Published in final edited form as:

ChemBiochem. 2011 April 11; 12(6): 868–873. doi:10.1002/cbic.201100048.

Design and Synthesis of Selenazole-Containing Peptides for Cocrystallization with P-Glycoprotein

Dr. Houchao Tao^a, Yue Weng^{a,b}, Dr. Rupeng Zhuo^c, Prof. Dr. Geoffrey Chang^a, Prof. Dr. Ina L. Urbatsch^c, and Prof. Dr. Qinghai Zhang^a

Qinghai Zhang: qinghai@scripps.edu

^aDepartment of Molecular Biology, The Scripps Research Institute, 10550 North Torrey Pines Road, CB265, La Jolla, CA 92037 (USA), Fax: (+ 1)858-784-9985

^cDepartment of Cell Biology and Biochemistry, Texas Tech University Health Sciences Center, 364 14th Street, Lubbock, TX 79430 (USA)

Keywords

cyclic peptides; glycoproteins; ligand design; selenium; X-ray diffraction

Over-expression of P-glycoprotein (Pgp) in some cancer cells is a primary cause of multidrug resistance (MDR) that results in chemotherapy failure. As a consequence, Pgp has been a long-sought target for drug development in the hope of circumventing MDR in clinical oncology.^[1,2] More than three decades of biochemical studies have indicated that Pgp might possess several distinct substrate-binding sites within a large, flexible region located between the two transmembrane domains; these sites recognize hundreds of chemically unrelated compounds, including a majority of traditional anti-cancer drugs.^[3–6] These compounds can be exported from cells by Pgp using energy released by self-catalyzed ATP hydrolysis in conjunction with substantial conformational changes. Our groups recently determined a structure of mouse Pgp at 3.8 Å resolution by X-ray diffraction.^[7] We also solved the cocrystal structures of Pgp with a pair of enantiomeric cyclic peptides, QZ59Se-*RRR* and QZ59Se-*SSS* (Scheme 1), in which the two enantiomers bind to Pgp with different stoichiometries and at distinct sites within a transmembrane portal open to the cytoplasmic side. These structures provided the first glimpse of the polyspecific drug-binding site of Pgp in atomic detail.

Scheme 1 outlines our strategies for the design and synthesis of selenium (Se)-labeled cyclic peptides that were crucial to the successful cocrystallization with Pgp for X-ray structure determination. Our early attempts to cocrystallize Pgp with several well-studied modulators, including cyclosporin A^[8,9] and the cyclosporin derivative PSC 833^[10] were unsuccessful. Cyclosporin A and PSC 833 are both macrocyclic undecamer peptides the solution-phase conformations of which are flexible.^[11,12] We assume that this conformational flexibility

Correspondence to: Qinghai Zhang, qinghai@scripps.edu.

^bPresent address: Y. Weng, College of Chemistry and Molecular Sciences, Wuhan University, Wuhan 430072 (China)

Supporting information for this article is available on the WWW under <http://dx.doi.org/10.1002/cbic.201100048>.

disfavored cocrystallization of these bound ligands within the flexible binding region of the highly dynamic Pgp molecule. Several smaller cyclic peptides such as dendroamide A^[13] and patellamide C^[14] isolated from marine cyanobacteria have been reported to have MDR reversal activity, although with less potency than cyclosporin peptides. Structurally, these natural products feature the presence of amino acid derived heterocycles, such as thiazoles, oxazoles, thiazolines, and oxazolines. These five-membered heterocycles can be regarded as constraining rings that further restrict the rotational freedom of bonds in the cyclopeptide backbone. We supposed that optimization of these rigid cyclopeptides might yield compounds more readily co-crystallizable with Pgp by reducing entropy cost for binding.

As with many other membrane proteins, highly diffracting crystals of Pgp had not been grown, which presented another significant challenge to ligand-binding site identification and accurate modeling of ligand position once cocrystals were obtained. Decorating the ligands with X-ray-anomalous scattering markers will provide fiducials useful for structure determination. Thus, we designed novel selenazole-containing peptide ligands for Pgp in which Se atoms replaced sulfur or oxygen atoms in the original thiazole or oxazole rings (Scheme 1). The identification of anomalous Fourier peaks in the density map corresponding to the three Se atoms in QZ59Se-*SSS* and QZ59Se-*RRR* enabled more accurate positioning of the bound ligands.

Although methodologies for oxazole^[15] and thiazole^[16–19] synthesis are well documented, reports of selenazole synthesis appear relatively rarely, and most have employed procedures analogous to the Hantzsch synthesis of thiazoles starting from selenamides.^[20,21] In our earlier report, we had synthesized racemic selenazole-containing amino acid **±3c** by refluxing selenamides with ethyl bromopyruvate (EBP) in the presence of pyridine in ethanol. Accordingly, QZ59Se-*RRR* and QZ59Se-*SSS* were isolated from a mixture of racemic products by a combination of reversed-phase and chiral HPLC purification.^[7] This purification procedure was time-consuming and yielded only small amounts of the final products. To expand our capacity to synthesize diverse Se-labeled peptides for ongoing structural studies directed at mapping the flexible binding sites of Pgp, developing a more efficient procedure for the preparation of optically pure selenazole-containing peptides was highly desirable.

We have found that synthesis of selenazole-containing peptides was hindered by low yield and irreproducibility even by using optimized Hantzsch-thiazole synthesis procedures.^[19,22,23] We overcame these problems by making several important modifications. Compared to thioamides, which can be readily prepared and isolated from the reaction of primary amides with Lawesson's reagent, aliphatic selenamides were unstable during purification^[24] after being prepared by refluxing the mixture of P₂Se₅ and the amino acid derived nitriles **1a–t** in ethanol–H₂O. The isolated yield was typically lower than 50% in our trials. Therefore, simple filtration and rapid concentration were carried out upon completion of the transformation. The crude selenamides **2a–t** were subsequently reacted with EBP for 5 min in the presence of excess KHCO₃ in DMF at –20°C. Dehydration was followed by addition of trifluoroacetic anhydride (TFAA) to the reaction mixtures to yield the aromatized selenazoles (Table 1). A major modification here, compared to literature procedures for thiazole synthesis,^[19,22,23] is the omission of an organic base (typically 2,6-

lutidine) during the dehydration step. This modification appeared to be important for suppressing racemization of the final products. For example, including 2,6-lutidine as a second base led to the synthesis of (*S*)-alanine-selenazole **3a** in enantiomeric excess (*ee*) of only 87%, whereas 97% *ee* was achieved under our optimized conditions (Table 1, entry 1). Most recently we have found that room temperature reactions in steps 2 and 3 gave similar results without compromising enantioselectivity (Table 1, entries 1 and 3), which is distinct from the previously reported thiazole synthesis in which it was critical to maintain the reaction of thioamides and EBP at low temperature ($< -15\text{ }^{\circ}\text{C}$).^[22,23]

We have applied the above procedure to the synthesis of all 20 natural amino acid derived selenazoles **3a–t** (Table 1). To confirm the *ee* value of each product (except for glycine derivative, entry 2), the corresponding racemic (or partially racemized) selenazoles were prepared according to a previously published procedure.^[7] Overall yields for the three-step and two-pot reactions were generally satisfactory for aliphatic and aromatic amino acid derivatives, but the yield was lower for several polar amino acid derivatives. Most importantly, little or no racemization occurred in the final products except for histidine-derived selenazole (79% *ee*, entry 19). It is notable that this procedure tolerated amino acid side chains with common protecting groups. In entry 19, the *tert*-butoxycarbonyl (*t*-Boc) protective group on the imidazole ring was found to be eliminated under conditions of selenation (the first step). Changing the *t*-Boc group to tosyl (Ts) also produced a deprotected product. The starting nitrile **1s** was enantiomerically pure according to chiral HPLC analysis (Supporting Information). Partial racemization in this case likely occurred during the dehydration step, which can be explained by the promotion of intramolecular deprotonation at the adjacent chiral α -carbon by the basic imidazole moiety. For arginine-derived substrate (entry 20), trifluoroacetylation of the guanidino group was observed in the final product, but this modification could be easily removed by dissolving the product in ethanol and allowing it to stand overnight.

Having used the above procedures to prepare optically pure (*S*)- and (*R*)-valine-selenazoles, we synthesized the cyclic tripeptides QZ59Se-*SSS* and QZ59Se-*RRR*, together with two other stereoisomers QZ59Se-*RRS* and QZ59Se-*SSR*, as well as two homochiral tetrameric cyclic peptides QZ60Se-*RRRR* and QZ60Se-*SSSS* (Scheme 2 and Figure 1A). Corresponding thiazole analogues were also prepared by similar means.^[25,26] The homochiral tri- and tetrapeptides were synthesized by one-step cyclization of the respective amino acid units. Other trimeric peptide stereoisomers were synthesized by recursive coupling of corresponding monomeric units and final macrocyclization (Scheme 2). By comparing with the individually prepared molecules from optically pure selenazole amino acids in this study, we further verified the identity and the absolute stereochemistry of QZ59Se-*RRR* and QZ59Se-*SSS* previously isolated from racemic mixtures for cocrystallization studies.^[7]

Next, we evaluated the inhibitory activity of Pgp for each of these selenazole/thiazole-containing cyclic peptides in Figure 1A by using a commonly used calcein acetoxymethyl ester (calcein-AM) transport assay in Pgp-overexpressing CR1R12 cells.^[7,27] The resulting IC₅₀ values determined for the selenazole-containing peptides were $2.1 \pm 0.1\text{ }\mu\text{M}$ (QZ59Se-*SSS*), $7.0 \pm 0.1\text{ }\mu\text{M}$ (QZ59Se-*RRR*), $13.1 \pm 2.9\text{ }\mu\text{M}$ (QZ59Se-*RRS*), $20.2 \pm 2.3\text{ }\mu\text{M}$ (QZ59Se-

SSR), $32.9 \pm 7.0 \mu\text{M}$ (QZ60Se-*RRRR*), and $67.8 \pm 8.8 \mu\text{M}$ (QZ60Se-*SSSS*; Figure 1B). For the thiazole peptides, the IC_{50} values were $2.7 \pm 0.2 \mu\text{M}$ (QZ59S-*SSS*), $8.4 \pm 0.8 \mu\text{M}$ (QZ59S-*RRR*), $15.9 \pm 0.9 \mu\text{M}$ (QZ59S-*RRS*), $13.8 \pm 0.7 \mu\text{M}$ (QZ59S-*SSR*), $19.7 \pm 3.6 \mu\text{M}$ (QZ60S-*RRRR*) and $26.7 \pm 3.9 \mu\text{M}$ (QZ60S-*SSSS*; Figure 1C). Overall, the potency of the selenazole and thiazole analogues followed a trend according to the ring size and stereochemistry, with the cyclic tripeptides QZ59Se/S-*SSS* being the most potent and tetrapeptides QZ60Se/S-*SSSS* being the least potent. However, there are noteworthy differences in IC_{50} values between S- and Se-cyclopeptides having the same stereoconfiguration. For instance, in the selenazole series, the *SSR* isomer is somewhat less potent than its *RRS*-counterpart, whereas in the thiazole series the *SSR*-isomer is slightly more potent than the *RRS*-isomer. With regards to the tetrapeptides, QZ60Se-*RRRR* is doubly as potent as its *SSSS* enantiomer, whereas the corresponding thiazole analogues show similar IC_{50} values. We currently do not have a clear explanation for the observed differences in the above IC_{50} measurements between S and Se analogues, which will be the subject of future studies. But overall, substituting Se for S in our cyclic peptides does not change the IC_{50} pattern very much, which indicated that this replacement could be an effective strategy for designing natural product analogues for X-ray structural studies.

The work described here is directed toward the development of compounds with which to study the polyspecific binding sites of Pgp by X-ray crystallography. By using the methodology that we have established for the synthesis of optically pure selenazoles, a large number of structurally diverse Se-labeled cyclopeptides including natural product mimetics could potentially be assembled. Structural and functional studies of these new compounds might help to further elucidate the molecular basis for polyspecific substrate binding and transport function of Pgp. Of note, this structure-based approach is clearly distinct from the majority of efforts to design new drug candidates that tend to emphasize criteria such as specificity and toxicity. On the other hand, studying Pgp–ligand interactions will facilitate the development of more potent and Pgp-specific modulators and ultimately more effective therapies to overcome devastating MDR. Because thiazole or oxazole structures are abundant in natural products that are leads for many drug development programs,^[28] the synthesis of unnatural selenazole analogues might also have direct applications in drug discovery.

Experimental Section

General procedure for the synthesis of selenazoles (3a–t)

H₂O (0.2 mL) was added dropwise to a refluxing solution of nitrile **1** (2.0 mmol) and powdered P₂Se₅ (460 mg, 1.0 mmol) in EtOH (10 mL). The mixture was stirred under N₂ for 2 h. To drive the transformation to completion, more P₂Se₅ (2×1.0 mmol) was usually added at 1 h intervals, followed by slow addition of H₂O (2×0.1 mL). **CAUTION!** H₂Se is produced in situ;^[20] this is highly toxic and can decompose into explosive H₂ and elemental Se. For safety concerns, we recommend wearing safety goggles and masks with respirator, and running the reactions in small scales (<5 mmol) in a well-ventilated fume hood. After cooling to RT, the reaction mixture was quickly filtered through a patch of silica gel and washed with EtOAc. The filtrate was then concentrated in vacuo. The residue **2** was

redissolved in dry DMF (12 mL), and the solution was cooled to -20°C , and KHCO_3 (4.6 g, 46 mmol) was added. The resulting suspension was stirred vigorously for 5 min, then ethyl bromopyruvate (90%, 0.5 mL, 3.2 mmol) was added. After an additional 5 min stirring, TFAA (2.8 mL, 20 mmol) was added in one portion (**CAUTION!** large amounts of CO_2 are released). The reaction was quenched by addition of H_2O (50 mL). The mixture was extracted with EtOAc (100 mL). The organic phase was washed with brine (50 mL), dried over Na_2SO_4 and filtered. After removal of solvent in vacuo, the residue was purified by silica gel chromatography to afford the product **3**.

Synthesis of cyclic peptides QZ59Se-RRS and QZ59Se-SSR

Treatment of **3c** ((*S*)-Val-selenazole, 188 mg, 0.5 mmol) with TFA (3 mL) in CH_2Cl_2 (9 mL) removed the *N*-*t*-Boc protective group to give the amine (**3c**-amine). The ethyl ester of *ent*-**3c** ((*R*)-Val-selenazole, 376 mg, 1.0 mmol) was hydrolyzed in a solution of NaOH (320 mg, 8 mmol) in THF/MeOH/ H_2O (10:2:3) [Eq. (2)]. The resulted acid (*ent*-**3c** acid) was dissolved in anhyd DMF (10 mL). Half of this solution (*ent*-**3c** acid in 5 mL DMF) was mixed with HOBt (0.21 g, 1.5 mmol), HBTU (0.51 g, 1.5 mmol), and **3c**-amine (dissolved in DMF). To this mixture was added $i\text{Pr}_2\text{EtN}$ (0.42 mL, 2.4 mmol) with stirring at RT under N_2 . The reaction was monitored by TLC. Upon completion, the reaction was quenched with aq HCl. The solution was diluted with EtOAc (50 mL), washed with sat. NaHCO_3 (50 mL) and brine (50 mL), dried over Na_2SO_4 and filtered. The solvent was then removed in vacuo, and the residue was subjected to column chromatography on silica gel to provide the dimer **4** (245 mg, 81% yield). $[\alpha]_{\text{D}}^{20} = +20.5$ ($c=0.73$, CHCl_3); ^1H NMR (300 MHz, CDCl_3): $\delta=8.76$ (s, 1H), 8.73 (s, 1H), 7.98 (d, $J=8.9$ Hz, 1H), 5.39 (d, $J=7.6$ Hz, 1H), 5.18 (dd, $J=8.6$, 6.4 Hz, 1H), 4.74 (brs, 1H), 4.31 (q, $J=7.0$ Hz, 2H), 2.72–2.50 (m, 1H), 2.32 (brm, 1H), 1.37 (s, 9H), 1.30 (t, $J=7.0$ Hz, 3H), 1.01–0.91 (m, 9H), 0.88 ppm (d, $J=6.9$ Hz, 3H); ^{13}C NMR (75 MHz, CDCl_3): $\delta=181.7$, 179.5, 161.6, 161.5, 155.7, 149.9, 148.2, 134.7, 130.8, 61.4, 60.6, 59.5, 36.7, 32.9, 32.6, 28.3 (3C), 19.9, 19.5, 17.7, 17.3, 14.4 ppm; MS (ESI): m/z : calcd for $\text{C}_{23}\text{H}_{34}\text{N}_4\text{O}_5\text{Se}_2\text{Na}$: 629.1 $[M+\text{Na}]^+$, found: 629.1.

The reaction of **4** (0.25 mmol) with TFA (5 mL) in CH_2Cl_2 (15 mL) afforded an amine in quantitative yield. The amine was then coupled with the remaining half of *ent*-**3c** acid under the same conditions used to synthesize **4**, which gave the linear trimeric peptide **5** (175 mg, 0.21 mmol). $[\alpha]_{\text{D}}^{20} = +4.4$ ($c=0.93$, CHCl_3); ^1H NMR (300 MHz, CDCl_3): $\delta=8.80$ (s, 1H), 8.78 (s, 1H), 8.76 (s, 1H), 8.01 (d, $J=9.1$ Hz, 1H), 7.91 (d, $J=9.2$ Hz, 1H), 5.28–5.21 (m, 3H), 4.78 (brm, 1H), 4.36 (q, $J=7.2$ Hz, 2H), 2.73–2.60 (m, 1H), 2.62–2.49 (m, 1H), 2.35–2.28 (m, 1H), 1.41 (s, 9H), 1.35 (t, $J=7.2$ Hz, 3H), 1.06–0.91 (m, 15H), 0.92 ppm (d, $J=6.7$ Hz, 3H); ^{13}C NMR (75 MHz, CDCl_3): $\delta=182.2$, 180.1, 179.8, 161.8, 161.61 (2C), 161.58, 155.7, 150.3, 148.5, 134.9, 131.2, 131.0, 61.6, 60.8, 59.7, 59.2, 36.9, 33.0, 32.8, 28.5 (3C), 24.9, 20.2, 19.9, 19.7, 17.9, 17.8, 17.6, 14.6 ppm; MS (ESI): m/z : calcd for $\text{C}_{31}\text{H}_{44}\text{N}_6\text{O}_6\text{Se}_3\text{Na}$: 859.1. $[M+\text{Na}]^+$, found: 859.1.

Peptide **5** was treated with NaOH to hydrolyze the ethyl ester, then with TFA to remove the *N*-*t*-Boc protective group. The residue was dissolved in mixed solvent ($\text{DMF}/\text{CH}_2\text{Cl}_2=2/1$, 30 mL), and a solution of PyBop (260 mg, 0.5 mmol) and DMAP (122 mg, 1.0 mmol) in

DMF/CH₂Cl₂ (2:1, 44 mL) was added slowly by using a syringe pump. The reaction mixture was washed successively with aq HCl (1N, 15 mL), sat. NaHCO₃ (50 mL) and brine (50 mL), dried over Na₂SO₄, and filtered. The solvent was then removed in vacuo, and the residue was subjected to column chromatography on silica gel to give QZ59Se-*RRS* (113 mg, 78%). $[\alpha]_D^{20} = +31.1$ ($c=1.0$, CHCl₃); ¹H NMR (500 MHz, CDCl₃): δ =8.91 (s, 1H), 8.82 (s, 1H), 8.81 (s, 1H), 8.39 (d, $J=9.6$ Hz, 1H), 8.33 (m, 2H), 5.67 (dd, $J=9.7$, 3.0 Hz, 1H), 5.57 (dd, $J=8.5$, 3.9 Hz, 1H), 5.29 (dd, $J=7.1$, 5.6 Hz, 1H), 2.49 (m, 1H), 2.37–2.27 (m, 1H), 2.22 (m, 1H), 1.11 (d, $J=6.7$ Hz, 3H), 1.08 (d, $J=6.9$ Hz, 3H), 0.94 (d, $J=6.8$ Hz, 3H), 0.90 (d, $J=6.9$ Hz, 3H), 0.86 (d, $J=6.8$ Hz, 3H), 0.83 ppm (d, $J=6.8$ Hz, 3H); ¹³C NMR (75 MHz, CDCl₃): δ =178.0, 174.9, 173.6, 160.8, 160.7, 160.6, 150.4, 149.8, 149.5, 132.1, 130.7, 130.5, 59.6, 58.9, 58.4, 38.2, 36.1, 33.6, 20.1, 19.7, 18.7, 18.1, 17.7, 15.9 ppm; HRMS (ESI-TOF): m/z : calcd for C₂₄H₃₁N₆Se₃O₃: 690.9948 [$M+H$]⁺, found: 690.9959.

Synthesis of QZ59Se-*SSR* was similar to that of QZ59Se-*RRS*. QZ59Se-*SSR*:

$[\alpha]_D^{20} = -31.8$ ($c=1.0$, CHCl₃). The ¹H and ¹³C NMR spectra of QZ59Se-*SSR* were identical to those of QZ59Se-*RRS*.

Synthesis of homochiral cyclic peptides QZ59Se-*SSS* and QZ60Se-*SSSS*

The ethyl ester of **3c** (800 mg, 2.13 mmol) was hydrolyzed in a solution of NaOH (800 mg, 20.0 mmol) in THF/MeOH/H₂O (10:2:3). The *N*-*t*-Boc protective group was subsequently removed by treatment with TFA (15 mL) in CH₂Cl₂ (45 mL). After removal of the solvent, the amino acid was directly dissolved in anhydrous MeCN (20 mL), and iPr₂EtN (1.4 mL, 8.5 mmol) and pentafluorophenol diphenylphosphinate (FDPP; 1.64 g, 4.3 mmol) were added to the solution at RT under N₂. The reaction was monitored by TLC. Upon completion of the reaction, the solvent was removed in vacuo and the residue was partitioned between CH₂Cl₂ (45 mL) and H₂O (30 mL). The organic phase was washed successively with aq HCl (2 M, 50 mL), aq NaOH (1 M, 2 × 50 mL), H₂O (50 mL), and brine (50 mL), dried over Na₂SO₄, and filtered. The solvent was then removed in vacuo, and the residue was subjected to column chromatography on silica gel to give QZ59Se-*SSS* (290 mg, 60% yield) and QZ60Se-*SSSS* (60 mg, 12% yield).

Data for the characterization of QZ59Se-*SSS* and QZ59Se-*RRR* have been reported.^[7]

QZ60Se-*SSSS*: $[\alpha]_D^{20} = -55.0$ ($c=1.0$, CHCl₃); ¹H NMR (300 MHz, CDCl₃): δ =8.81 (s, 4H), 7.87 (d, $J=9.3$ Hz, 4H), 5.31 (dd, $J=9.3$, 7.0 Hz, 4H), 2.63–2.40 (m, 4H), 1.122 (d, $J=6.8$ Hz, 12H), 1.119 ppm (d, $J=6.6$ Hz, 12H); ¹³C NMR (75 MHz, CDCl₃): δ =176.4, 160.9, 149.1, 132.1, 58.1, 32.9, 20.2, 18.5 ppm; HRMS (ESI-TOF): m/z : calcd for C₃₂H₄₁N₈Se₄O₄: 920.9906 [$M+H$]⁺, found: 920.9917.

QZ59Se-*RRR* and QZ60Se-*RRRR* were synthesized by analogous procedures. QZ60Se-*RRRR*: $[\alpha]_D^{20} = +55.1$ ($c=1.0$, CHCl₃). The ¹H and ¹³C NMR spectra of QZ60Se-*RRRR* are identical to those of QZ60Se-*SSSS*.

Calcein-AM transport assay

Calcein acetoxymethyl ester (Calcein-AM) is a substrate for export activity of Pgp, and Calcein-AM transport is a commonly used fluorescence-based functional assay. Chinese hamster ovary cells CR1R12 that over-express high levels of Pgp, which accounts for ~20% of the total plasma membrane protein were previously selected in colchicine. CR1R12 and their parental control cells AuxB1 were cultured as described.^[27] Briefly, 5×10^5 CR1R12 cells were pre-incubated with increasing concentrations of cyclopeptide inhibitors for 15 min at RT in culture medium (100 μ L). Non-fluorescent calcein-AM (0.25 mM) was added, and the fluorescence intensity of calcein ($\lambda_{\text{ex}}=494$ nm, $\lambda_{\text{em}}=517$ nm), the hydrolysis product of calcein-AM in the cytosol, was monitored after 30 min incubation at RT in a FluoroLog-3 fluorimeter (Jobin Yvon Horiba, Edison, USA) equipped with a 96-well plate reader. 100% was the maximum fluorescence seen upon full inhibition of Pgp in CR1R12 cells. The means and standard deviations obtained from two independent, quadruplet experiments were shown in Figure 1, and each line represented a fit to the Hill equation. The compounds had no effect on calcein-AM accumulation in the parental AuxB1 control cells and the relative fluorescence intensities remained constant (100%) over the entire concentration range assayed (data not shown).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We thank P. Dawson for comment and E. Hildebrandt for editorial assistance.

References

1. Szakacs G, Paterson JK, Ludwig JA, Booth-Genthe C, Gottesman MM. Nat Rev Drug Discovery. 2006; 5:219. [PubMed: 16518375]
2. Eckford PD, Sharom FJ. Chem Rev. 2009; 109:2989. [PubMed: 19583429]
3. Shapiro AB, Ling V. Eur J Biochem. 1997; 250:130. [PubMed: 9432000]
4. Loo TW, Bartlett MC, Clarke DM. J Biol Chem. 2003; 278:39706. [PubMed: 12909621]
5. Loo TW, Clarke DM. Biochem Biophys Res Commun. 2005; 329:419. [PubMed: 15737603]
6. Martin C, Berridge G, Higgins CF, Mistry P, Charlton P, Callaghan R. Mol Pharmacol. 2000; 58:624. [PubMed: 10953057]
7. Aller SG, Yu J, Ward A, Weng Y, Chittaboina S, Zhuo R, Harrell PM, Trinh YT, Zhang Q, Urbatsch IL, Chang G. Science. 2009; 323:1718. [PubMed: 19325113]
8. Twentymen PR, Fox NE, White DJG. Br J Cancer. 1987; 56:55. [PubMed: 3040060]
9. Slater LM, Sweet P, Stupecky M, Gupta S. J Clin Invest. 1986; 77:1405. [PubMed: 3457021]
10. Loo F. Expert Opin Invest Drugs. 1999; 8:807.
11. Ko SY, Dalvit C. Int J Pept Protein Res. 1992; 40:380. [PubMed: 1483832]
12. O'Donohue MF, Burgess AW, Walkinshaw MD, Treutlein HR. Protein Sci. 1995; 4:2191. [PubMed: 8535256]
13. Ogino J, Moore RE, Patterson GML, Smith CD. J Nat Prod. 1996; 59:581. [PubMed: 8786364]
14. Fu X, Do T, Schmitz FJ, Andrushevich V, Engel MH. J Nat Prod. 1998; 61:1547. [PubMed: 9868162]
15. Yeh VSC. Tetrahedron. 2004; 60:11995.

16. Wipf P. Chem Rev. 1995; 95:2115.
17. You SL, Razavi H, Kelly JW. Angew Chem. 2003; 115:87. Angew Chem Int Ed. 2003; 42:83.
18. Riedrich M, Harkal S, Arndt HD. Angew Chem. 2007; 119:2755. Angew Chem Int Ed. 2007; 46:2701.
19. Boden CDJ, Pattenden G, Ye T. Synlett. 1995; 417
20. Geisler K, Pfeiffer WD, Kunzler A, Below H, Bulka E, Langer P. Synthesis. 2004; 875
21. Hennen WJ, Hinshaw BC, Riley TA, Wood SG, Robins RK. J Org Chem. 1985; 50:1741.
22. Merritt EA, Bagley MC. Synthesis. 2007; 3535
23. Aguilar E, Meyers AI. Tetrahedron Lett. 1994; 35:2473.
24. Ogawa A, Miyake J, Karasaki Y, Murai S, Sonoda N. J Org Chem. 1985; 50:384.
25. Bertram A, Hannam JS, Jolliffe KA, de Turiso FGL, Pattenden G. Synlett. 1999; 11:1723.
26. Bertram A, Maulucci N, New OM, Nor SMM, Pattenden G. Org Biomol Chem. 2007; 5:1541. [PubMed: 17571182]
27. al-Shawi MK, Senior AE. J Biol Chem. 1993; 268:4197. [PubMed: 8095047]
28. Bagley MC, Dale JW, Merritt EA, Xiong X. Chem Rev. 2005; 105:685. [PubMed: 15700961]

Figure 1.

A) Stereospecific chemical structures of trimeric and tetrameric cyclic peptides comprised of valine-selenazole/thiazole units. B) Inhibition of Pgp-mediated calcein-AM transport by selenium-labeled cyclopeptides, or C) by their sulfur analogues, occurred with similar IC_{50} values. The means and standard deviations obtained from two independent, quadruplet experiments are shown, and each line represents a fit to the Hill equation.

Scheme 1.

Constraining the conformation of cyclic peptide ligands to facilitate cocrystallization with Pgp. Structures of macrocyclic peptides cyclosporin A and PSC 833 are shown on the left. In the middle are natural products dendroamide A and patellamide C, wherein the presence of heterocycles further constrains the flexibility of these smaller cyclopeptides. On the right are enantiomeric cyclic peptides QZ59Se-SSS and QZ59Se-RRR in which three selenium atoms (in dark circles) were decorated as X-ray-anomalous scattering markers to facilitate the identification and modeling of bound ligands.

Scheme 2.

Synthesis of trimeric and tetrameric cyclic peptides from optically pure selenazole amino acids in the solution phase. Synthetic routes were illustrated for QZ59Se-RRS (bottom) and the homochiral cyclic peptides QZ59Se-SSS and QZ60Se-SSSS (top). The synthesis of QZ59Se-SSR, QZ59Se-RRR, and QZ60Se-RRRR was similar to that of QZ59Se-RRS, QZ59Se-SSS and QZ60Se-SSSS, respectively. d) HOBt, HBTU, DIEA, DMF, 84%; e) PyBop, DMAP, 78%. a) NaOH, THF/MeOH/H₂O; b) TFA/CH₂Cl₂; c) FDPP, DIEA, CH₃CN; d) +HOBt, HBTU, DIEA, DMF (84%); e) PyBop, DMAP (78 %).

Table 1

Synthesis of optically pure selenazole-containing amino acids.

R	Product	Yield [%][a]	<i>ee</i> [b]	R	Product	Yield [%][a]	<i>ee</i> [b]
1 Me	3a	51 46	97 96[c]	11	3k	53	97
2 H	3b	52	–	12	3l	51	99
3 <i>i</i> Pr	3c	73 68	>99 >99[c]	13	3m	49	>99
4 <i>t</i> Bu	3d	72	99	14	3n	71	>99
5	3e	71	>99[de]	15	3o	32	>99[d]
6	3f	71	>99	16	3p	39	99
7	3g	72	98	17	3q	32	99
8	3h	67	99	18	3r	74	99
9	3i	72	100[f]	19	3s	48	79[d,g]

R	Product	Yield [%][a]	ee[b]
10			
74		74	>99
20			
3j			
98		98	
47		47	
3a-3i			

[a] Isolated yield for three steps.

[b] *ee* values were determined by using a ChiralPak AD-H column unless otherwise noted. The eluent was 1:9 iPrOH/hexane with a flow rate of 1 mL/min, unless otherwise noted.

[c] Steps 2 and 3 were carried out at RT.

$[d]$ Optical purity was determined by ChiralPak OD-H column.

[e] By using an eluent of 4% *n*-PrOH in hexane.

[f] Very minor diastereomer was removed by silica gel chromatography.

$[g]$ Gradient elution from 4% to 10% of *i*-PrOH in hexane.

$[h]_{\text{EtOH}}$, overnight.