Analogs Incorporating *trans*-4-Hydroxy-L-proline That Reverse Multidrug Resistance Better Than Hapalosin

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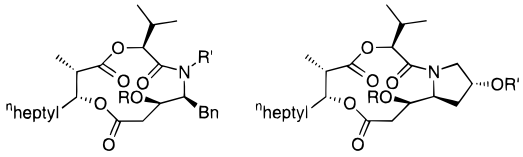
Chemotherapy often fails over time due to multidrug resistance (MDR), one of whose principal mechanisms is expulsion of a wide spectrum of drugs from tumor cells by P-glycoprotein (P-gp).[2] The cyclic depsipeptide hapalosin, **1**, reverses MDR presumably by inhibiting P-gp.[3] Computation with the aid of ^{1}H,^{1}H-NOESY data revealed that the major *s-cis* rotamer of hapalosin[4] and the single *s-trans* rotamer of the non-*N*-Me analog **2** have very different conformations, whereas the minor *s-trans* rotamer of hapalosin and analog **2** have very similar conformations.[5] Analog **2** and the PMB ether of hapalosin (**3**) were found to possess substantially lower anti-MDR activity than hapalosin. Four proline analogs of hapalosin, **4**−**7**, were also synthesized. Analogs **4** and **7** reverse MDR better than hapalosin, while analogs **5** and **6** were less effective than hapalosin.



1 R = H, R' = Me
2 R = H, R' = H
3 R = PMB, R' = Me

4 R = PMB, R' = H
5 R = PMB, R' = C(O)NHBn
6 R = H, R' = H
7 R = H, R' = C(O)NHBn

The synthesis of the four proline analogs of hapalosin, **4**−**7**, is illustrated in Scheme 1. *trans*-4-Hydroxy-L-Pro (**8**) was tris-protected as ester **9**. The ester was converted to an aldehyde that underwent Brown allylboration[6] to produce homoallylic alcohol **10** in >90% de. The alcohol was protected with *p*-methoxybenzyl 2,2,2-trichloroacetimidate (PMBTCAI),[7] and the olefin was transformed to acid **11**. The acid was coupled to alkenol **12**[5] using 1-ethyl-3-[3-(dimethylamino)propyl]carbodiimide (EDC), and the resulting olefin was oxidized to acid **13**. After the acid was coupled to alcohol **14**,[5] the Cbz carbamate and benzyl ester were selectively deprotected in the presence of the PMB ether by hydrogenation over W-2 Raney nickel.[8] Cycloamidation of the crude amino acid with bis(2-oxo-3-oxazolidinyl)phosphinic chloride (BOP-Cl) and ^{i}Pr$_2$NEt (DIPEA) in PhMe at 85 °C[9] provided **15** in a good yield of 58% for the two steps. The four proline analogs **4**−**7** were easily generated subsequently. The hydroxyl group of analog **4** was unreactive with benzyl 2,2,2-trichloroacetimidate/TfOH[10] and with DIAD/phenol/PPh$_3$.[11] The synthesis was designed to be linear so that an alcohol different than **12** or **14** could be introduced at the particular position.

The conformational ratios of the five macrolactams in Scheme 1 are interesting. The three compounds with a β-PMB ether—**15**, **4**, and **5**—have a conformational ratio from 1.1:1 to 1.5:1 in $CDCl_3$ at 25 °C. By contrast, the two analogs with a free β-hydroxyl group—**6** and **7**—exist as a mixture of about 6:1 conformers. Applying distance constraints (1.5−5.0 Å) to protons exhibiting NOESY crosspeaks, computation with Macromodel (v. 4.5)[12] using the AMBER* force field and GB/SA chloroform solvation[13] resulted in seven possible conformations for analog **6**, all containing an *s-cis* amide bond. Analog **7** exhibits NOESY correlations similar to those for **6**.

The anti-MDR activities of synthetic hapalosin and its analogs (modulators) were determined by cytotoxicity and drug accumulation assays using MCF-7/ADR cells, which overexpress P-gp. In the cytotoxicity assay (Figure 1), cells were exposed to a modulator alone or in the presence of actinomycin D, daunomycin, or cisplatin. Cisplatin was included as a non-P-gp substrate to demonstrate selective enhancement of killing by actinomycin D and daunomycin, which are transported by P-gp. In the drug accumulation assay (Figure 2), MCF-7/ADR cells were treated with a modulator and then incubated with [^{3}H]-vinblastine, which is also transported by P-gp. Reversal of MDR is indicated if minimally cytotoxic doses of a modulator selectively increase cell killing by actinomycin D and daunomycin and increase the intracellular concentration of [^{3}H]vinblastine.

Comparing the ability of the analogs to potentiate the cytotoxicity of actinomycin D and daunomycin to that of synthetic hapalosin (Figure 1), analogs **4** and **7** are better, analogs **5** and **6** are significantly worse, and the non-*N*-Me analog **2** is about two times worse. The PMB ether of hapalosin (**3**) has only marginal activity. With regard to the modulators' enhancement of the intracellular concentration of [^{3}H]vinblastine (Figure 2), analogs **4** and **7** are better than hapalosin, while analogs **2**, **3**, **5**, and **6** are significantly worse. None of the modulators altered the sensitivity of MCF-7/ADR cells to cisplatin or the sensitivity of MCF-7 cells, which do not express P-gp, to any of the drugs.

The bioassay results suggest that a free hydroxyl and an aromatic group may be important for the anti-MDR activity of hapalosin and its analogs.[14] Except for the non-*N*-Me analog **2**, all the analogs less potent than

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(14) NMR experiments on the conformations of modulators in solvent systems more biologically pertinent than $CDCl_3$ are being conducted.

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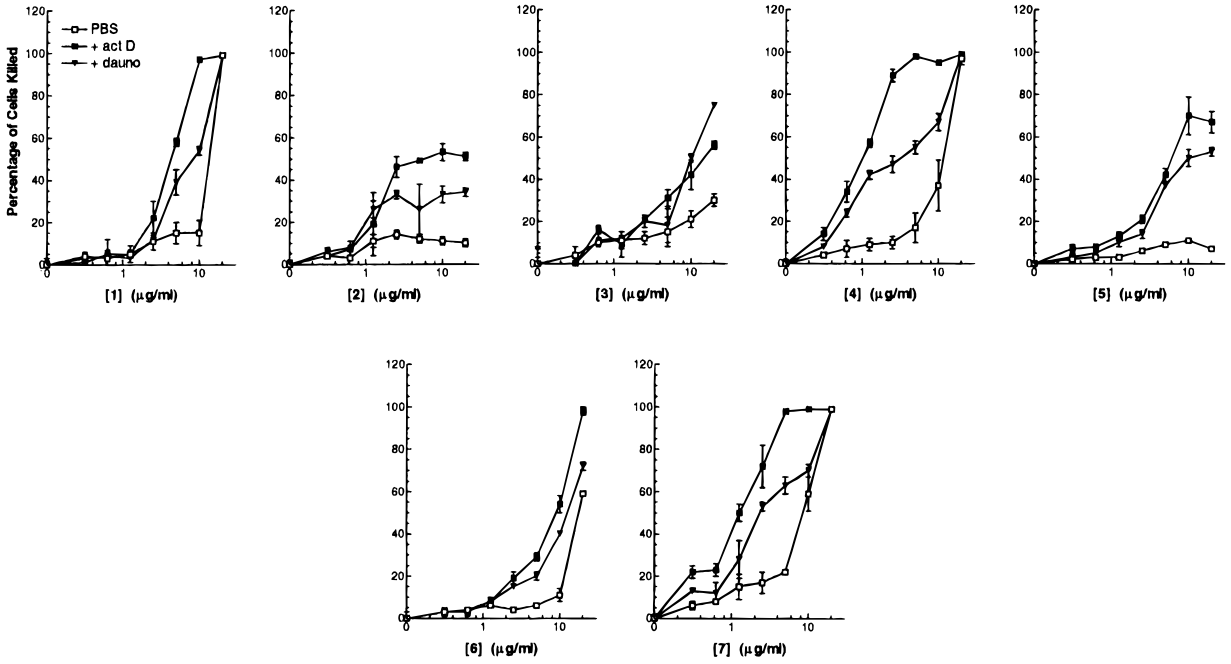
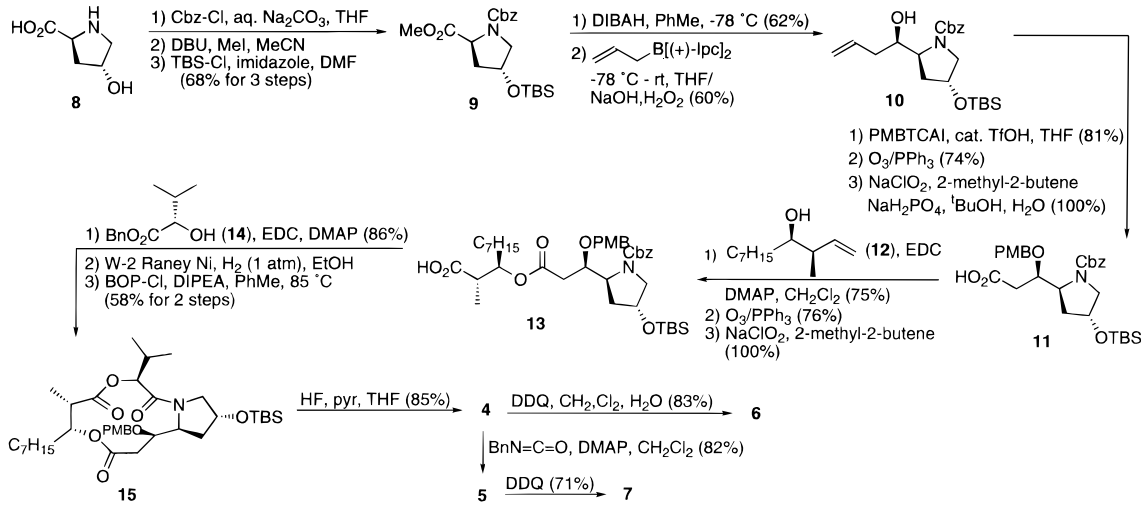


Figure 1. Reversal of MDR by hapalosin analogs. MCF-7/ADR cells were incubated with the indicated concentrations of analogs **1**−**7** (left to right) in the presence of phosphate-buffered saline (as a control) (□), 25 nM actinomycin D (■), or 2 μM daunomycin (▼). Cell survival after 48 h was determined as indicated in the Supporting Information. Values represent the mean ± SD of triplicate samples.

Scheme 1



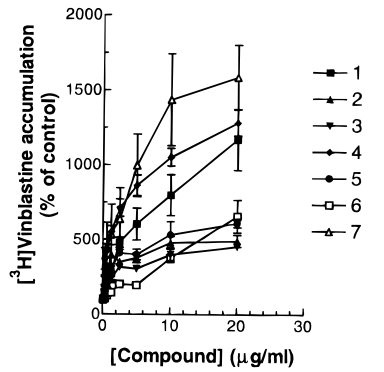


Figure 2. Effects of hapalosin analogs on [^{3}H]vinblastine accumulation by MDR cells. MCF-7/ADR cells were incubated with the indicated concentrations of analogs **1** (■), **2** (▲), **3** (▼), **4** (◆), **5** (●), **6** (□), or **7** (△). The intracellular accumulation of [^{3}H]vinblastine was then determined as indicated in the Supporting Information. Values represent the mean ± SD of triplicate samples.

hapalosin (**3**, **5**, and **6**) have either two free hydroxyl or two aromatic groups. By contrast, hapalosin and the two analogs more potent than it (**4** and **7**) have a free hydroxyl and an aromatic group. An objective is to synthesize analogs that are more potent and water soluble than **4** and **7** but are less intrinsically cytotoxic.

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Supporting Information Available: Experimental procedures for all the steps in Scheme 1; ^{1}H and ^{13}C NMR spectra and characterization data for compounds **3**−**7**, **9**−**11**, **13**, and **15**; and protocol for the cytotoxicity and drug accumulation assays (32 pages).

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