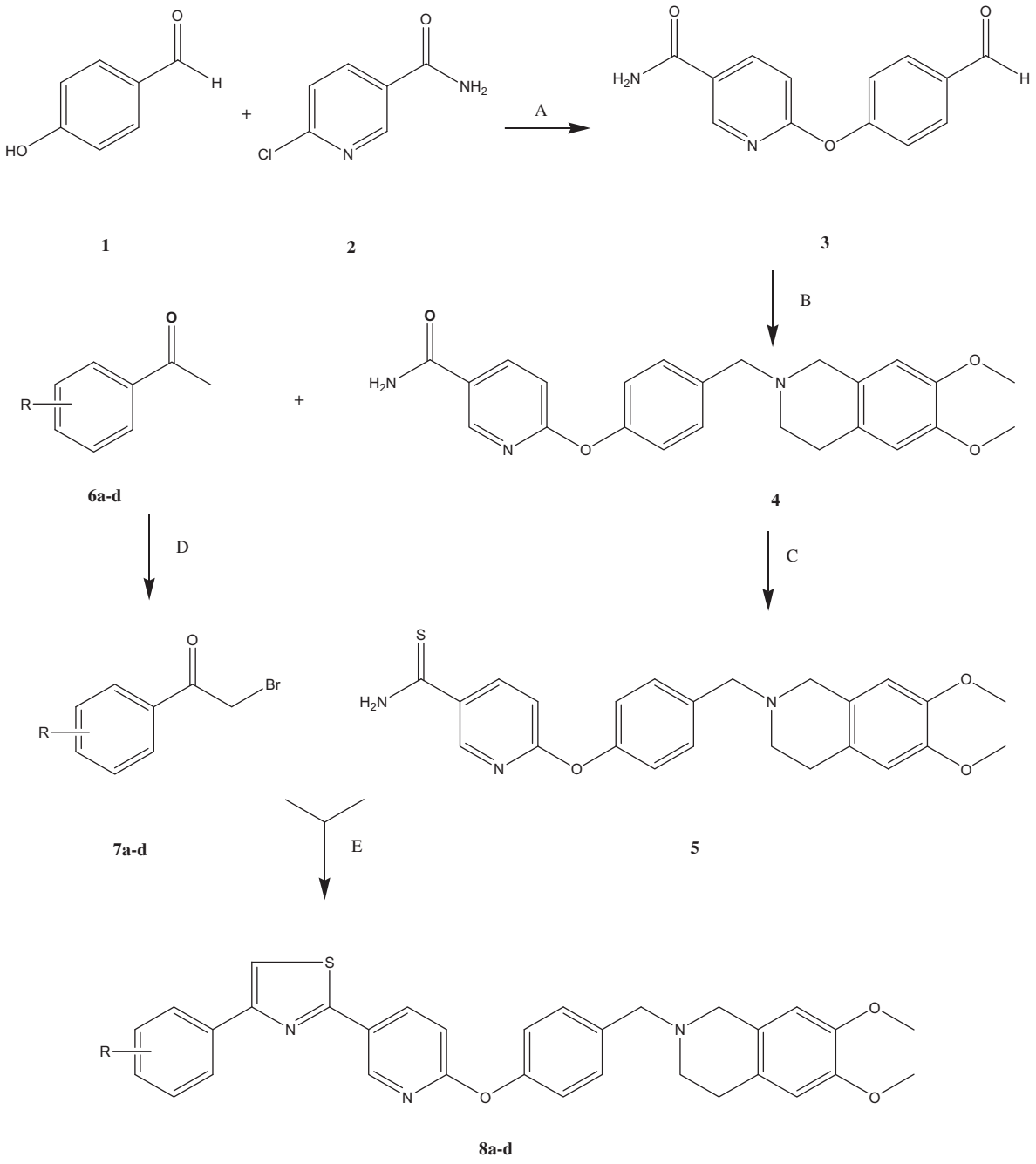


Figure 4. General formula of arylthiazole derivatives **8a–d**. R = 3-OH, 3-OCH_3, 4-NO_2, 4-F.



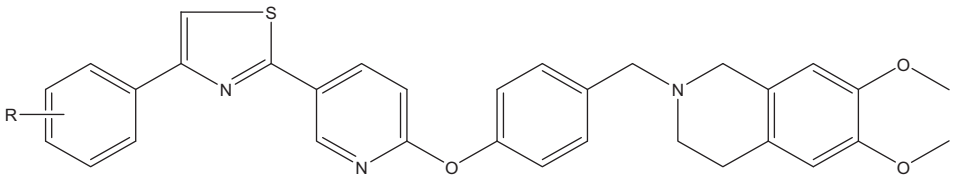
Scheme 1. Reagents and conditions: K_2CO_3, DMF (A); 6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline, $NaBH_4$, CH_2Cl_2 (B); Lawesson's reagent THF (C); $Br_2{\cdot}CHCl_3$ (D); EtOH (E).

8a–d displayed submicromolar P-gp activity ranging from 0.25 to 0.95 μM. Moreover, all ligands were inactive towards MRP1 (EC_{50} >100 μM). Surprisingly, compound **8a** was more active towards BCRP (EC_{50} = 0.018 μM) than towards P-gp (EC_{50} = 0.25 μM). Compound **8b** displayed superimposed P-gp/BCRP activities whereas compounds **8c** and **8d** were moderately more potent towards P-gp than towards BCRP.

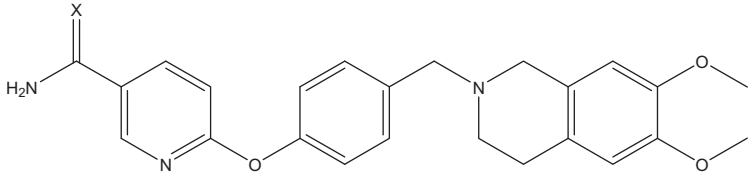
The derivatives **4** and **5**, lacking arylthiazole nucleus, were about 100-fold less potent than thiazoles **8a–d** confirming that this moiety plays a crucial role in P-gp activity. Apparent Permeability (P_{app}) and ATP-ase activity for each compound indicated that compounds **8a–d** could be considered 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 [^{11}**C**]**MC113**[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.[7] Since P-gp density at the human blood-brain barrier is in nanomolar range,[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

Table 1
Biological evaluation of arylthiazole **8a**–**d**, amide (**4**) and thioamide (**5**) derivatives



Compound	R	P-gp	BCRP	MRP1	Apparent Permeability BA/AB	ATP-ase
		EC_{50}[a] (μM)				
8a	3-OH	0.25 ± 0.02	0.018 ± 0.005	>100	0.9	Y (20%)[b]
8b	3-OCH_3	0.95 ± 0.04	0.50 ± 0.02	>100	2.5	Y (20%)
8c	4-NO_2	0.51 ± 0.03	10 ± 0.5	>100	2.7	Y (16%)[b]
8d	4-F	0.41 ± 0.02	1.0 ± 0.2	>100	4.5	Y (18%)[b]
MC18[c]		1.50	90	2.80	1.64	
MC113		0.6 ± 0.03	15 ± 3	21 ± 2	2.6	
MC90[c]		1.94	30	2.7	6.1	
Tariquidar		0.044 ± 0.001	0.010 ± 0.005	Not tested	22	Y (30%)[b]



Compound	X	P-gp	MRP1	Apparent Permeability BA/AB	ATP-ase
		EC_{50} μM[a]			
4	O	19 ± 2.5	>100	NT	NT
5	S	11 ± 1.2	>100	NT	NT

[a] The result is the mean of three independent experiments sample in duplicate.
[b] The percentage at 50 μM of the effect is in parenthesis.
[c] See Ref. 8 for **MC18** and Ref. 11 for **MC18** and **MC90**.

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 bioisosters 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.

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13. Experimental section: *3-(2-(6-(4-((3,4-Dihydro-6,7-dimethoxyisoquinolin-2-(1H)-yl)methyl)phenoxy)pyridin-3-yl)thiazol-4-yl)phenol* (**8a**). Yellow oil, 60% yield from column chromatography ($CHCl_3$). ESI^+/MS *m*/*z* 552 (M^++1, 8) 549 (13), 359 (100). ^1H NMR δ 2.78–2.84 (m, 4H, NCH_2CH_2), 3.60 (s, 2H, $OC_6H_4CH_2N$), 3.70 (s, 2H, $CH_2CH_2NCH_2$), 3.82 (s, 3H, OCH_3), 3.84 (s, 3H, OCH_3), 6.50–8.80 (m, 15H, aromatic and OH). Anal. ($C_{32}H_{29}N_3O_4S$·2HCl) C, H, N (hydrochloride salt, white solid).
2-(4-(5-(4-(3-Methoxyphenyl)thiazol-2-yl)pyridin-2-yloxy)benzyl)-1,2,3,4-tetrahydro-6,7-dimethoxyisoquinoline (**8b**). Yellow oil, 65% yield from column chromatography (eluent $CHCl_3$/MeOH 19:1). ESI^+/MS *m*/*z* 566 (M^++1, 40) 562 (82) 551 (100). ^1H NMR δ 2.65–2.95 (m, 4H, NCH_2CH_2), 3.58 (s, 2H, $OC_6H_4CH_2N$), 3.75 (s, 2H, $CH_2CH_2NCH_2$), 3.82 (s, 3H, OCH_3), 3.84 (s, 3H, OCH_3), 3.88 (s, 3H, OCH_3), 6.75–8.83 (m, 14H, aromatic). ($C_{33}H_{31}N_3O_4S$·2HCl) C, H, N (hydrochloride salt, white solid).
2-(4-(5-(4-(4-Nitrophenyl)thiazol-2-yl)pyridin-2-yloxy)benzyl)-1,2,3,4-tetrahydro-6,7-dimethoxyisoquinoline (**8c**). Yellow oil, 32% yield from column chromatography (eluent $CHCl_3$ ESI^+/MS *m*/*z* 581 (M^++1, 7) 579 (74) 491 (100). ^1H NMR δ 2.68–2.84 (m, 4H, NCH_2CH_2), 3.60 (s, 2H, $OC_6H_4CH_2N$), 3.70 (s, 2H, $CH_2CH_2NCH_2$), 3.82 (s, 3H, OCH_3), 3.84 (s, 3H, OCH_3), 6.40–8.83 (m, 14H,

aromatic). Anal. ($C_{32}H_{28}N_4O_5S{\cdot}2HCl{\cdot}H_2O$) C, H, N (hydrochloride salt, white solid).

*2-(4-(5-(4-(4-Fluorophenyl)thiazol-2-yl)pyridin-2-yloxy)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 δ 2.75–2.84 (m, 4H, NCH_2CH_2), 3.58 (s, 2H, $OC_6H_4CH_2N$), 3.70 (s, 2H, $CH_2CH_2NCH_2$), 3.82 (s, 3H, OCH_3), 3.84 (s, 3H, OCH_3), 6.51-8.80 (m, 14H, aromatic). Anal. ($C_{32}H_{28}FN_3O_3S{\cdot}2HCl$) C, H, N (hydrochloride salt, white solid).

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