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Analogs of a 4-aminothieno[2,3-*d*]pyrimidine lead (QB13) as modulators of P-glycoprotein substrate specificity

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ABSTRACT

P-glycoprotein (P-gp) is an important factor in the development of multidrug resistance (MDR) in cancer cells. In literature reports, a thieno[2,3-*d*]pyrimidine (QB13) was described as P-gp modulator and opposed effects on the cell accumulation of distinct P-gp substrates were postulated. On the basis of this lead structure, a series of 2-alkylthio-4-aminothieno[2,3-*d*]pyrimidines was prepared and tested in a daunorubicin accumulation assay. Modulation of substrate specificity was shown for selected compounds in cytotoxicity (MTT) assays.

Lack of response to chemotherapy is a serious impediment to cancer treatment. The frequently observed development of multidrug resistance (MDR) leads to a reduced sensibility of cancer cells toward structurally unrelated cytostatics. The predominant mechanism of MDR is the overexpression of ATP binding cassette (ABC) transporters.[1] P-glycoprotein (P-gp) was the first discovered member of these membrane-spanning efflux pumps. This 170 kDa protein is encoded by the *ABCB1* gene mapped to chromosome 7q21 and consists of two transmembrane domains as well as two nucleotide binding sites.[2,3] The three-dimensional structure of mouse P-gp (87% sequence homology to human P-gp) was solved recently by means of X-ray crystallography. Distinct binding sites in the internal cavity of two additional P-gp structures with cocrystallized cyclic peptide inhibitors could be identified.[4]

There is a wide variety of published compounds that can reverse MDR mediated by ABC transporters. These inhibitors or P-gp modulators belong to different chemical and pharmacological classes, for example, representatives of calcium channel blockers, immunosuppressants, calmodulin antagonists, steroids, flavonoids, and anthranilamides.[2,5–7] Particularly for P-gp, in silico models have been successfully applied to identify new lead structures.[8,9] However, a smaller number of substances showed a reversed effect. They were able to protect cells from distinct cytostatics by stimulation of the efflux pump. Shapiro and Ling[10] identified two positively cooperative drug-binding sites of P-gp. Hoechst 33342 and rhodamine 123 activated the P-gp-mediated transport of each other. Moreover, different groups of cytostatics stimulated either Hoechst 33342 or rhodamine 123 transport. A comparable effect has also been discussed for flavonoids.[11–13] Recently, erlotinib, an inhibitor of the epidermal growth factor receptor tyrosine kinase, was found to be a substrate-dependent bidirectional modulator of P-gp.[14]

Kondratov et al.[15] investigated a diverse set of p53 inhibitors in a cell-based assay using different P-gp substrates, for example, doxorubicin, daunorubicin, rhodamine 123, vinblastine, and taxol. The addition of these small molecules improved survival of Con A cells in the presence of certain cytotoxic substrates, while pump activity was decreased for others, thus changing the cross-resistance pattern of P-gp. For example, QB102, a tricyclic tetrahydrobenzimidazo[2,1-*b*]thiazole, increased resistance to the anthracyclines doxorubicin and daunorubicin, but showed the opposite effect for taxol and vinblastine. On the basis of daunorubicin accumulation, other active substances such as the triazacyclopentaindene QB11 and the thieno[2,3-*d*]pyrimidine QB13 (Scheme 1) were supposed to behave similar to QB102. This group of compounds was also included in the derivation of a pharmacophore pattern for the Hoechst 33342 binding site of P-gp.[16] In accordance with the biological results, QB11 and QB13 produced similar overlays as QB102. QB13 complied

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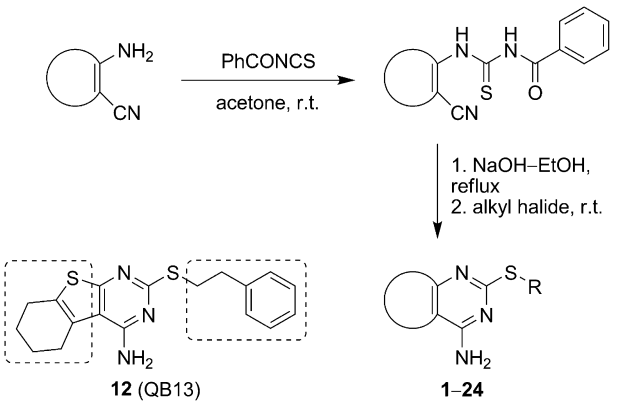


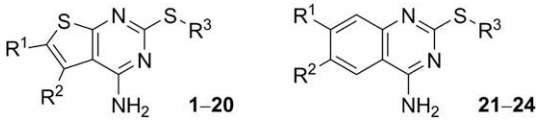
Scheme 1. Structure of the lead compound **12** (QB13) and synthetic route to compounds **1**–**24**.

with the pharmacophore in four hydrophobic aromatic centers, one hydrogen bond (HB) acceptor point, and one HB donor point.

In continuation of our recent investigations on modulators of ABC efflux pumps,[17–19] we intended to modify the lead structure QB13 to further characterize the interaction of the analog substances with P-gp. Therefore, the annelated tetrahydrobenzothieno moiety and the phenethylsulfanyl residue of QB13 were varied to compose two sets of target compounds (Scheme 1, dashed boxes).

In the first set (Table 1, **1**–**14**), the sulfanyl substituent in position 2 of the heterosystem was diversified for dimethyl-substituted (**1**–**7**) and tetrahydrobenzothieno[2,3-*d*]pyrimidines (**8**–**14**). Besides the phenethyl residue of **12** (QB13), small alkyl (Me, Et), polar aliphatic ($(CH_2)_2OH$, CH_2CO_2Et) and aromatic substituents (CH_2COPh, $CH(Ph)CO_2H$) were included. The second set (**15**–**24**) was intended to discover possible influences of the ring system fused with the aminopyrimidine. Three tricyclic thienopyrimidine templates (**15**–**20**) as well as two quinazolines (**21**–**24**) were combined with the parent phenethylsulfanyl residue ($(CH_2)_2Ph$) or the polar ester function (CH_2CO_2Et) in position 2.

Table 1
Thieno[2,3-*d*]pyrimidines **1**–**20** and quinazolines **21**–**24** with corresponding EC_{50} values in the daunorubicin accumulation assay



Compds	R^1	R^2	R^3	EC_{50} (μM)[a]
1	|	|	Me	1.31 (0.49)
2	|	|	Et	4.59 (0.94)
3	|	|	$(CH_2)_2OH$	nd[b]
4	CH_3	CH_3	CH_2CO_2Et	0.90 (0.27)
5	|	|	$(CH_2)_2Ph$	2.25 (0.89)
6	|	|	CH_2COPh	1.31 (0.63)
7	|	|	$CH(Ph)CO_2H$	>100
8	|		Me	2.65 (0.80)
9	|		Et	0.46 (0.14)
10	|		$(CH_2)_2OH$	1.92 (0.14)
11	$-(CH_2)_4-$		CH_2CO_2Et	2.24 (0.43)
12	|		$(CH_2)_2Ph$	1.02 (0.48)
13	|		CH_2COPh	0.64 (0.12)
14	|		$CH(Ph)CO_2H$	>100
15	$-(CH_2)_3-$		CH_2CO_2Et	4.15 (1.59)
16	$-(CH_2)_3-$		$(CH_2)_2Ph$	0.97 (0.21)
17	$-CH_2-O-(CH_2)_2-$		CH_2CO_2Et	1.55 (0.42)
18	$-CH_2-O-(CH_2)_2-$		$(CH_2)_2Ph$	0.56 (0.04)
19	$-CH_2-N(Bn)-(CH_2)_2-$		CH_2CO_2Et	0.64 (0.19)
20	$-CH_2-N(Bn)-(CH_2)_2-$		$(CH_2)_2Ph$	nd
21	H	H	CH_2CO_2Et	3.98 (1.02)
22	H	H	$(CH_2)_2Ph$	1.10 (0.33)
23	OCH_3	OCH_3	CH_2CO_2Et	1.21 (0.60)
24	OCH_3	OCH_3	$(CH_2)_2Ph$	1.52 (0.35)

[a] Values are means of at least three experiments, standard deviations are given in parentheses.
[b] nd, not determined due to limited solubility.

The synthesis of the target compounds (Scheme 1) was accomplished applying a recently published procedure.[20] Aminothiophenes accessible by Gewald synthesis[21] or commercially available *ortho*-aminobenzonitriles were reacted with benzoyl isothiocyanate to afford benzoylthioureas. Thermal ring closure of these intermediates in aqueous sodium hydroxide solution was followed by the reaction with suitable alkyl halides to obtain **1**–**24** (Table 1, synthetic procedures and analytical data for new compounds are given in the Supplementary data).[20,22]

Structure and purity of the products were confirmed by NMR and elemental analyses. Two-dimensional NMR experiments (HMQC, HMBC) were carried out for the thieno[2,3-*d*]pyrimidine **2** and the quinazoline **21** (see Supplementary data). To the best of our knowledge, ^{13}C assignments for thieno-annelated 4-aminopyrimidines have not been reported in the literature so far. C=N signals given for 4-amino-2-(2-thienyl)quinazoline[23] and 4-aminopyrazolo[3,4-*d*]pyrimidines[24] were in accordance with the values obtained, for example, for compound **21** (C-4, 161.3 ppm; C-2, 165.6 ppm). In the thieno[2,3-*d*]pyrimidines (e.g., **2**), the signal of C-4 was shifted downfield (164.3 ppm), whereas the chemical shift of the C-2 signal remained nearly constant (165.7 ppm). Furthermore, the structure of **19** was confirmed by X-ray crystallography (Fig. 1).[25]

The effects of the target compounds on P-gp were investigated in a daunorubicin accumulation assay with the human ovarian cancer cell line A2780 and its P-gp overexpressing counterpart A2780 Adr.[26] Most compounds showed effects in the accumulation assay. Those reduced intracellular daunorubicin fluorescence with EC_{50} values between 0.5 and 5 μM (Table 1, Fig. 2), thus behaved similarly as activators of P-gp. This is in accordance with the effect of QB13 on the accumulation of daunorubicin in Con A cells.[15]

When considering pairs with the same sulfanyl moiety within the first set (**1**–**14**), a clear effect of the dimethyl–tetramethylene exchange (**1**–**7** vs **8**–**14**) in the thieno[2,3-*d*]pyrimidines was not observed. Three out of seven compounds with aliphatic side chains (**1**, 1.31 μM, **4**, 0.90 μM, and **9**, 0.46 μM) had activities better or comparable to the lead compound **12** (1.02 μM). The two phenacyl derivatives **6** (1.31 μM) and **13** (0.64 μM) were the most potent aromatic representatives. Substituted phenylacetic acids (**7**, **14**) showed no effect on daunorubicin accumulation. This is in agreement with literature data, as P-gp generally does not transport negatively charged compounds.

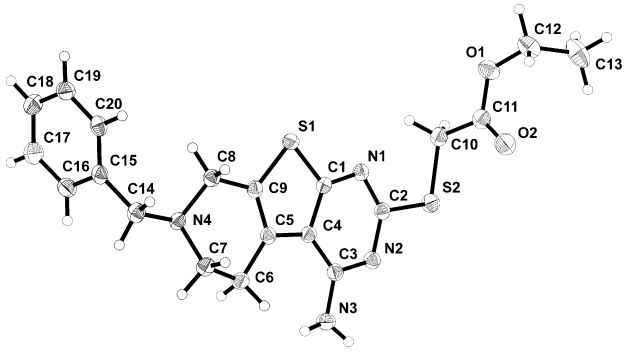


Figure 1. Molecular plot of **19** showing the atom-labeling scheme and displacement ellipsoids at the 30% probability level for the non-H atoms. H atoms are depicted as small circles of arbitrary radii.[25]