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Solid phase synthesis of tariquidar-related modulators of ABC transporters preferring breast cancer resistance protein (ABCG2)

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ABSTRACT

Aiming at structural optimization of potent and selective ABCG2 inhibitors, such as UR-ME22-1, from our laboratory, an efficient solid phase synthesis was developed to get convenient access to this class of compounds. 7-Carboxyisatoic anhydride was attached to Wang resin to give resin bound 2-aminoterephthalic acid. Acylation with quinoline-2- or -6-carbonyl chlorides, coupling with tetrahydroisoquinolinylethylphenylamine derivatives, cleavage of the carboxylic acids from solid support and treatment with trimethylsilydiazomethane gave the corresponding methyl esters. Among these esters highly potent and selective ABCG2 modulators were identified (inhibition of ABCB1 and ABCG2 determined in the calcein-AM and the Hoechst 33342 microplate assay, respectively). Interestingly, compounds bearing triethyleneglycol ether groups at the tetrahydroisoquinoline moiety (UR-COP77, UR-COP78) were comparable to UR-ME22-1 in potency but considerably more efficient (max inhibition 83% and 88% vs 60%, rel. to fumitremorgin c, 100%) These results support the hypothesis that solubility of the new ABCG2 modulators and of the reference compounds tariquidar and elacridar in aqueous media is the efficacy-limiting factor.

Whereas numerous inhibitors of *p*-glycoprotein (ABCB1) are reported in the literature,[1–9] the number of available modulators of the breast cancer resistance protein (ABCG2) is very limited.[10–15] The multidrug resistance (MDR) modulators tariquidar[16] and elacridar[17] (Fig. 1) are among the most potent inhibitors of ABC transporters. They are known to inhibit both *p*-glycoprotein (ABCB1) and the breast cancer resistance protein (ABCG2) with a preference for ABCB1 in case of tariquidar. Recently, we synthesized a series of tariquidar analogues in which, at the benzamide core, the hetarylcarboxamido residue was shifted to the meta-position and the two methoxy groups were replaced with a carboxylic acid methyl ester. Surprisingly, compounds such as UR-ME22-1 (Fig. 1) turned out to be potent and highly selective modulators of ABCG2.[18] It is noteworthy that compared to the considerably less potent but more efficient reference compound fumitremorgin c the maximum inhibitory effect was by 40–60% lower[18] (cf. concentration response curve of UR-ME22-1 in Fig. 2), presumably due to limited water solubility. Regardless of the lack of drug-like properties, UR-ME22-1 and analogues are considered of potential value as PET-ligands[19] and as pharmacological tools in proof-of-concept studies, for example, to overcome the blood-brain barrier by analogy with the modulation of ABCB1.[20] Although the synthesis of the new ABCG2 inhibitors reported before[18] is relatively simple, only moderate to low yields were obtained due to solubility and purification problems.

With respect to structural optimization of the lead compound UR-ME22-1 and solving the aforementioned problems, we developed a solid phase synthesis (SPS) (Scheme 1) to get more convenient chemical access to a broader variety of analogues including more soluble ABCG2 modulators.[21]

Wang resin was selected as polymer support, and 7-carboxyisatoic anhydride[22] as a "key" building block, because it can be easily linked to the resin giving a solid phase bound aminoterephthalic derivative, which is the central core structure of the target compounds. The best conditions to attach 7-carboxyisatoic anhydride **1** to the solid support were found when the resin, previously swollen in DMF, was heated overnight at 98 °C with 5 equiv of 7-carboxyisatoic anhydride and 3 equiv of DMAP.

The second combinatorial step involved acylation of the resin bound aminoterephthalic derivative **2** with quinoline-2- or quinoline-6-carboxylic acids. Although different peptide coupling conditions were tested to link the heterocyclic carboxylic acids (HBTU,

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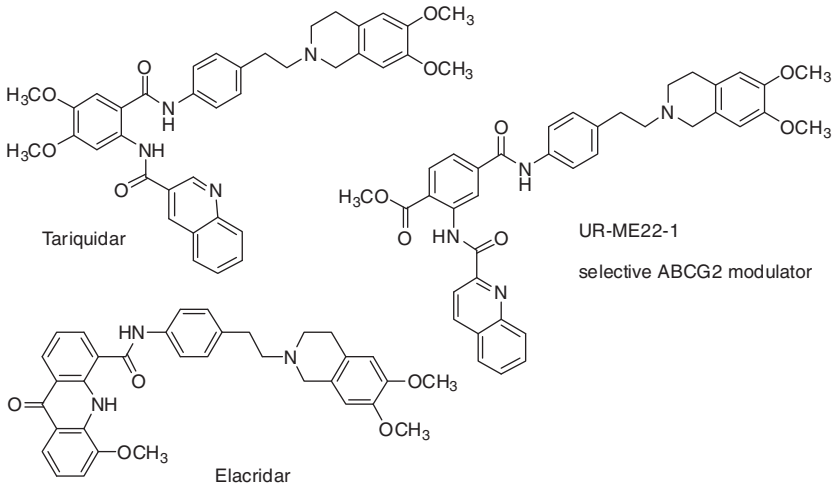


Figure 1. Structures of tariquidar (ABCB1 preferring), elacridar (combined ABCB1 and ABCG2 modulator) and the selective ABCG2 modulator UR-ME22-1.

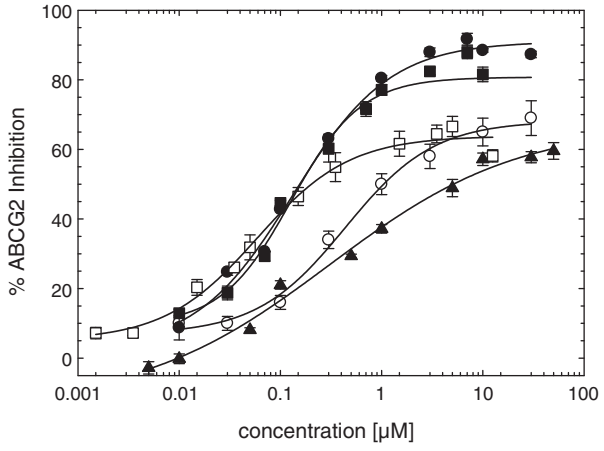
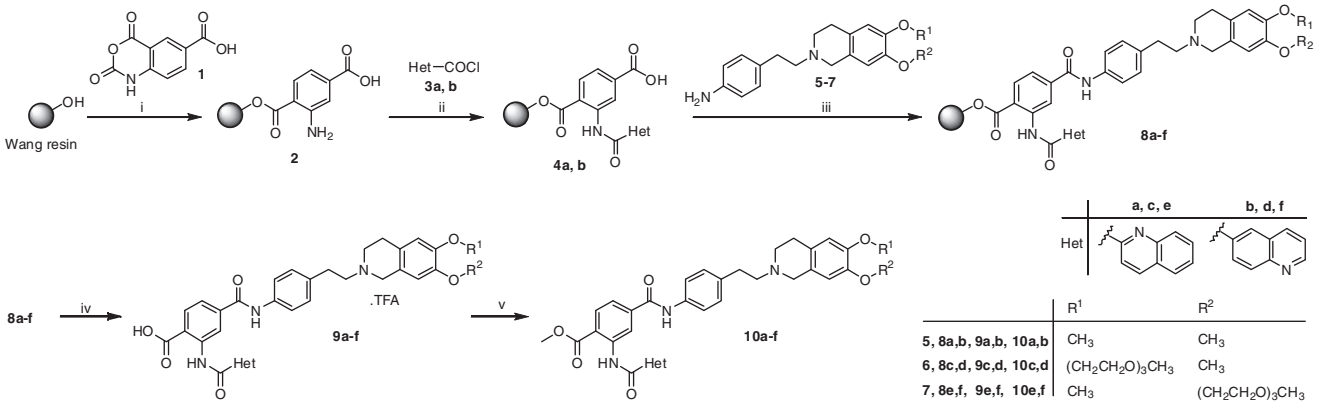


Figure 2. Concentration dependent inhibition of the ABCG2 transporter in MCF-7/Topo cells (Hoechst 33342 assay) by tariquidar (open circles) and the tariquidar analogues **10a** (UR-ME22-1; open squares), **10c** (filled squares), **10e** (UR-COP78; filled circles) and **10f** (filled triangles). The maximal inhibition is expressed as % relative to the maximum inhibition of ABCG2 by fumitremorgin c (at a concentration of 10 mM)).

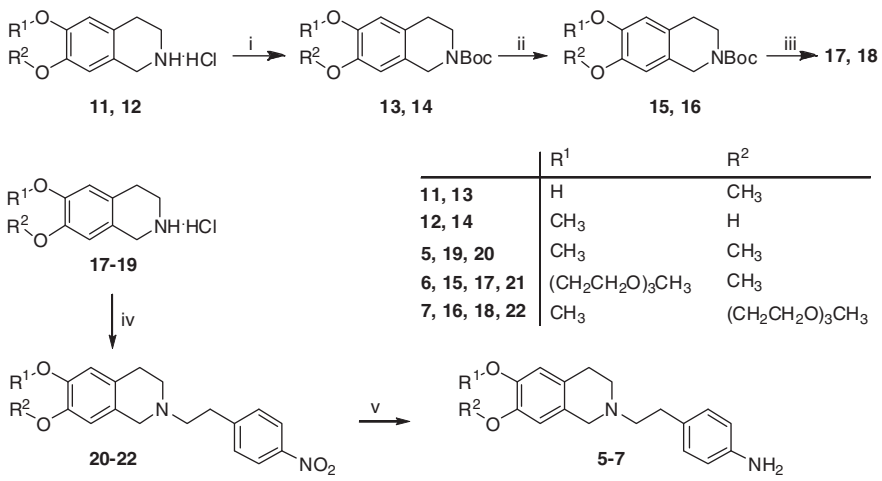
DIPEA; HOBt, EDC, DMAP; HOAt, DCC), only a mixture of 40% the desired amide and 60% of 2-aminoterephthalic acid was obtained as detected by ^{1}H NMR spectra of products released for analysis from solid support. However, acylation was successfully achieved, when a mixture of the resin, freshly prepared acid chloride (**3a**,**b**) and DIPEA was shaken in DCM during 12 h at room temperature (this procedure was repeated once).

In the next synthetic step the tetrahydroisoquinolinylethylphenylamine derivatives **5**–**7** were linked to the carboxylic acids **4a**,**b**. Aiming at more hydrophilic analogues of UR-ME22-1, we attached a triethyleneglycol chain to the tetrahydroisoquinoline motif (building blocks **6** and **7** cf. Scheme 2). The synthesis of the resin bound tariquidar analogues **8a**–**f** was accomplished when **4a**,**b** were reacted with **5**–**7**, HBTU, and DIPEA in DMF for 24 h (Scheme 1). Cleavage of the resin with a "cocktail" of TFA/DCM/TES (1:1:0.05) gave the carboxylic acids **9a**–**f**, which were transformed into the methyl esters **10a**–**f** using trimethylsilyldiazomethane ($TMSCHN_2$).

For the synthesis of the building blocks **5**–**7**, the required tetrahydroisoquinolines **17** and **18** (Scheme 2) were prepared from methoxytetrahydroisoquinolinols **11** and **12** according to the procedure described by Bobbitt et al.[23] and were N-protected using di-*tert*-butyl dicarbonate ($(BOC)_2O$). The *N*-Boc protected tetrahydroisoquinolines **13** and **14** were allowed to react with 2-[2-(2-methoxyethoxy)ethoxy]ethyl 4-methylbenzenesulfonate[24] to give **15** and **16** which were deprotected with HCl in anhydrous ether yielding **17** and **18**. Finally, compounds **6** and **7** as well as the



Scheme 1. SPS of tariquidar analogues **9a**–**f** and **10a**–**f**. Reagents and conditions: (i) 7-carboxyisatoic anhydride **1**, DMAP, DMF, 98 °C, overnight; (ii) quinolinecarbonyl chlorides **3a**,**b**, DIPEA, DCM, rt, 12 h (twice); (iii) tetrahydroisoquinolinylethylphenylamines **5**–**7**, HBTU, DIPEA, DMF, rt, 24 h; (iv) TFA/DCM/TES (1:1:0.05), rt, 30 min (twice); (v) $TMSCHN_2$, PhH/MeOH (1:1), rt, 1 h.



Scheme 2. Synthesis of tetrahydroisoquinolinylethylphenylamine derivatives **5**–**7**. Reagents and conditions: (i) $(BOC)_2O$, TEA, DCM, rt, overnight; (ii) 2-[2-(2-methoxyethoxy)ethoxy]ethyl 4-methylbenzenesulfonate, KOH, THF, reflux, 6 h; (iii) HCl/Et_2O, DCM, overnight; (iv) 4-nitrophenethyl bromide, K_2CO_3, CH_3CN, reflux, 18 h; (v) EtOH, Pd/C, H_2, 5 bar, rt, 24 h.

dimethoxy-substituted analogue **5**[25] were obtained by refluxing **17**–**19** with 4-nitrophenethyl bromide and reduction of the nitro group by catalytic hydrogenation.

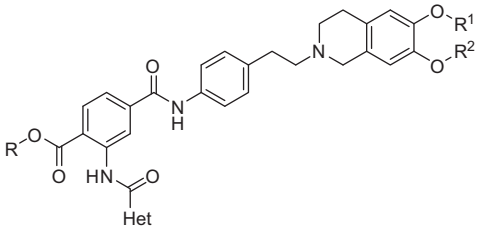
As shown in Table 1, a set of 12 tariquidar analogues was obtained in moderate to high yields. Compounds **10a** and **10b** were obtained in significantly better yields compared the previously described route,[18,19] illustrating that the SPS methodology is superior to the synthesis in solution.

The synthesized modulators and the reference compounds tariquidar and elacridar were investigated for inhibition of ABCB1 and ABCG2 in a calcein-AM (ABCB1)[26] and a Hoechst 33342 (ABCG2) microplate assay[27] using ABCB1-overexpressing KBv1 and ABCG2-overexpressing MCF-7/Topo cells. The data are summarized in Table 2.

Tariquidar and elacridar show IC_{50} values in the high nanomolar range and are almost equipotent at ABCB1, whereas elacridar is approximately four times more potent as an ABCG2 inhibitor. The carboxylic acids **9a**–**f** are inactive at both transporters, confirming preliminary results on such compounds as potential cleavage products of modulators such as **10a**.[18] By contrast, the methyl esters **10b**[18] and **10c**,**e** selectively modulate ABCG2 with IC_{50} values comparable to that of elacridar, being two to three fold less potent than the reference compound, UR-ME22-1 (**10a**). Interestingly, the regioisomeric triethylene glycol ethers **10c** and **10e**, bearing a quinoline-2-carboxamido substituent at the benzamide core, are superior to **10a** with respect to the maximal inhibitory effect: 83% and 88% versus 61% (Fig. 2). This strongly supports the hypothesis that limited water solubility is the major reason for submaximal efficacy of UR-ME22-1 (**10a**) and related potent ABCG2 modulators.

The solid phase synthesis presented in this contribution proved to be a convenient method for the preparation of small libraries of

Table 1
Tariquidar analogues synthesized on solid-phase Wang resin



Compound	R	R^1	R^2	Het.	Yield (%)
9a	H	CH_3	CH_3	2-Quinol.	81[a]
9b	H	CH_3	CH_3	6-Quinol.	97[a]
9c	H	$(CH_2CH_2O)_3CH_3$	CH_3	2-Quinol.	73[a]
9d	H	$(CH_2CH_2O)_3CH_3$	CH_3	6-Quinol.	87[a]
9e	H	CH_3	$(CH_2CH_2O)_3CH_3$	2-Quinol.	65[a]
9f	H	CH_3	$(CH_2CH_2O)_3CH_3$	6-Quinol.	95[a]
10a (UR-ME22-1)	CH_3	CH_3	CH_3	2-Quinol.	75 (10)[b] (53)[c]
10b (UR-ME19-2)	CH_3	CH_3	CH_3	6-Quinol.	95 (14)[b] (86)[c]
10c (UR-COP77)	CH_3	$(CH_2CH_2O)_3CH_3$	CH_3	2-Quinol.	90
10d	CH_3	$(CH_2CH_2O)_3CH_3$	CH_3	6-Quinol.	53
10e (UR-COP78)	CH_3	CH_3	$(CH_2CH_2O)_3CH_3$	2-Quinol.	95
10f (UR-COP134)	CH_3	CH_3	$(CH_2CH_2O)_3CH_3$	6-Quinol.	50

[a] Overall yield based on the loading of the resin.
[b] Kühnle et al.[18]
[c] Wang et al.[19]

Table 2
Inhibition of ABC transporters by reference compounds and the tariquidar analogues **9a–f**, **10a–f** determined in the calcein-AM (ABCB1) and Hoechst 33342 (ABCG2) microplate assay unless otherwise indicated

Compd	ABCB1 IC_{50} (nM)	ABCG2 IC_{50} (nM)
Tariquidar	223 ± 8[a]	526 ± 85[b]
Elacridar	193 ± 18[a]	127 ± 41[b]
9a	>50,000	>5000
9b	>1000	>100,000
9c	>100,000	>50,000
9d	>50,000	>50,000
9e	>100,000	6200
9f	>50,000	>50,000
10a[c]	>29000[a]	59 ± 11[b]
10b[d]	>10000[a]	172 ± 45[b,e]
10c[f]	>50,000	183 ± 32
10d	>100,000	390 ± 57[g]
10e[h]	>50,000	130 ± 29
10f[i]	>50,000	508 ± 191[j]

[a] Ref. 15: data from flow cytometric calcein-AM assay.
[b] Ref. 15: data from flow cytometric mitoxantron assay (% maximal inhibitory effect, relative to fumitremorgine c): IC_{50} values (% max. effect), Tariquidar: 916 ± 197 nM (39%), Elacridar 250 ± 45 nM (46%), **10a**: 60 ± 10 nM (56%); **10b**: 179 ± 35 nM (25%).
[c] UR-ME22-1.
[d] UR-ME19-2.
[e] 55% maximal inhibitory effect.
[f] UR-COP77.
[g] 41% maximal inhibitory effect.
[h] UR-COP78.
[i] UR-COP134.
[j] 61% maximal inhibitory effect. 179 ± 35 nM (25%).

this class of ABCG2 inhibitors due to high conversion efficiency in all reaction steps resulting in good to high yields. In particular, intrinsic difficulties in solution chemistry, due to low solubility of intermediates and target molecules, were circumvented.

Compounds **10c**,**e** are among the most potent and selective ABCG2 modulators reported so far. The increased solubility and ABCG2 inhibitory efficacy of these compounds is very promising with respect to the development of pharmacological tools for in vivo proof-of-concept studies, provided that the drug-like properties can be further improved. Thereby, automated parallel solid phase synthesis resulting in larger substance libraries should be helpful to optimize the lead structures.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2011.04.094.

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21. *General procedure for SPS of tariquidar analogues* **9**. A polypropylene 2.0-mL fritted syringe was charged with 50 mg of Wang resin (1.1 mmol/g loading) and the resin was swollen in 1 mL of DMF during 1 h. The resin was transferred to an eppendorf tube and a mixture of 7-carboxyisatoic anhydride (57 mg, 0.27 mmol, 5 equiv) and DMAP (19 mg, 0.16 mmol, 3 equiv) in 1 mL of DMF was added. The resin was heated at 98 °C overnight, then transferred to a polypropylene 2.0-mL syringe and washed three times with 5% AcOH/DCM, DCM, MeOH, DMF and DCM. The syringe was fritted and a solution of DIPEA (47 μL, 0.27 mmol, 5 equiv) in DCM was added and the resin was shaken during 5 min, after that, quinoline-2- or -6-carbonyl chloride (freshly prepared, 52 mg, 0.27 mmol, 5 equiv) was added and the resin was shaken at room temperature during 12 h, washed three times with DCM, MeOH, DMF, MeOH and DMF (this coupling was repeated once more). The resin was cooled down and a solution of DIPEA (95 μL, 0.55 mmol, 10 equiv) and HBTU (102 mg, 0.27 mmol, 5 equiv) in 1 mL of DMF was added, the resin was shaken for 2 min and compound **5**, **6** or **7** (0.27 mmol, 5 equiv) was added. The resin was shaken at room temperature for 24 h and then washed three times with DCM, MeOH and DMF. Cleavage: the resin was dried under vacuum and a mixture of TFA/DCM/TES 1:1:0.05 was added (1 mL). The resin was shaken for 1 h, the cleavage cocktail was collected and the content of the syringe was washed two times with fresh 50% TFA in DCM (this procedure was repeated once more). Combined washes were evaporated and residual oil was washed with fresh diethyl ether, the precipitated solid was filtered and dried. General procedure for the esterification of compounds **9**. The carboxylic acid derivative (1 equiv) was dissolved in 3 mL of a mixture PhH/MeOH 2:1 and trimethylsilyldiazomethane solution (2 M in diethyl ether) was added dropwise until no evolution of N^2 was observed. The reaction was stirred during 1 h at room temperature. The solvent was evaporated and the solid was purified by flash chromatography ($CHCl_3$/MeOH 5% or 10%).
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