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A Gene-Expression Inhibitor that Targets an α-Helix-Mediated Protein Interaction

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Protein−protein interactions are harder to target by small organic molecules than by enzymes or nuclear hormone receptors. Protein−protein binding typically occurs over a relatively large surface area, and the binding surfaces between two proteins tend to be flat and often lack in pockets that might provide binding sites suited for small organic molecules. Nevertheless, protein−protein interfaces vary widely in nature from one to another, and some are likely to present better druggability than others.[1] A good example is the interaction between the somatostatin receptor and β-turn peptide ligands. The success of β-turn-mimicking molecules provided a basis for the notion that the protein−protein interactions mediated by a β-turn peptide are generally more druggable than others.[2]

Recent studies suggest that the protein−protein interactions that are mediated by short α-helical segments of proteins are similarly tractable to inhibition by small nonpeptidic molecules.[3] Helical peptide segments of proteins are responsible for a number of biologically important protein associations in the fields of signal transduction and gene transcription. For our case study, we focused on the interaction between the two cancer-linked nuclear proteins, ESX (an epithelial-specific transcription factor) and Sur-2/DRIP130 (a Ras-linked subunit of the human mediator complex).[4] This recently identified interaction is important for the overexpression of the *Her2* oncogene in malignant breast cancer cells (Figure 1)[4] and thus serves as a potential therapeutic target for *Her2*-positive breast cancers amounting to 60 000 cases per year in the U.S.

The interaction is mediated by one face of an eight-amino acid α-helical region in the transcriptional activation domain of ESX (Ser-**Trp**-Ile-Ile-Glu-Leu-Leu-Glu), and the tryptophan residue in the hydrophobic face of the helix makes a unique contribution to the specificity of the interaction.[4] The relatively small size of the interface and the importance of the tryptophan residue suggested the existence of small-molecule inhibitors in a chemical library enriched in the structural families of indole, benzimidazole, and benzodiazepin − indole-mimicking π-electron-rich pharmacores found in bioavailable drugs. We screened 2422 indolelike compounds by cell-based assays and found the compound that we named adamanolol (Figure 1).[5] The druglike pindolol derivative impaired the ability of the ESX activation domain to stimulate transcription in cells, whereas it had little effect on those of the activation domains of VP16 and NF-κB p65, two functionally irrelevant activation domains structurally similar to the ESX activation domain (Figure 2A).[6] Adamanolol impaired the viability of Her2-positive breast cancer cell lines (SK-BR3, MDA-MB453, and MCF-7), but had much milder effects on MDA-MB468 with no detectable levels of Her2 (Figure 2B).[4] Western blot analyses of drug-treated cells showed that the expression of Her2 protein, but not that of α-tubulin, was significantly reduced by adamanolol in the Her2-positive SK-BR3 cells (Figure 2C). Although the expression of other proteins may be influenced by adamanolol, the impaired expression of Her2 is likely to be an important driving force for the selective cell death in Her2-positive cells.

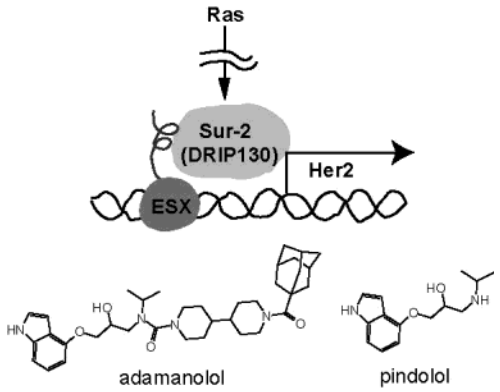

Figure 1. The interaction of the activation domain of ESX with the Ras-linked coactivator Sur-2/DRIP130 is required for the overexpression of the *Her2* oncogene. We found adamanolol, a derivative of pindolol, as an inhibitor of the interaction.

The cellular effects of adamanolol are strikingly similar to those observed when an α-helical peptide inhibitor derived from ESX ($ESX_{129-145}$) was introduced into cells,[4] implicating that adamanolol directly inhibits the ESX−Sur-2 interaction. To demonstrate that, we examined if adamanolol competes with fluorescein isothiocyanate (FITC)-labeled $ESX_{129-145}$ for the interaction with Sur-2 *in vitro*. As shown in Figure 2D, adamanolol inhibited the ESX−Sur-2 interaction in a dose-dependent manner.[7] The interaction was severely impaired at an IC_{50} of ~8 μM, consistent with the results of the cell-based assays. These results support the notion that adamanolol exerts its biological activity by directly disrupting the ESX−Sur-2 interaction.

It remains unclear how adamanolol inhibits the α-helix-mediated interaction. Two close analogues of adamanolol, in which the adamantane group was replaced by a thiophene or toluene group, had little biological activity, suggesting the importance of the bulky, hydrophobic adamantane group. NMR analyses of adamanolol showed signal broadening of the protons around the urea linker at room temperature and the absence of NOEs between the isopropyl protons and the piperidine even with a 500 ms NOESY mixing time.[8] A reasonable explanation for these NMR characters is a rigid conformation around the urea linker with the preference for the *Z* conformer imposed by the bulky isopropyl group (Figure 3). The *Z* conformation perhaps plays a role in bringing the adamantan group and the indole ring into proximity and forming a helixlike surface for the interaction (Figure 3).[9]

Complete analysis of human genome is anticipated to identify an unprecedented number of potential drug targets. Among these genomic pseudotargets, the "relatively easy" targets such as nuclear hormone receptors or enzymes will certainly be an immediate focus in pharmaceutical industries. However, more challenging genomic targets including protein−protein interactions in transcriptional regulation need to be assessed to extend the scope of druggable

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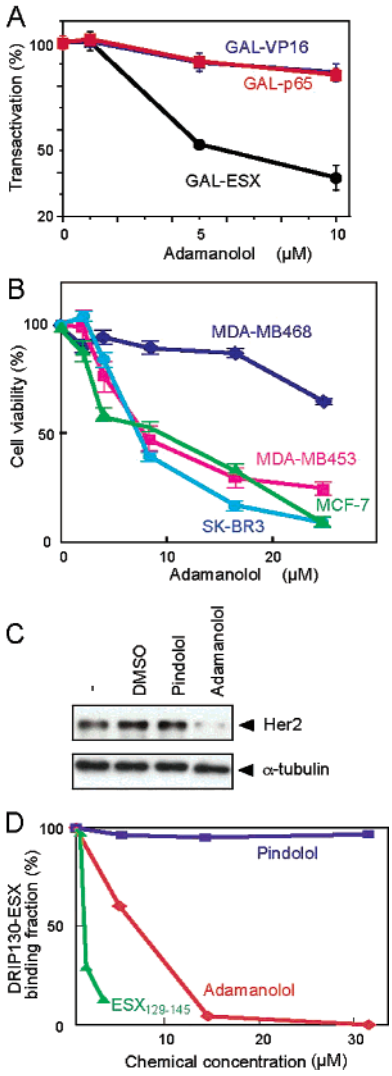

Figure 2. Biological activity of adamanolol. (A) Adamanolol selectively inhibits the ability of the ESX activation domain to activate transcription of the secreted alkaline phosphatase reporter gene in HEK293T cells. (B) Adamanolol selectively impaires the viability of Her2-positive breast cancer cell lines (MDA-MB453, MCF-7, and SK-BR3) but has milder effects on MDA-MB468 with no detectable levels of Her2. Cells were treated by adamanolol for 24 h, and cell viability was estimated by WST metabolic assay. (C) Inhibition of Her2 protein expression by adamanolol. SK-BR3 cells were treated by adamanolol (7.3 μM) for 24 h, and cell lysates were analyzed by Western blots. (D) Adamanolol competes with fluorescein isothiocyanate (FITC)-labeled $ESX_{129-145}$ (10 nM) for the interaction with Sur-2 (50 μM) in vitro. Pindolol, a truncated derivative of adamanolol, had no effects even at 30 μM.

targets for future drug discovery. Continued efforts targeting the ESX−Sur-2 interaction may serve as a unique case study.

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Supporting Information Available: Details of the experimental procedures and Figures S1 and S2 (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

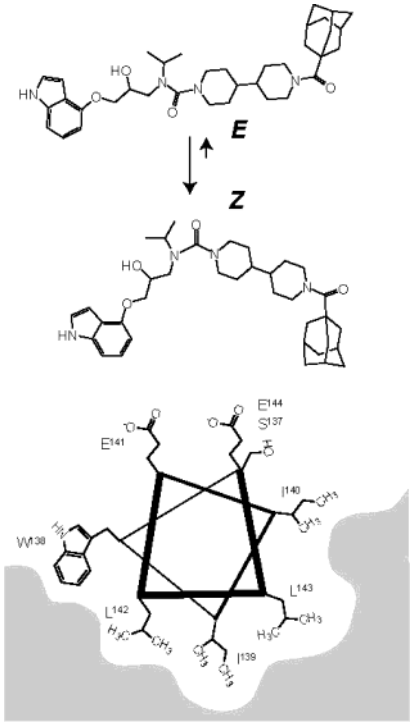

Figure 3. Two possible conformers of adamanolol. NMR analysis of adamanolol suggested its preference of the *Z* conformer imposed by the bulky isopropyl group. The *Z* conformation may play a role in forming a helixlike interface as indicated in the helical wheel presentation of the natural ligand, $ESX_{137-144}$.

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(5) Poor cell-permeability is often a problem of inhibitors of protein−protein interactions. In our study, we performed cell-based screens to eliminate non-cell-permeable molecules. Details of the cell-based screens are shown in the Supporting Information.
(6) The activation domains of VP16 and NF-κB p65 served as an excellent control because both have been reported to be α helical [Uesugi, M.; Nyanguile, O.; Lu, H.; Levine, A. J.; Verdine, G. L. *Science* **1997**, *277*, 1310−1313. Schmitz, M. L.; Silva, M. A.; Altman, H.; Czisch, M.; Holak, T. A.; Baeuerle, P. A. *J. Biol. Chem.* **1994**, *269*, 25613−25620] and are as potent as ESX is in cells. However, it is possible that adamanolol is not completely specific for the ESX activation domain.
(7) The estimated dissociation constant of FITC-labeled $ESX_{129-145}$ for Sur-2 was 12 μM under the same condition (Figure S1).
(8) Complete assignments of the proton signals of adamanolol were achieved through TOCSY, DQF-COSY, and NOESY experiments. A summary of key NOE connectivities is shown in Figure S2.
(9) A less constrained adamanol derivative that lacks the isopropyl group had no detectable activity in SK-BR3 cells even at 100 μM, supporting the notion that the *Z* conformation is important for the activity (Uesugi, M.; Shimogawa, H.; Kigoshi, H., unpublished results).

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