AD_____

Award Number: DAMD17-00-1-0274

TITLE: Structural-Based Design of ErbB-2 Selective Small Molecule Kinase Inhibitors

PRINCIPAL INVESTIGATOR: Shaomeng Wang, Ph.D.

CONTRACTING ORGANIZATION: Georgetown University Medical Center
Washington, DC 20057

REPORT DATE: July 2002

TYPE OF REPORT: Annual

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

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 074-0188

1. AGENCY USE ONLY (Leave blank)	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	July 2002	Annual (1 Jul 01 - 30 Jun 02)

4. TITLE AND SUBTITLE
Structural-Based Design of ErbB-2 Selective Small Molecule Kinase Inhibitors

5. FUNDING NUMBERS
DAMD17-00-1-0274

6. AUTHOR(S)
Shaomeng Wang, Ph.D.

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
Georgetown University Medical Center
Washington, DC 20057

E-Mail: shaomeng@umich.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)*
No abstract provided

14. SUBJECT TERMS
Breast cancer

15. NUMBER OF PAGES
10

16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
Unclassified	Unclassified	Unclassified	Unlimited

Table of Contents

Task 1.a. Further refinement of the erbB-2 and EGFR models

During the first year, this task has been successfully completed. A summary on this task has been included in the 2001 Annual Report submitted to the DOD. We have used three template protein structures all with high resolution to model the active conformations of erbB-2 and EGFR. These template protein structures include the structure of the kinase domain of the insulin receptor kinase bound to an ATP analog and a peptide substrate, which was determined with X-ray crystallography to an accuracy of 1.9 Å (PDB code: 1IR3), the kinase domain of the human FGFR1, either alone or in the complex with a small molecule kinase inhibitor SU4984, or SU5402, or PD173074, whose structures were determined with X-ray crystallography to an accuracy of from 2.0 to 2.5 Å (PDB codes: 1FGK, 1AGW, 1FGI and 2FGI), and the structure of the human tyrosine protein C (SRC), whose structure was determined with X-ray crystallography to an accuracy of 1.5 Å (PDB code: 1FMK). These proteins share the highest degree of homology with erbB-2 and EGFR in their kinase domains among all the structures in the Protein Databank (PDB). We have then performed extensive refinement of these modeled structures of erbB-2 and EGFR through molecular dynamics simulations using the CHARMM program and its latest version of the force field in explicit water environment.

Task 1.b. Performance of structure-based database searching on both the ACD and NCI 3D-database to identify potential kinase inhibitors

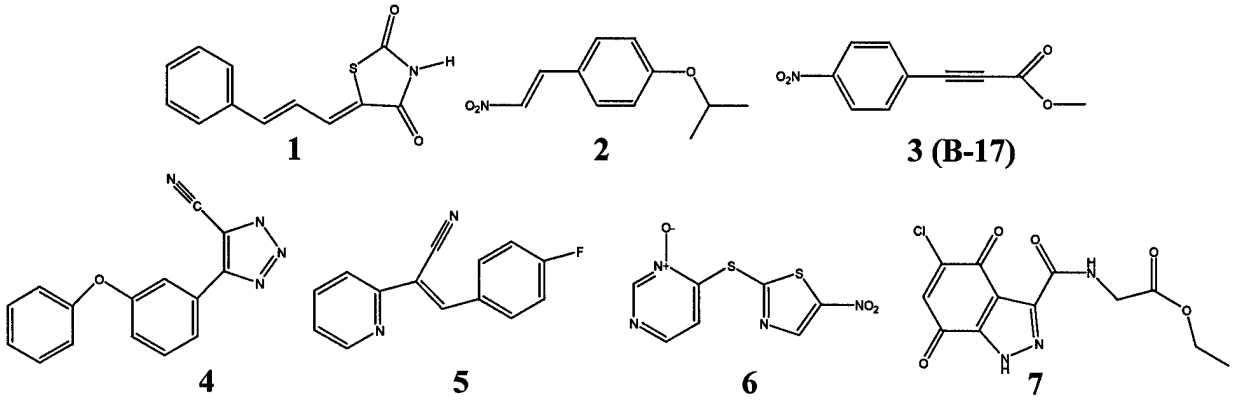
During the first two years, this task has been successfully accomplished. Using our extensively refined erbB-2 three-dimensional (3D) structure, we have performed structure-based 3D-database searching over two 3D-structural databases containing approximately 500,000 compounds we have built in our laboratories. These include the National Cancer Institute's 3D-database of 250,00 structurally diverse synthetic compounds and natural products and the ACD (Available Chemical Directory)'s 3D-database of 275,000 compounds.

We identified the top 2000 compounds with most favorable docking score as potential erbB-2 inhibitors that bind to the ATP binding site in erbB-2. We then identified 200 compounds that are structurally diverse, have small molecular weight (<800) and simple chemical structures as most promising candidates for subsequent biochemical and biological testing and confirmation for their activity.

Using a panel of biochemical and cell-based assays, we have confirmed 7 different chemical classes as quite potent erbB-2 small molecule inhibitors with selectivity against EGFR (see summary of data in **Task**

2). The chemical structures of these lead compounds are provided in Chart I. The summary of their activity is provided in Table 1.

Char I. Chemucal Structures of novel erbB-2 small molecule inhibitors discovered from structure-based 3D-database searching.



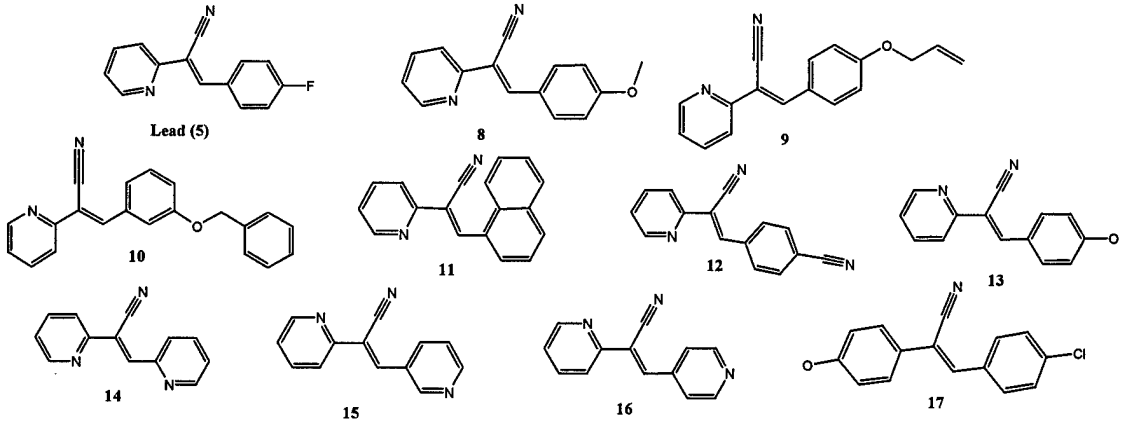
Task 1.c, Task 1.d and Task 2. Optimization of the most promising lead compounds to further improve their potency and computational docking studies of lead compounds to erbB-2 and biochemical and biological testing of new analogues **(1-24 months)**

Based upon these lead compounds, we have performed further optimization aiming at further improving their activity. To date, we have focused on optimization of two promising lead compounds **5** and **3 (B-17).**

Identification and Biological Evaluation of New Analogues of Lead Compound 5

A number of analogs (**8-17**) of lead **5** were identified by structure searching of its analogues in the NCI

Chart 2. Chemical structures of new analoges of lead compound **5.**



and in the ACD databases. These new analogues were evaluated for their ability in inhibition of erbB-2 phosphorylation in MDA-453 cell line. Analogs **8** and **9** were found to be more active than **5** in inhibition of

erbB-2 phosphorylation, while other compounds (**10-17**) are substantially less active than the lead. As compared to **5**, **8** and **9** have a methoxy or an allyloxy group in the para position.

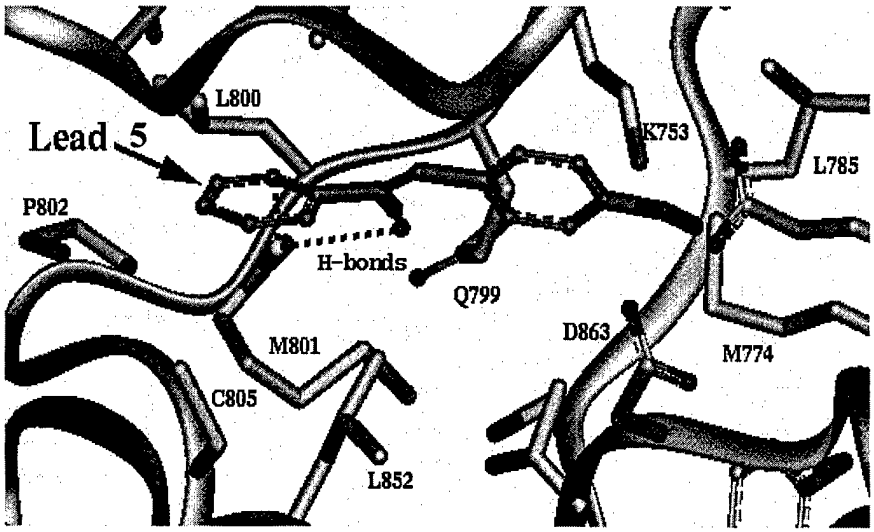


Figure 1. The predicted binding model of lead **5**. The lead interacts with a number of hydrophobic residues (M774, M801, L852, L800) and forms 2 H-bonds with backbone amide group of M801 in erbB-2.

We have further performed extensive computational docking of lead compound **5** to gain an insight on its interaction with erbB-2 using program AutoDock, followed by extensive molecular dynamics simulation. The predicted binding model is shown in Figure 1. Our predicted binding model shows that lead compound 5 forms two specific hydrogen bonds with Met 801 in erbB-2 and has several hydrophobic contacts with several hydrophobic residues Met 774, Leu 800 Met 801 and L852.

Our predicted binding model for **5** showed that the 4-fluorine group in **5** interacts with M774 in erbB-2. There is enough room available to accommodate a larger group and a larger hydrophobic group should enhance the hydrophobic interactions with M774. The improved activity of **8** and **9** appears to confirm our predicted model for the lead **5**. However, the space available in this region is quite limited and a too bulky group is prohibited. The decreased activity of **10** and **11** appeared to confirm this. Furthermore, a hydrophilic group at the para position would have detrimental effect to the activity due to decreased hydrophobic interactions and the decreased activity of **12** and **13** seems in support the prediction. To test the influence of the electronic factor, we have evaluated **14**, **15** and **16**. While **14** is equally potent as the lead compound, **15** and **16** are much less potent than the lead, suggesting that the location of the nitrogen in the pyridine ring in these compounds play an important role for their activity. Compound **17** is more potent than

the lead, suggesting that a pyridine ring in the lead may be replaced by a substituted phenyl group, such as hydroxyl phenyl. In summary, our preliminary SAR studies of the lead compound **5** have identified 2 more potent analogs and the data are in good agreement with our predicted model for lead **5**.

Therefore, in addition to provide an insight into the interaction of lead compound **5** with erbB-2, our predicted binding model provides a structural basis for the design of new analogues to further improve its activity.

Design, Synthesis and Biological Evaluation of New Analogues of Lead Compound 5

B-17 appears to be the most potent and selective erbB-2 inhibitor among these novel lead compounds we have discovered. Therefore, we have focused our optimization efforts on this class of compounds through computational design and chemical synthesis of new compounds. Although the chemical synthesis was not budgeted in the original proposal, we felt that it was essential to achieve our ultimate goal in designing a potent and selective erbB-2 small molecule inhibitor as a potential new therapy for breast cancer treatment.

To date, we have designed and synthesized over 20 new analogues of lead compound **3** (**B-17**). The results are summarized in Table 1 and Figure 1. The anti-proliferation activity of a drug was measured by treatment of MDA-453 human breast cancer cells for 5 days. MDA-453 human breast cancer cells have high erbB-2 expression. Based upon our data, it was found that the position of the nitro group on the phenyl ring is important. New analog **B17-A28-1** with a nitro group in the *meta* position is at least 20-times less potent than **B17** with a nitro group in the *para* position. As predicted based upon the modeled structure of **B17** in complex with erbB-2, additional hydrophobic interactions can be achieved by another fused aromatic ring to the phenyl ring in **B17**, which may lead to an improvement in potency. Indeed, **B17-A82** is 3-times more potent than **B17**. In fact, **B17-A82** is a very potent inhibitor with an anti-proliferative activity in the sub-micromolar range (IC_{50} = 350 nM). Thus, design and synthesis of new analogs of **B17** have lead to the identification of a potent analog **B17-A82** with an IC_{50} value of 350 nM in inhibition of cancer cell growth in human breast cancer cell line MDA-MB-453 with erbB-2 everexpression.

Table 1. Anti-proliferative activity of **B17** and new analogs in human breast cancer cell line MDA-453 with erbB-2 overexpression.

	Chemical Structure	**IC_{50} (μM)**

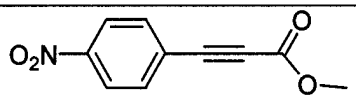
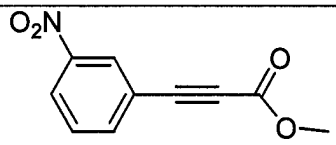
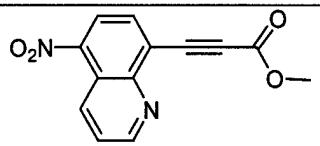
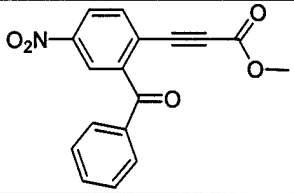
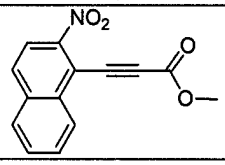
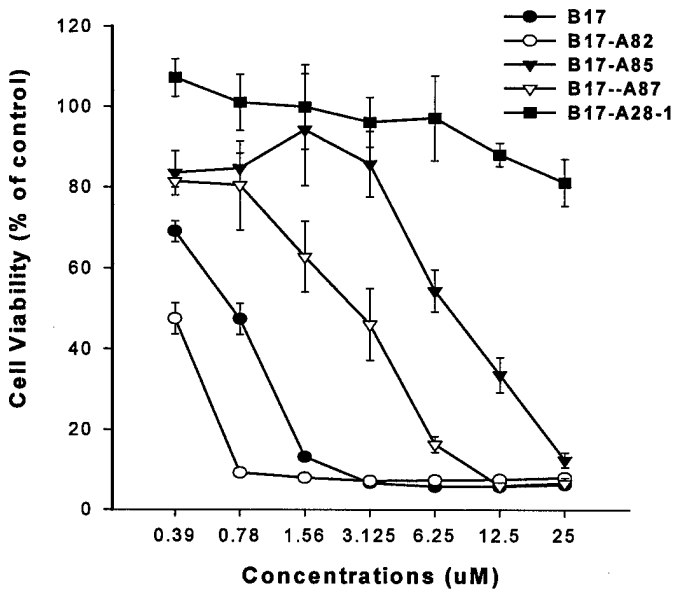
B17		**1.2**
B17-A28-1		**>25**
B17-A82		**0.35**
B17-A87		**3.5**
B17-A85		**8.0**

Figure 2. Anti-proliferation activity of **B-17** and new analogs in human breast cancer cell MDA-453 with erbB-2 everexpression.



Task 3. *In vivo* testing of most promising lead compounds **(24-36 months)**

In our original proposal, we have planned to conduct *in vivo* studies of antitumor activity of most promising erbB-2 small molecule inhibitors in the third year. However, due to rapid progress we have made in discovery of promising lead compounds, we have begun our first *in vivo* experiment in year 2, ahead of our original schedule.

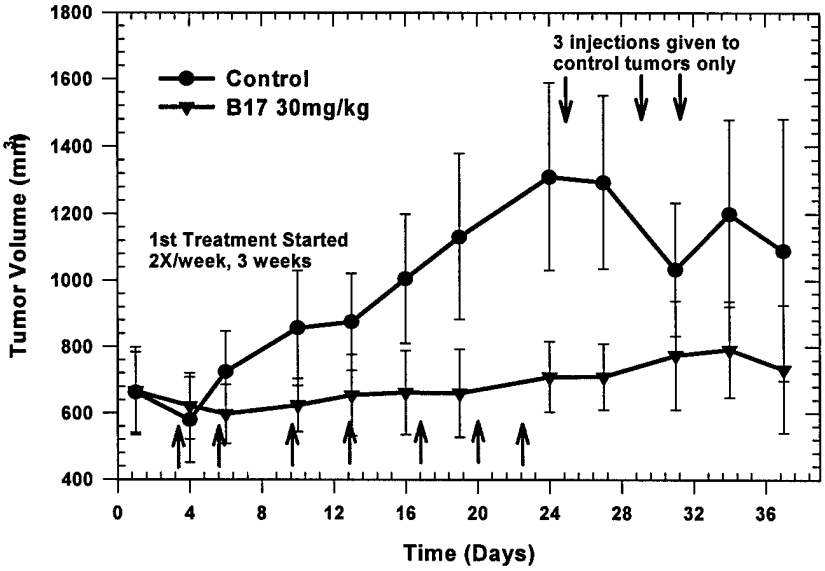


Figure 3. Antitumor activity of **B-17** in BT-474/M1 xenograft model of human breast cancer in nude mice.

B-17 has a potent activity in inhibition of erbB-2 kinase activity and in inhibition of cell proliferation in human breast cancer cells with a high level of erbB-2 kinase activity. Furthermore, **B-17** also displays more than 100-fold selectivity in human breast cancer cells with a high level of EGFR kinase activity but a low level of erbB-2 kinase activity. Therefore, **B-17** appears to be a promising quite potent and selective erbB-2 inhibitor. Thus, we have evaluated the anti-tumor activity of **B-17** *in vivo* using BT-474/M1 xenograft model. BT-474/M1 is a human breast cancer cell line with a high level of erbB-2 kinase activity. Tumors were allowed to grow to approximately 600 mm^3 in volume before the treatment started with a dose schedule of 30 mg/kg twice weekly. As shown in **Figure 3**, tumor growth was inhibited over 90% while tumors in untreated mice continuously grown to a size of more than 1200 mm^3 in volume in 3 weeks. To test whether **B-17** can inhibit the growth of very large tumors, we began to treat mice bearing large tumors

in the previously untreated group after 3 weeks. After only 3 treatment of 30 mg/kg, not only had the tumors stopped the growth, but also those very large tumors begun to show appreciable reduction in tumor volume. Statistically analysis showed that the inhibition of tumor growth in the treated group of mice is highly statistically significant ($p < 0.01$). Furthermore, **B17** had no effects on the tumor growth using A431 xenografts model, which In has a high level of EGFR kinase activity. It is noted that none of the mice showed weight loss or any other overt signs of toxicity at the dose regime used.

Therefore our *in vivo* antitumor activity data demonstrated that inhibition of the erbB-2 kinase activity using a potent and erbB-2 specific small molecule kinase inhibitor has the great potential for the treatment of cancers overexpressed erbB-2.

Project Plan for Year 3.

- We will continue to perform further structure-based design and optimization based upon promising lead compounds.

- Our first *in vivo* experiment showed that **B-17** has a potent antitumor activity in human breast cancer in anima model of human breast cancer. In year 3, we will perform more extensive experiments to evaluate the antitumor activity of most promising small molecule inhibitors of erbB-2 using more than one xenograft model of human breast cancer (such as BT-474/M1 and MDA-316) (**Task 3**).

- One manuscript on the discovery and *in vitro* studies of potent and selective erbB-2 inhibitor is virtually completed and will be submitted within a month. A second manuscript more structure-based design and optimization, in vitro and in vivo studies is currently in preparation. Preparation of manuscripts for scientific publications is a major task in year 3.