ACS Medicinal Chemistry Letters

Letter

pubs.acs.org/acsmedchemlett

Benzanilide−Biphenyl Replacement: A Bioisosteric Approach to Quinoline Carboxamide-Type ABCG2 Modulators

Cristian Ochoa-Puentes,[†,‡,⊥] Stefanie Bauer,[§,⊥] Matthias Kühnle,[§] Günther Bernhardt,[§] Armin Buschauer,*[,§] and Burkhard König*[,†]

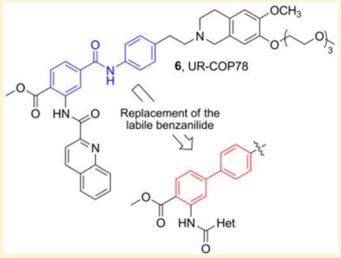
[†]Institute of Organic Chemistry, University of Regensburg, D-93040 Regensburg, Germany

[‡]Departamento de Química, Universidad Nacional de Colombia, Bogotá D.C., Colombia

[§]Institute of Pharmacy, University of Regensburg, D-93040 Regensburg, Germany

Supporting Information

ABSTRACT: Recently reported compounds such as UR-COP78 (**6**) are among the most potent and selective ABCG2 modulators known so far but are prone to rapid enzymatic cleavage at the central benzanilide moiety. In search for more stable analogues, according to a bioisosteric approach, a series of *N*-(biphenyl-3-yl)quinoline carboxamides was prepared by solid phase and solution phase synthesis. The biphenyl moiety was constructed by Suzuki coupling. Inhibition of ABCB1 and ABCG2 was determined in a calcein-AM and a Hoechst 33342 microplate assay, respectively. Most synthesized compounds selectively inhibited the ABCG2 transporter at submicromolar concentrations with a maximal inhibitory effect (I_{max}) over 90% (e.g., UR-COP228 (**22a**), IC_{50} 591 nM, I_{max} 109%; UR-COP258 (**31**), IC_{50} 544 nM, I_{max} 112%), though with lower potency and selectivity than **6**. The biphenyl analogues are considerably more stable and demonstrate that the benzanilide core is not a crucial structural feature of quinoline carboxamide-type ABCG2 modulators.



KEYWORDS: *ABC transporter, breast cancer resistance protein, MCF-7 cells, solid phase synthesis, Suzuki coupling*

Expression of ATP-binding cassette (ABC) transporters such as ABCB1 (p-glycoprotein, p-gp), ABCC1 (MRP1), or ABCG2 (breast cancer resistance protein, BCRP) is associated with multidrug resistance (MDR).[1,2] Additionally, as numerous cytostatics are substrates, the chemotherapy of malignant brain tumors compromises efflux pumps at the blood−brain barrier, especially ABCB1 and ABCG2. By analogy with an approach described for ABCB1,[3,4] coadministration of ABCG2 inhibitors and appropriate cytostatics might be useful to overcome MDR and to improve the chemotherapy of malignancies in the CNS.[5]

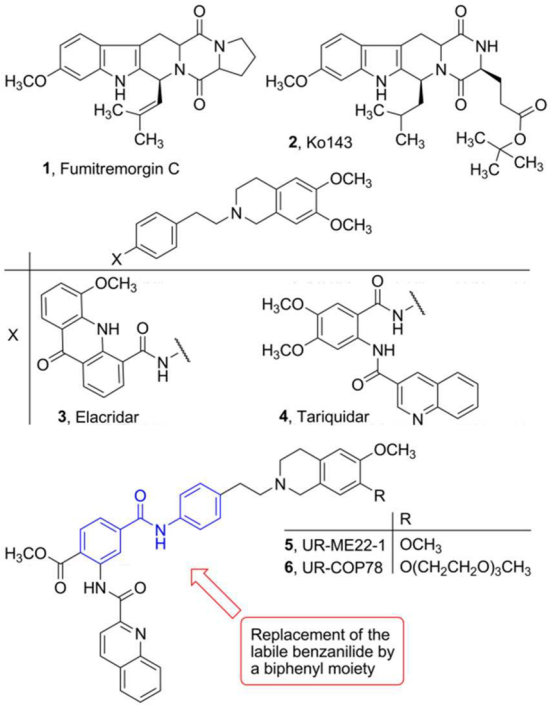
Compared to ABCB1 modulators, the number of reported ABCG2 inhibitors is still limited. An analogue of the natural compound fumitremorgin C (**1**),[6] Ko143 (**2**),[7] is known as one of the most potent and selective ABCG2 inhibitors (Chart 1), whereas elacridar (**3**, GF120918)[8] and tariquidar (**4**, XR9576)[9,10] are dual ABCB1 and ABCG2 modulators. Recently, we described the synthesis of a new class of potent and selective ABCG2 modulators derived from **4** (Chart 1).[11,12] Compound **5**, in which the quinoline-carboxamido residue was shifted to the meta-position at the benzamide core and the two methoxy groups were replaced by a methyl carboxylate, is highly selective for ABCG2 with an IC_{50} value in the low nanomolar range (65 nM) and a maximal inhibitory effect of 63% (Hoechst 33342 assay) relative to fumitremorgin C (100%).[13] The maximal response increased to about 90% when one of the methoxy groups on the tetrahydroisoquinoline moiety was replaced by a triethylenglycol chain (**6**),[12] indicating that water solubility was limiting the efficacy in this class of compounds. When characterized in more detail with respect to studies on human tumor xenograft models in nude mice, surprisingly, compounds **5** and **6** proved to be unstable in mouse plasma, due to rapid enzymatic cleavage at the benzanilide group, whereas the ester group remained unchanged under these conditions.[13]

As part of a project aiming at more stable ABCG2 inhibitors, the replacement of the labile benzanilide core in **5** and **6** was considered to be a promising bioisosteric approach. Here, we report on *N*-biphenylyl quinoline carboxamides, which were synthesized by analogy with a previously developed solid phase synthesis protocol.[12]

As shown in Scheme 1, 4-bromo-2-nitrobenzoic acid **7** was attached to Wang resin using EDC·HCl. Reduction of the nitro group and acylation with quinoline-2- or quinoline-6-carbonyl chloride **10a,b** (freshly prepared) led to the resin bound amides **11a,b**. In the next step, the biphenyl system was constructed via Suzuki coupling between **11a,b** and commercially available 4-(hydroxymethyl)phenylboronic acid. Biphenyl derivatives **12a,b** were mesylated at the hydroxy group and substituted by the tetrahydroisoquinolines **14**−**18**.[12] Finally, cleavage of the resin with TFA/DCM (1:1) and treatment of the obtained

Received: December 7, 2012
Accepted: March 4, 2013
Published: March 4, 2013

Chart 1. Structures of ABCG2 Modulators



carboxylic acids with trimethylsilyldiazomethane ($TMSCHN_2$) led to the desired methyl esters **19a,b–21a,b**, **22a**, and **23a**.

The synthesized compounds were obtained in acceptable to low overall yields, mainly due to incomplete coupling between **11a,b** and the boronic acid as confirmed by NMR analysis of byproducts. All analogues bearing a tetrahydroisoquinoline with a triethylenglycol chain showed increased water solubility.

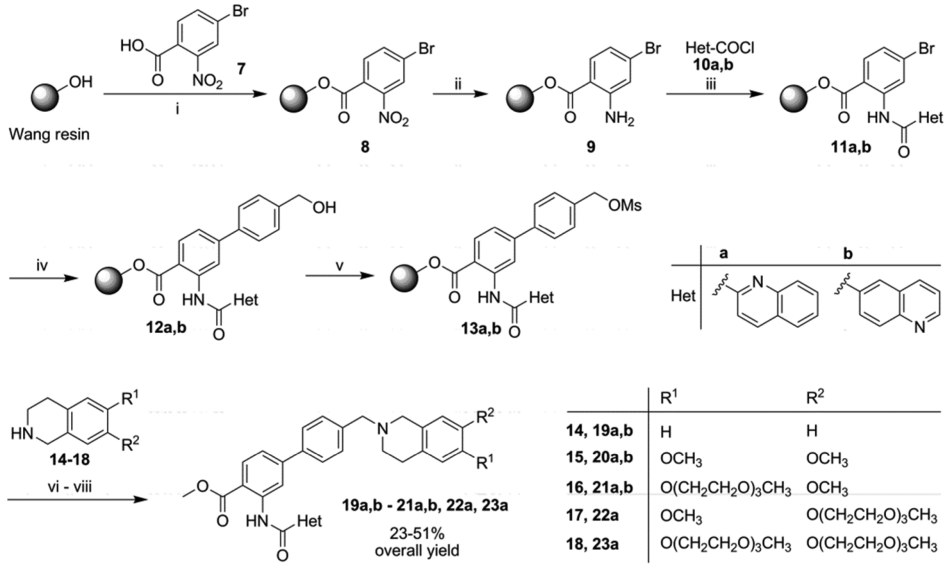
In addition, several homologues were synthesized retaining the methyl ester and the quinoline-2-carboxamido substituents as characteristic features of compounds **5** and **6**.[11,12] Compounds **28–31**, in which the length of the linker between the tetrahydroisoquinoline moiety and the biphenyl motif was extended by one methylene group, were synthesized in solution starting from methyl 2-amino-4-bromobenzoate **24** and 4-[2-(*tert*-butyldimethylsilyloxy)ethyl]phenylboronic acid[14] (Scheme 2).

ABCB1 and ABCG2 inhibitory activity of compounds **19a,b–21a,b**, **22a**, **23a**, and **28–31** as well as of reference compounds were investigated in a calcein-AM (ABCB1)[15] and a Hoechst 33342 (ABCG2) microplate assay[13] using ABCB1-overexpressing Kb-V1 and ABCG2-overexpressing MCF-7/Topo cells. The data are summarized in Table 1.

Elacridar strongly inhibited both transporters without preference for one of the two targets, whereas tariquidar was almost equipotent with elacridar at ABCB1, but about four times less potent at ABCG2. The potent ABCG2 inhibitor Ko143 (**2**)[7] was inactive at the ABCB1 transporter and showed an IC_{50} of 117 nM with a maximal inhibitory effect comparable to that of fumitremorgin C (**1**). Compounds **5** (UR-ME22-1) and **6** (UR-COP78) were comparable to Ko143 regarding potency and selectivity but produced a lower maximal response, especially in the case of the poorly soluble dimethoxytetrahydroisoquinoline **5**.

All newly synthesized compounds showed a marked preference for ABCG2 inhibition. Compounds **19a**, **20a**, **21a**, and **23a** were even inactive at the ABCB1 transporter. Whereas the IC_{50} values (~600 nM,) of compounds **19b** and **22a** were comparable to that of tariquidar, the maximal inhibitory effects were significantly higher, achieving 94% and 109% relative to fumitremorgin C (**1**). Except for **21b**, all modulators showed a maximal ABCG2 inhibition of about 90% or higher. Compound **22a**, the most potent modulator in this series, only showed a marginal maximum inhibition of 20% at the ABCB1 transporter.

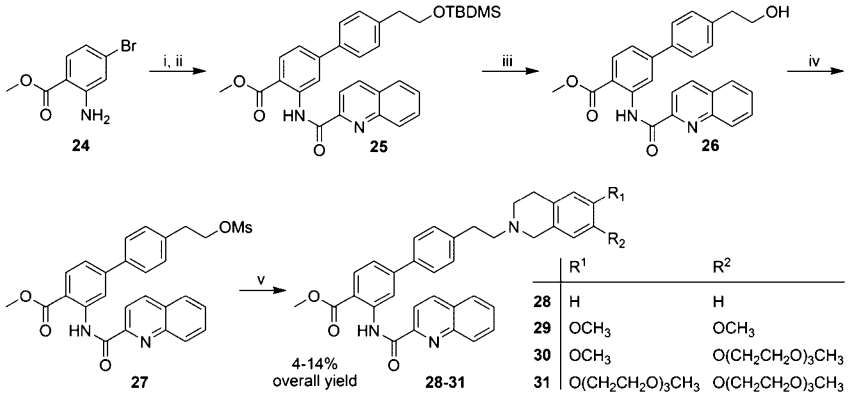
Scheme 1. Solid Phase Synthesis of *N*-(Biphenyl-3-yl)-Substituted Quinoline Carboxamides 19a,b–21a,b, 22a, and 23a[a]



[a]Reagents and conditions: (i) 4-bromo-2-nitrobenzoic acid **7**, EDC·HCl, DMAP, DMF/DCM 1/1, rt, overnight; (ii) $SnCl_2 \cdot 2H_2O$, DMF, 80 °C, overnight; (iii) quinoline carbonyl chlorides **10a,b**, DIPEA, DCM, rt, 12 h (twice); (iv) 4-(hydroxymethyl)phenylboronic acid, $Pd(PPh_3)_4$, K_3PO_4, DME, 80 °C, 21 h; (v) MsCl, DIPEA, DCM, rt, 6 h; (vi) tetrahydroisoquinolines **14–18**, THF, 80 °C, 21 h; (vii) TFA/DCM (1:1), rt, 30 min (twice); (viii) $TMSCHN_2$, PhH/MeOH (1:1), rt, 1 h.

dx.doi.org/10.1021/ml4000832 | ACS Med. Chem. Lett. 2013, 4, 393–396

Scheme 2. Solution Phase Synthesis of the *N*-(Biphenyl-3-yl)quinoline Carboxamides 28−31[a]



[a]Reagents and conditions: (i) methyl 2-amino-4-bromobenzoate **24**, 4-[2-(*tert*-butyldimethylsilyloxy)ethyl]phenylboronic acid, $Pd(PPh_3)_4$, K_3PO_4, THF, 80 °C, 15 h; (ii) quinoline-2-carbonyl chloride **10a**, TEA, DCM, 40 °C, overnight; (iii) TBAF, THF, rt, 3 h; (iv) MsCl, TEA, DCM, rt, 5 h; (v) tetrahydroisoquinolines **14**, **15**, **17**, and **18**, CH_3CN, 80 °C, overnight.

Table 1. Inhibition of ABC Transporters by Reference Compounds 1-6 and Quinoline Carboxamides 19a,b−21a,b, 22a, 23a, and 28−31

	ABCB1[a]	ABCG2[b]	
compd	IC_{50} (nM)[c]	IC_{50} (nM)[c]	I_{max}[d] (%)
1, fumitremorgin C	n.d.	731 ± 92	100
2, Ko143[e]	inactive[f]	117 ± 53	103 ± 7
3, elacridar[e]	193 ± 18	127 ± 41	63 ± 7
4, tariquidar[e]	223 ± 8	526 ± 85	69 ± 5
5, UR-ME22-1[e]	>29000[g]	65 ± 8	63 ± 2
6, UR-COP78[h]	>50000	130 ± 29	88 ± 3
19a	inactive[f]	1461 ± 169	85 ± 7
19b	4490 ± 160	641 ± 175	94 ± 2
20a	inactive[f]	943 ± 79	87 ± 5
20b	10900 ± 170	1540 ± 110	92 ± 6
21a	inactive[f]	1031 ± 170	114 ± 20
21b	18000 ± 1560	237 ± 65	67 ± 1
22a, UR-COP228	1230 ± 30[i]	591 ± 87	109 ± 8
23a	inactive[f]	839 ± 105	103 ± 3
28	inactive[f]	1100 ± 156	90 ± 2
29	inactive[f]	3214 ± 1050	126 ± 15
30	5420 ± 230[j]	760 ± 67	98 ± 6
31, UR-COP258	5990 ± 520[j]	544 ± 53	112 ± 19

[a]Calcein-AM microplate assay (unless otherwise indicated) using ABCB1-overexpressing Kb-V1 cells. [b]Hoechst 33342 microplate assay using ABCG2-overexpressing MCF-7/Topo cells. [c]Mean values ± SEM calculated from two to three independent experiments performed in triplicate, unless otherwise indicated; n.d.: not determined. [d]Maximal inhibitory effects are expressed as percental inhibition caused by the highest concentration of the compound tested (70 or 100 μM, respectively) relative to fumitremorgin C (**1**) at a concentration of 10 μM (100% inhibition). [e]Ref 13. [f]No effect up to a concentration of 100 μM. [g]Data from flow cytometric calcein-AM assay.[13] [h]Ref 12. [i]Maximum inhibition corresponds to 20% of the maximal response to tariquidar (1 μM). [j]A 50% maximal inhibitory effect relative to tariquidar (1 μM).

The potency of compounds **28−31** was not increased compared to that of the lower homologue **22a**. The results in the Hoechst 33342 assay were comparable to those for compounds **19a,b−21a,b**, **22a**, and **23a**, suggesting that the distance between the tetrahydroisoquinoline core and the biaryl moiety may be varied within this class of ABCG2 modulators.

Compounds **22a**, **30**, and **31** showed higher IC_{50} values compared to all the reference compounds, but with respect to the maximal inhibitory effects, they were superior to tariquidar, elaquidar, and compounds **5** and **6**, and comparable to Ko143 (**2**) (Figure 1). In general, the addition of one or two

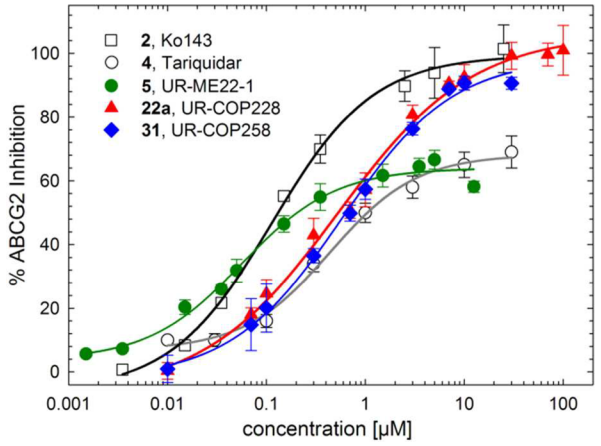


Figure 1. Concentration dependent inhibition of the ABCG2 transporter in MCF-7/Topo cells (Hoechst 33342 assay) by Ko143 (**2**), tariquidar (**4**), UR-ME22-1 (**5**), UR-COP228 (**22a**), and UR-COP258 (**31**). The inhibition is expressed as % relative to the maximal inhibition of ABCG2 by 10 μM of fumitremorgin C (**1**).

triethylene glycol chains at the tetrahydroisoquinoline core increased the inhibitory activity compared to tariquidar, elacridar, and compound **5** due to higher water solubility.

Investigations on the stability under physiological conditions (mouse plasma) revealed that compounds **5**[13] and **6** were enzymatically completely degraded at the benzanilide within 30 min. To increase the stability, the benzanilide was replaced by the biphenyl system. In this new series of modulators, the ester group is prone to enzymatic cleavage resulting in the inactive carboxylic acid. However, hydrolysis of the ester occurs much slower than cleavage of the benzanilide: regarding stability in mouse plasma (cf. Supporting Information), all biphenyl-type modulators had a half-life of about 24 h compared to 10 min in the cases of compounds **5** and **6**. Additionally, the quinoline carboxamide group was cleaved, though to a very small extent.

dx.doi.org/10.1021/ml4000832 | ACS Med. Chem. Lett. 2013, 4, 393−396

In conclusion, the presented solid and solution phase syntheses are straightforward methods to give convenient access to *N*-biphenylyl-substituted quinoline carboxamides. Among the prepared compounds, **22a** and **31** were the most potent and selective ABCG2 inhibitors. A prominent feature of these ABCG2 modulators is their higher maximal inhibitory effect, by far surpassing that of tariquidar-related compounds and even surmounting that of the reference substance fumitremorgin C. This again supports the hypothesis that water solubility is the limiting factor with respect to efficacy of previously reported ABCG2 modulators. Additionally, taking into consideration the improved stability, the results demonstrate that the benzanilide core structure is not essential but can be replaced by a biphenyl moiety. This suggests that optimization of the drug-like properties is possible according to bioisosteric concepts to obtain potent and selective ABCG2 modulators for coadministration with cytostatics in orthotopic brain tumor xenograft models in nude mice.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Synthetic methods, analytical data for compounds **19a,b**−**21a,b**, **22a**, **23a**, and **28**−**31**, investigations on the stability of **6**, **22a**, and **30** in mouse plasma, and methods for in vitro assays. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*E-mail: armin.buschauer@chemie.uni-regensburg.de (A.B.); burkhard.koenig@chemie.uni-regensburg.de (B.K.).

Present Address

∥(C.O.-P.) Departamento de Química, Universidad Nacional de Colombia Carrera 45 No 26-85, Edificio Uriel Gutierréz Bogotá DC, Colombia.

Author Contributions

⊥These authors (C.O.-P. and S.B.) contributed equally to this work. All authors have given approval to the final version of the manuscript.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

We are grateful to Manuel Bause for the synthesis of compound **25**, to Maria Beer-Krön for excellent technical assistance, to Dr. Susan Bates from the NIH (Bethesda, MD) for the reference compound **1** (fumitremorgin C), and to Dr. A. H. Schinkel, Netherlands Cancer Institute (Amsterdam), for kindly providing **2** (Ko143). C.O.P. thanks the German Academic Exchange Service (DAAD) for a graduate fellowship.

■ ABBREVIATIONS

ABCB1, ATP-binding cassette transporter, subfamily B, member 1; ABCC1, ATP-binding cassette transporter, subfamily C, member 1; ABCG2, ATP-binding cassette transporter, subfamily G, member 2; BCRP, breast cancer resistance protein (= ABCG2); CNS, central nervous system; IC_{50}, concentration of inhibitor required to give 50% inhibition of activity; MDR, multidrug resistance; MRP1, multidrug resistance associated protein 1 (= ABCC1); p-gp, p-glycoprotein (= ABCB1); SEM, standard error of the mean

■ REFERENCES

(1) Han, B.; Zhang, J. T. Multidrug resistance in cancer chemotherapy and xenobiotic protection mediated by the half ATP-binding cassette transporter ABCG2. *Curr. Med. Chem. Anticancer Agents* **2004**, *4* (1), 31−42.

(2) Gottesman, M. M.; Fojo, T.; Bates, S. E. Multidrug resistance in cancer: role of ATP-dependent transporters. *Nat. Rev. Cancer* **2002**, *2* (1), 48−58.

(3) Fellner, S.; Bauer, B.; Miller, D. S.; Schaffrik, M.; Fankhänel, M.; Spruss, T.; Bernhardt, G.; Graeff, C.; Färber, L.; Gschaidmeier, H.; Buschauer, A.; Fricker, G. Transport of paclitaxel (Taxol) across the blood−brain barrier in vitro and in vivo. *J. Clin. Invest.* **2002**, *110* (9), 1309−1318.

(4) Hubensack, M.; Müller, C.; Höcherl, P.; Fellner, S.; Spruss, T.; Bernhardt, G.; Buschauer, A. Effect of the ABCB1 modulators elacridar and tariquidar on the distribution of paclitaxel in nude mice. *J. Cancer Res. Clin. Oncol.* **2008**, *134* (5), 597−607.

(5) Breedveld, P.; Beijnen, J. H.; Schellens, J. H. Use of P-glycoprotein and BCRP inhibitors to improve oral bioavailability and CNS penetration of anticancer drugs. *Trends Pharmacol. Sci.* **2006**, *27* (1), 17−24.

(6) Rabindran, S. K.; Ross, D. D.; Doyle, L. A.; Yang, W.; Greenberger, L. M. Fumitremorgin C reverses multidrug resistance in cells transfected with the breast cancer resistance protein. *Cancer Res.* **2000**, *60* (1), 47−50.

(7) Allen, J. D.; Loevezijn, A. v.; Lakhai, J. M.; Valk, M. v. d.; Tellingen, O. v.; Reid, G.; Schellens, J. H. M.; Koomen, G.-J.; Schinkel, A. H. Potent and specific inhibition of the breast cancer resistance protein multidrug transporter in vitro and in mouse intestine by a novel analogue of fumitremorgin C. *Mol. Cancer Ther.* **2002**, *1*, 417−425.

(8) de Bruin, M.; Miyake, K.; Litman, T.; Robey, R.; Bates, S. E. Reversal of resistance by GF120918 in cell lines expressing the ABC half-transporter, MXR. *Cancer Lett.* **1999**, *146* (2), 117−126.

(9) Robey, R. W.; Steadman, K.; Polgar, O.; Morisaki, K.; Blayney, M.; Mistry, P.; Bates, S. E. Pheophorbide a is a specific probe for ABCG2 function and inhibition. *Cancer Res.* **2004**, *64* (4), 1242−1246.

(10) Kannan, P.; Telu, S.; Shukla, S.; Ambudkar, S. V.; Pike, V. W.; Halldin, C.; Gottesman, M. M.; Innis, R. B.; Hall, M. D. The "specific" P-glycoprotein inhibitor tariquidar is also a substrate and an inhibitor for breast cancer resistance protein (BCRP/ABCG2). *ACS Chem. Neurosci.* **2011**, *2* (2), 82−89.

(11) Kühnle, M.; Egger, M.; Müller, C.; Mahringer, A.; Bernhardt, G.; Fricker, G.; König, B.; Buschauer, A. Potent and selective inhibitors of breast cancer resistance protein (ABCG2) derived from the p-glycoprotein (ABCB1) modulator tariquidar. *J. Med. Chem.* **2009**, *52*, 1190−1197.

(12) Puentes, C. O.; Höcherl, P.; Kühnle, M.; Bauer, S.; Bürger, K.; Bernhardt, G.; Buschauer, A.; König, B. Solid phase synthesis of tariquidar-related modulators of ABC transporters preferring breast cancer resistance protein (ABCG2). *Bioorg. Med. Chem. Lett.* **2011**, *21*, 3654−3657.

(13) Kühnle, M. *Experimental Therapy and Detection of Glioblastoma: Investigation of Nanoparticles, ABCG2 Modulators and Optical Imaging of Intracerebral Xenografts*. Doctoral thesis, University of Regensburg, 2010. http://epub.uni-regensburg.de/13008/.

(14) Pizzirani, D.; Roberti, M.; Cavalli, A.; Grimaudo, S.; Cristina, A. D.; Pipitone, R. M.; Gebbia, N.; Tolomeo, M.; Recanatini, M. Antiproliferative agents that interfere with the cell cycle at the G1 S transition: further development and characterization of a small library of stilbene-derived compounds. *ChemMedChem* **2008**, *3*, 345−355.

(15) Höcherl, P. *New Tariquidar-Like ABCB1 Modulators in Cancer Chemotherapy: Preclinical Pharmacokinetic/Pharmacodynamic Investigations and Computational Studies*. Doctoral thesis, University of Regensburg, 2010. http://epub.uni-regensburg.de/15219/.