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Award Number: DAMD17-02-1-0277

TITLE: Inhibition of Her2 Transcription by Small Organic
Molecules

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REPORT DATE: April 2005

TYPE OF REPORT: Annual Summary

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for Public Release;
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REPORT DOCUMENTATION PAGEForm Approved
OMB No. 074-0188

Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing this collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden to Washington Headquarters Services, Directorate for Information Operations and Reports, 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302, and to the Office of Management and Budget, Paperwork Reduction Project (0704-0188), Washington, DC 20503

1. AGENCY USE ONLY (Leave blank)		2. REPORT DATE April 2005	3. REPORT TYPE AND DATES COVERED Annual Summary (18 Mar 2004 - 17 Mar 2005)	
4. TITLE AND SUBTITLE Inhibition of Her2 Transcription by Small Organic Molecules			5. FUNDING NUMBERS DAMD17-02-1-0277	
6. AUTHOR(S) Yongmun Choi Motonari Uesugi				
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) Baylor College of Medicine Houston, Texas 77030 <i>E-Mail:</i> ychoi@bcm.tmc.edu			8. PERFORMING ORGANIZATION REPORT NUMBER	
9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES) U.S. Army Medical Research and Materiel Command Fort Detrick, Maryland 21702-5012			10. SPONSORING / MONITORING AGENCY REPORT NUMBER	
11. SUPPLEMENTARY NOTES				
12a. DISTRIBUTION / AVAILABILITY STATEMENT Approved for Public Release; Distribution Unlimited				12b. DISTRIBUTION CODE
13. ABSTRACT (Maximum 200 Words) Overexpression of the Her2 protein has been found in ~30% of breast tumors, and the inhibition of Her2 expression may be an effective way to treat Her2-positive patients. Recently, the P.I. and co-workers reported identification of chemical inhibitors of Her2 transcription. The compounds that we named adamanolol and wrencholol inhibited Her2 transcription by disrupting the interaction of two cancer-linked nuclear proteins, ESX and Sur-2. Affinity purification revealed that wrencholol binds to the Sur-2 subunit of the human mediator complex by mimicking the potent activation domain of transcription factor ESX. In the third year of funding, we designed a STF1 (synthetic transcription factor), taking advantage of the ability of wrencholol to bind to the Sur-2 subunit and the specific DNA-binding affinity of a hairpin polyamide molecule. The hybrid compound of these two molecules activated transcription of a reporter gene <i>in vitro</i> in a promoter-dependent manner through simultaneous contacts with DNA and Sur-2. Our results indicate that wrencholol serves as an activation domain mimic, and that it is possible to generate a transcription factor out of completely organic components.				
14. SUBJECT TERMS Adamanolol, Her2, breast cancer, wrencholol, STF1				15. NUMBER OF PAGES 8
				16. PRICE CODE
17. SECURITY CLASSIFICATION OF REPORT Unclassified	18. SECURITY CLASSIFICATION OF THIS PAGE Unclassified	19. SECURITY CLASSIFICATION OF ABSTRACT Unclassified	20. LIMITATION OF ABSTRACT Unlimited	

NSN 7540-01-280-5500

Standard Form 298 (Rev. 2-89)
Prescribed by ANSI Std. Z39-18
298-102

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Introduction

Overexpression of Her2 is associated with poor overall survival and enhances malignancy and the metastatic phenotypes in breast cancers (1-5). Recently, the P.I. and co-workers reported identification of chemical inhibitors of Her2 transcription. The compounds that we named adamanolol and wrenchnolol inhibited Her2 transcription by disrupting the interaction of two cancer-linked nuclear proteins, ESX and Sur-2 (DRIP130). The drug-like molecules showed strong cytotoxicity against Her2-overexpressing breast cancer cells, but had much milder effects on breast cancer cells with no detectable levels of Her2. We also demonstrated direct interaction of wrenchnolol with Sur-2 in a cellular context, and proposed that wrenchnolol is a chemically tractable bioactive compound that is suited for further application (6, 7). The study in the third year is focused on the application of chemistry of wrenchnolol to the design of synthetic modulator of gene transcription.

Body

Naturally occurring transcription factors usually have two separable domains for activating selective genes: one for binding to specific promoters and the other for activating transcription through protein-protein interactions (8, 9). Our designed molecule **1** (STF1: synthetic transcription factor 1) comprises two functional domains (see scheme 1 in the attached paper): a hairpin polyamide molecule that binds specifically to 5'-TGACCAT sites in DNA (10) and a wrench-shaped synthetic compound (wrenchnolol) that binds to the Sur-2 protein (6, 7), a subunit of human mediator complex that links transcription activators to RNA polymerase II in human cells (11, 12). STF1 was synthesized by amide couplings of the hairpin polyamide, wrenchnolol, and a polyethylene linker. An internal pyrrole residue was chosen as an attachment site in the hairpin polyamide because previous studies had suggested that an extension at this position provides effective projection of an activation module (13, 14). The ability of STF1 to activate transcription was evaluated *in vitro* by using a reporter construct in which transcription of the reporter gene is controlled by six repeats of the hairpin-polyamide-binding sites (see Figure 1 in the attached paper). STF1 activated transcription of the reporter gene in a dose-dependent manner (lanes 2-5), whereas the control molecule **2** lacking the wrenchnolol moiety had no detectable activity (lane 6). Addition of excess amounts of a dominant negative Sur-2 protein fragment (lane 7) or immunodepletion of endogenous Sur-2 protein from nuclear extracts (lane 8) rendered the activity of STF1 undetectable,

consistent with our previous observation that wrencholol binds selectively to Sur-2 protein. A reporter construct with point mutations in the hairpin-polyamide-binding sites was unresponsive to STF1 (lanes 9-11), suggesting a high degree of selectivity of STF1. To verify the biochemical mechanism of the STF1-mediated gene activation, the promoter region of the reporter construct was labeled with a biotin molecule and bound to an avidin-agarose resin after incubation with nuclear extracts (see Figure 2 in the attached paper). Western blot analysis of the bound proteins showed that no detectable levels of Sur-2 or RNA polymerase II were recruited to the promoter in the absence of STF1 or in the presence of the control molecule **2**. In the presence of STF1, in contrast, Sur-2 protein and RNA polymerase II were recruited to the promoter. These results support our notion that STF1 stimulates transcription by recruiting human mediator complex to the promoter through simultaneous contacts with Sur-2 and DNA.

We are now testing wrencholol in animal models in collaboration with Dr Hung at MD Anderson Cancer center (Houston, TX). His laboratory has nude mice with ovarian cancer orthotopic tumor xenografts derived from SK-OV-3-ip1, a Her2-overexpressing cell line. These animals are being used to examine whether our drug lead suppresses tumor development in animal and prolong animal survival.

Key research accomplishments

1. Development of synthetic modulator of gene transcription.

Reportable outcomes

1. Asada, S., **Choi, Y.**, and Uesugi, M. (2003) A gene-expression inhibitor that targets an α -helix-mediated protein interaction. *J. Am. Chem. Soc.* **125**, 4992-4993
2. Shimogawa, H., Kwon, Y., Mao, Q., Kawazoe, Y., **Choi, Y.**, Asada, S., Kigoshi, H., and Uesugi, M. (2004) A wrench-shaped synthetic molecule that modulates a transcription factor-coactivator interaction. *J. Am. Chem. Soc.* **126**, 3461-3471
3. Kwon, Y., Arndt, H., Mao, Q., **Choi, Y.**, Kawazoe, Y., Dervan, P. B., and Uesugi, M. (2004) Small molecule transcription factor mimic. *J. Am. Chem. Soc.* **126**, 15940-15941

Conclusion

Our designed molecule **1** (STF1) comprises two functional domains: a hairpin polyamide molecule that binds specifically to 5'-TGACCAT sites in DNA and a wrench-shaped synthetic compound (wrenchnolol) that binds to the Sur-2 protein. The hybrid compound of these two molecules activates transcription of a reporter gene *in vitro* in a promoter-dependent manner through simultaneous contacts with DNA and Sur-2. Our results indicate that wrenchnolol serves as an activation domain mimic, and that it is possible to generate a transcription factor out of completely organic components.

References

1. D. J. Slamon *et al.*, *Science* **244**, 707 (1989).
2. C. T. Guy *et al.*, *Proc. Natl. Acad. Sci USA* **89**, 10578 (1992).
3. D. H. Yu *et al.*, *Oncogene* **6**, 1191 (1991).
4. B. A. Gusterson *et al.*, *J. Clin. Oncol.* **10**, 1049 (1992).
5. D. Yu *et al.*, *Oncogene* **16**, 2087 (1998).
6. S. Asada *et al.*, *J. Am. Chem. Soc.* **125**, 4992 (2003).
7. H. Shimogawa *et al.*, *J. Am. Chem. Soc.* **126**, 3461 (2004).
8. M. Ptashne *et al.*, *Nature* **386**, 569 (1997).
9. R. Tjian *et al.*, *Cell* **77**, 5 (1994).
10. T. Best *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **100**, 12063 (2003).
11. J. L. Stevens *et al.*, *Science* **296**, 755 (2002).

Appendix: attached

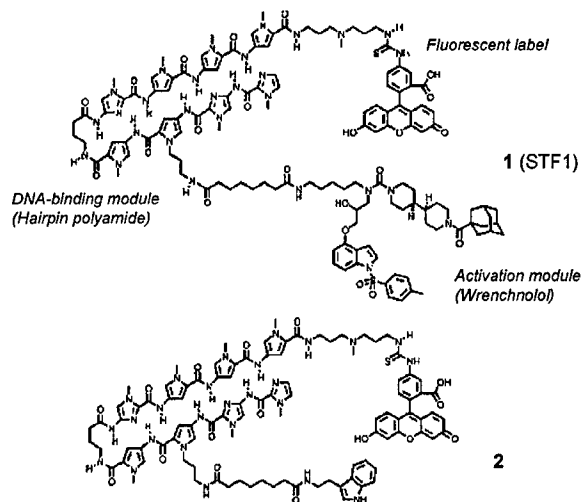
Small Molecule Transcription Factor Mimic

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Peter B. Dervan,^{*,§} and Motonari Uesugi^{*,†}*The Verna and Marrs McLean Department of Biochemistry and Molecular Biology, Baylor College of Medicine, Houston, Texas 77030, and Division of Chemistry and Chemical Engineering, California Institute of Technology, Pasadena, California 91125*

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Regulation of gene expression by transcription factors touches many aspects of eukaryotic biology, and its systematic, external control by organic molecules represents a challenge in chemistry.^{1,2} A number of attempts have been made to mimic transcription factors by synthetic molecules,^{3–9} including those utilizing DNA-binding hairpin polyamide attached to peptide activation domains.^{3,4} These studies indicate that it is possible to reproduce the gene-activation function of transcription factors by synthetic molecules. However, the presence of peptide regions in these molecules may limit their future applications. Here we report the design of a nonpeptidic small molecule that mimics a transcription factor. Naturally occurring transcription factors usually have two separable domains for activating selective genes: one for binding to specific promoters and the other for activating transcription through protein–protein interactions.^{10,11} Our designed molecule **1** (STF1: synthetic transcription factor 1) comprises two functional domains (Scheme 1): a hairpin polyamide molecule that binds specifically to 5'-TGACCAT sites in DNA¹² and a wrench-shaped synthetic compound (wrenchnolol) that binds to the Sur-2 protein,^{13,14} a subunit of human mediator complex that links transcription activators to RNA polymerase II in human cells.^{15,16}

Scheme 1



STF1 was synthesized by amide couplings of the hairpin polyamide, wrenchnolol, and a polyethylene linker. An internal pyrrole residue was chosen as an attachment site in the hairpin polyamide because previous studies had suggested that an extension at this position provides effective projection of an activation

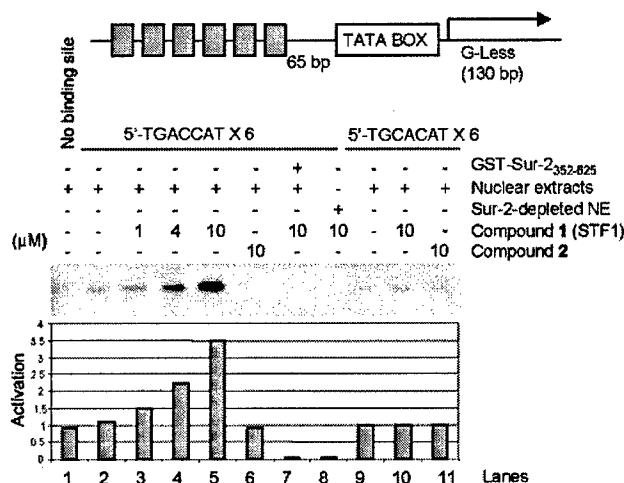


Figure 1. STF1 (**1**) activates transcription of a reporter gene in vitro. STF1 (0, 1, 4, or 10 μ M) was incubated with HeLa cell nuclear extracts and a reporter DNA construct in which a G-less reporter gene is controlled by six repeats of the hairpin-polyamide-binding site (5'-TGACCAT) or those of its mutant (5'-TGACAT). The 130-base mRNA of the reporter gene was detected by ³²P autoradiography and quantified by a phosphorimager. Addition of dominant negative Sur-2 protein fragment (GST-Sur-2₃₅₂₋₆₂₅) or immunodepletion of Sur-2 protein from nuclear extracts abolished the gene activation. It is evident that the reporter gene with mutated polyamide-binding sites is completely unresponsive to STF1 (**1**). Preparation of GST-Sur-2₃₅₂₋₆₂₅ and immunodepleted nuclear extracts is described in Supporting Information. A schematic diagram of the design of the reporter gene constructs is shown in the top. A gray square indicates a DNA sequence of the hairpin-polyamide-binding site.

module.^{4,5} The ability of STF1 to activate transcription was evaluated in vitro by using a reporter construct in which transcription of the reporter gene is controlled by six repeats of the hairpin-polyamide-binding sites (Figure 1). STF1 activated transcription of the reporter gene in a dose-dependent manner (lanes 2–5), whereas the control molecule **2** lacking the wrenchnolol moiety had no detectable activity (lane 6). Addition of excess amounts of a dominant negative Sur-2 protein fragment (lane 7) or immunodepletion of endogenous Sur-2 protein from nuclear extracts (lane 8) rendered the activity of STF1 undetectable, consistent with our previous observation that wrenchnolol binds selectively to Sur-2 protein. A reporter construct with point mutations in the hairpin-polyamide-binding sites was unresponsive to STF1 (lanes 9–11), suggesting a high degree of selectivity of STF1.

To verify the biochemical mechanism of the STF1-mediated gene activation, the promoter region of the reporter construct was labeled with a biotin molecule and bound to an avidin–agarose resin after incubation with nuclear extracts (Figure 2). Western blot analysis of the bound proteins showed that no detectable levels of Sur-2 or RNA polymerase II were recruited to the promoter in the absence

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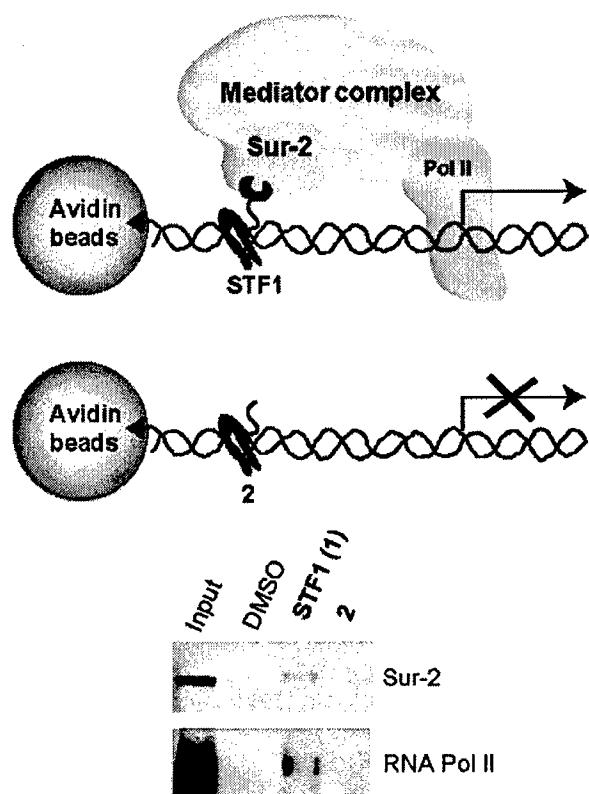


Figure 2. STF1 recruits Sur-2 and RNA polymerase II to the promoter. The promoter region of the reporter DNA construct was labeled by a biotin molecule and incubated with HeLa cell nuclear extracts in the presence of **1**, **2**, or solvent (DMSO) alone. The biotinylated DNA was recovered by using Neutravidin agarose, and the bound proteins were analyzed by Western blots with antibodies against Sur-2 and RNA polymerase II.

of STF1 or in the presence of the control molecule **2**. In the presence of STF1, in contrast, Sur-2 protein and RNA polymerase II were recruited to the promoter. These results support our notion that STF1 stimulates transcription by recruiting human mediator complex to the promoter through simultaneous contacts with Sur-2 and DNA.

Our results indicate that it is possible to generate a transcription factor out of nonpeptidic components.¹⁷ The fluorescein moiety of STF1 permitted evaluation of its cell permeability.¹² Unfortunately, STF1 had limited cell permeability, even though the hairpin-

polyamide-FITC conjugate and the wrenchnolol molecule are cell permeable as separate compounds.^{1,12,14,18} By decreasing the size of the molecule and optimizing its physical properties, it may be possible to increase its cell permeability for biological studies.

Acknowledgment. We thank H. Shimogawa and H. Kigoshi for supplying synthetic intermediates, Y. Kang for a plasmid construct, and J. H. Wilson for critical review of the manuscript. Supported by NIH Grants GM071800-01 (M.U.) and GM51747 (P.B.D.), Welch Foundation Grant Q-1490 (M.U.), and U.S. Army Grant DAMD17-02-1-0277 (Y.C.). Supported by DAAD postdoctoral fellowship (H.D.A.).

Supporting Information Available: Detailed experimental procedures. This material is available free of charge via the Internet at <http://pub.acs.org>.

References

- (1) Gottesfeld, J. M.; Neely, L.; Trauger, J. W.; Baird, E. E.; Dervan, P. B. *Nature* **1997**, *387*, 202–205.
- (2) For a review: Denison, C.; Kodadek, T. *Chem. Biol.* **1998**, *5*, R129–R145.
- (3) Mapp, A. K.; Ansari, A. Z.; Ptashne, M.; Dervan, P. B. *Proc. Natl. Acad. Sci. U.S.A.* **2000**, *97*, 3930–3935.
- (4) Ansari, A. Z.; Mapp, A. K.; Nguyen, D. H.; Dervan, P. B.; Ptashne, M. *Chem. Biol.* **2001**, *8*, 583–592.
- (5) Arora, P. S.; Ansari, A. Z.; Best, T. P.; Ptashne, M.; Dervan, P. B. *J. Am. Chem. Soc.* **2002**, *124*, 13067–13071.
- (6) Nyanguile, O.; Uesugi, M.; Austin, D. J.; Verdine, G. L. *Proc. Natl. Acad. Sci. U.S.A.* **1997**, *94*, 13402–13406.
- (7) Kuznetsova, S.; Ait-Si-Ali, S.; Nagibneva, I.; Troalen, F.; Le Villain, J. P.; Harel-Bellan, A.; Svinarchuk, F. *Nucleic Acids Res.* **1999**, *27*, 3995–4000.
- (8) Liu, B.; Han, Y.; Corey, D. R.; Kodadek, T. *J. Am. Chem. Soc.* **2002**, *124*, 1838–1839.
- (9) Liu, B.; Han, Y.; Ferdous, A.; Corey, D. R.; Kodadek, T. *Chem. Biol.* **2003**, *10*, 909–916.
- (10) Ptashne, M.; Gann, A. *Nature* **1997**, *386*, 569–577.
- (11) Tjian, R.; Maniatis, T. *Cell* **1994**, *77*, 5–8.
- (12) Best, T. P.; Edelson, B. S.; Nickols, N. G.; Dervan, P. B. *Proc. Natl. Acad. Sci. U.S.A.* **2003**, *100*, 12063–12068.
- (13) Asada, S.; Choi, Y.; Uesugi, M. *J. Am. Chem. Soc.* **2003**, *125*, 4992–4993.
- (14) Shimogawa, H.; Kwon, Y.; Mao, Q.; Kawazoe, Y.; Choi, Y.; Asada, S.; Kigoshi, H.; Uesugi, M. *J. Am. Chem. Soc.* **2004**, *126*, 3461–3471.
- (15) Stevens, J. L.; Cantin, G. T.; Wang, G.; Shevchenko, A.; Berk, A. J. *Science* **2002**, *296*, 755–758.
- (16) Asada, S.; Choi, Y.; Yamada, M.; Wang, S. C.; Hung, M. C.; Qin, J.; Uesugi, M. *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99*, 12747–12752.
- (17) Minter, A. R.; Brennan, B. B.; Mapp, A. K. *J. Am. Chem. Soc.* **2004**, *126*, 10504–10505.
- (18) Dudouet, B.; Burnett, R.; Dickinson, L. A.; Wood, M. R.; Melander, C.; Belitsky, J. M.; Edelson, B.; Wurtz, N.; Brichn, C.; Dervan, P. B.; Gottesfeld, J. M. *Chem. Biol.* **2003**, *10*, 859–867.

JA0445140