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論　文　名	「Molecular Design of Small Organic Molecules as Mimics for Biologically Active Peptides with α-Helical Conformation（α -ヘリックス構造をもつ生理活性ペプチドに関する低分子ミミック化合物の分子設計)」
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論文要旨

Chapter 1: Introduction

In the fields of medicine, biotechnology and pharmacology, **Drug Discovery** is the process by which drugs are discovered and/or designed.

The first step in designing new ligands is to discover a lead compound which is a chemical compound that has pharmacological or biological activity and whose chemical structure is used as a starting point for chemical modifications in order to improve potency, selectivity, and/or pharmacokinetic parameters. Despite the advances in technology and understanding of biological systems, development of new strategies for the design and synthesis of small organic compounds that targeting the interfaces of protein-protein interactions is still challenging. Thus, the development of new strategies for the rapid design and synthesis of such ligands (lead compounds) is essential to analyze and control the biological processes of each protein.

Therefore, the aim of this work is to use the directed evolution and the structure based drug design for development of a new strategy for the design of small organic compounds that target the protein-protein interactions using Granulocyte-Colony Stimulation Factor (**G-CSF**) receptor as a model protein. G-CSF is one of the important cytokines that stimulates the bone marrow to release granulocytes and stem cells into the blood. The interaction of G-CSF with its receptor stimulates the survival, proliferation, differentiation, and function of neutrophil precursors and mature neutrophils. So ligands, which can inhibit the interaction between G-CSF and its receptor, would be interested as cytotoxic agents for treatment of cancer.

Chapter 2: Molecular Design and Synthesis of the Target Ligands

In this work, a new strategy for the ligand design has been developed which uses conformationally constrained peptide libraries. Screening of the libraries provides rigidly folding peptides that have high binding affinity to the target proteins. In addition, the rigid structure of the selected peptide provides the information about the spatial orientation of the pharmacophore which facilitate the structure based design of the

peptidomimetics.

Our group has examined the directed evolution of peptides in a phage-displayed library and has successfully synthesized a de novo designed **helix-loop-helix** peptide consisting of 35 amino acids. Previously, screening of the peptide library has isolated a peptide, named **P8-2KA**, which can specifically bind to G-CSF receptor.

2-1. Design of tri-Substituted Ligands

The structure modeling and alanine scanning have shown that amino acids leu^{28}, lys^{29} and glu^{32}or Arg^{35} are essential for the receptor binding and the biological activity and based on information of the pharmacophore several small molecule peptidomimetics have been designed (Figure 1).

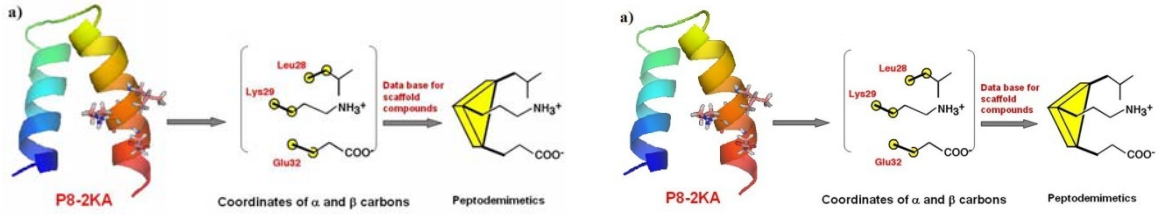
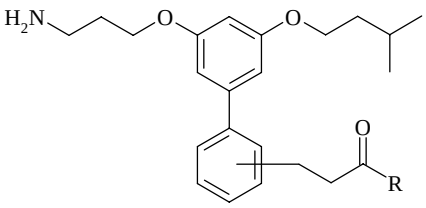


Figure 1: Structure based design from active peptide to peptidomimetic scaffold. a) Design based on amino acids Leu^{28}, Lys^{29} and Glu^{32}. b) Design based on amino acids Leu^{28}, Lys^{29} and Arg^{35}.

2-2. Synthesis and Evaluation of tri-Substituted Ligands

Literature survey revealed that the biphenyl framework could act as an effective scaffold for the design of small ligands for G-CSF receptor. Therefore, I designed and synthesized tri-substituted biphenyl ligands **I-VI** with three residues, which simulate the side chains of the effective amino acids of **P8-2KA**, two of them are at 3- and 5- positions and the third residue at variable position of the other benzene ring at 2`-, 3`- or 4` positions.



Compound	Position	R	Compound	Position	R
I	2`-	OH	**IV**	3`-	NH_2
II	2`-	NH_2	**V**	4`-	OH
III	3`-	OH	**VI**	4`-	NH_2

After synthesis and confirmation of the structures using the different spectral analyses, the binding affinity toward the G-CSF receptor using the **SPR Biacore** technology has been evaluated and pleasantly, compound **IV** could bind to G-CSF receptor with a *Kd* around 110 μM and this result was supported by PyMol Modeling studies (Figure 2).

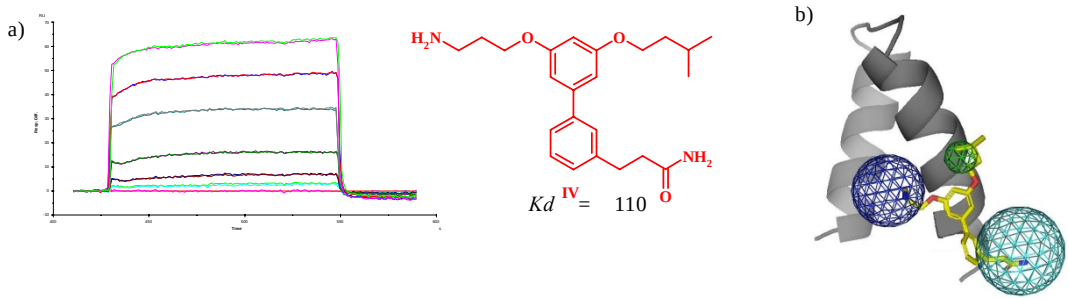
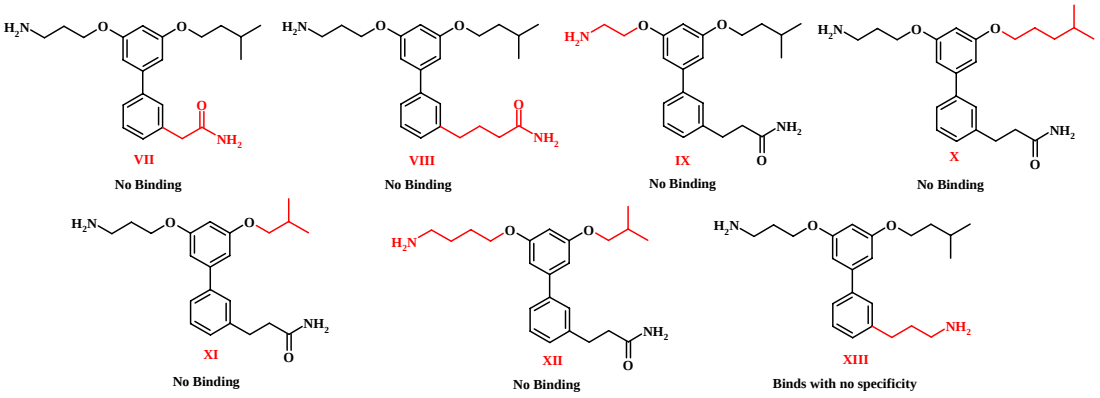
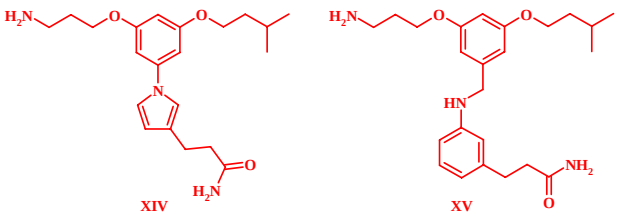


Figure 2: a) SPR sensorgram of the **IV**. b) Superimposed model of compound **IV** and **P8-2KA**.

Unfortunately, all changes applied to optimize the activity of ligand **IV** resulted in disappearance of the activity except only when the amide side chain was changed to amine side chain, the resultant compound **XIII** could bind to the receptor but with no specificity.



Although changing the biphenyl scaffold with the phenyl-pyrrol scaffold resulted in inactive compound, **XIV**, the *N*-benzyl aniline scaffold gives active compound **XV** with a *Kd* around 370 μM.



2-3. Design of tetra-Substituted Ligands

Motivated by the studies that showed the importance of amino acids Leu[28], Lys[29], Glu[32] and/or Arg[35] to receptor binding and the biological activity, I decided to design and synthesize peptidomimetics that contain four substituents simulating the side chains of these important amino acids (Figure 3). Thus compound **XVI** was designed by addition of a fourth substituent that simulates Glu[32] to the active compound **IV**. As expected the activity was increased more than five folds as the *Kd* was around 20 μM (Figure 3).

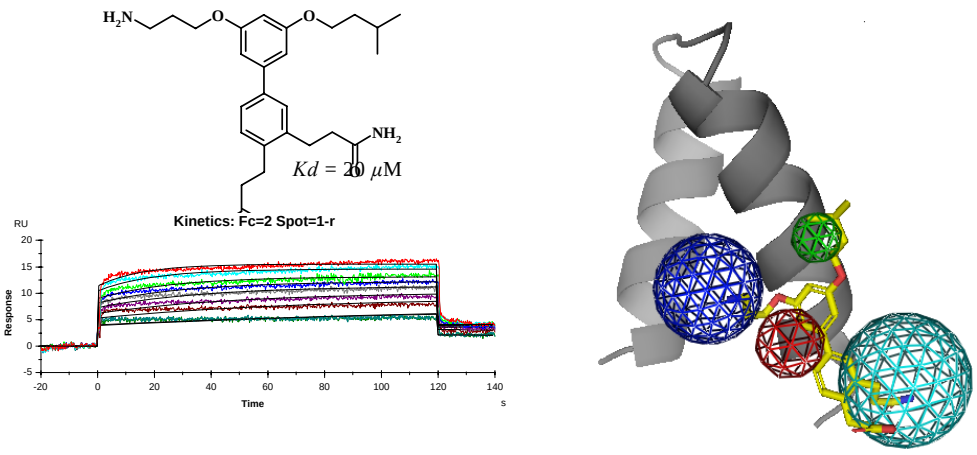
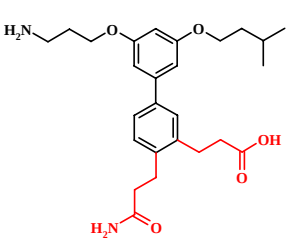


Figure 3: a) SPR sensorgram of the **XVI**. b) Superimposed model of compound **XVI** and **P8-2KA**.

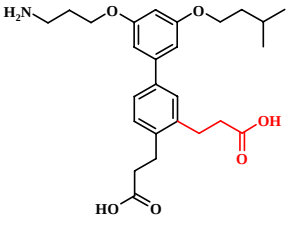
2-4. Synthesis and Evaluation of tetra-Substituted Ligands

Once more, all modifications applied to compound **XVI** for optimizing the activity resulted in inactive compounds **XVII-XX**



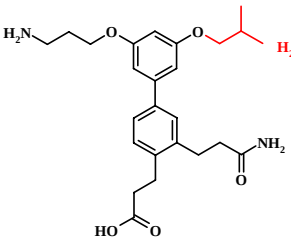
XVII

No Binding



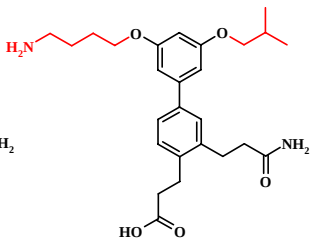
XVIII

No Binding



XIX

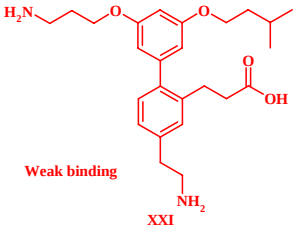
No Binding



XX

No Binding

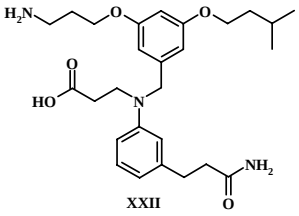
In addition, changing the position of the four substituents was interested and **XXI** was chosen as next target, but it showed a weak binding towards G-CSF receptor.



Weak binding

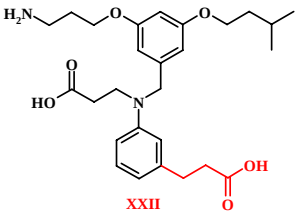
XXI

Next, the addition of the fourth chain to the *N*-benzyl aniline scaffold afforded compound **XXII** which binds to the receptor with *Kd* = 47 μM, however, modifications applied to compound **XXII** gave inactive compounds **XXIII-XXV**.



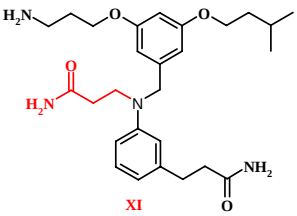
XXII

Kd = 47 μM



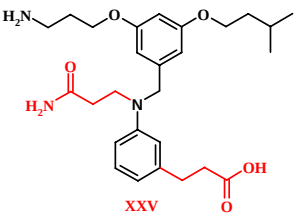
XXII

No Binding



XI

No Binding



XXV

No Binding

Chapter 3: Binding Affinity

The binding affinities of compounds **I-XXVII** were evaluated by SPR with BIAcore S51 apparatus and, in conclusion, we were able to apply a novel methodology to design and synthesize small molecules binding to G-CSF receptor based on conformationally constrained helix-loop-helix peptide libraries. The biphenyl scaffold shows better binding affinity than the *N*-benzyl aniline scaffold and, in the both scaffolds, the tetra-substituted ligands showed better binding affinity to the receptor than the tri-substituted ligands.

審査結果の要旨

藤井研究室は,ファージ表層ディスプレイ技術と有機合成化学を組み合わせることにより，従来のとは異なった独創的な新しいリード化合物設計法を提案した。この方法は２つのステップから構成される。第１ステップでは，立体構造モチーフ（α-ヘリックス）をもつペプチドのファージ表層提示ライブラリーを構築し,標的タンパク質に作用するペプチドをスクリーニングする。このライブラリーから得られるペプチドは，強固な立体構造を持っているのでファーマコフォアとその空間配置を容易に決定することができる。そこで,第２ステップでは,ペプチドから得られた立体構造情報をもとに低分子リード化合物を設計する。申請者は，この第２ステップを担当し，顆粒球コロニ−刺激因子（G-CSF）受容体に結合するα−ヘリックス構造ペプチドから受容体結合性低分子化合物の創出に成功した。

G-CSF 受容体結合α−ヘリックスペプチドでは，i, i + 2, i + 4, i + 5 の位置の４つのアミノ酸が受容体結合に関与する。そこで，申請者は，これらのアミノ酸の空間配置を再現できる低分子化合物として，２つのビフェニル誘導体を設計した。これらの分子を土台として対応するアミノ酸の官能基を導入し，受容体結合低分子の設計に成功した。また，官能基変換による構造活性相関を検討し，設計の妥当性を証明した。

以上のように，申請者は，ファージ表層ディスプレイ技術と有機合成化学を組み合わせることにより，新しいリード化合物設計法を開発した。本法では，手間のかかる標的タンパク質の立体構造解析や高価な低分子ライブラリーを必要としないので，迅速かつ低コストのリード化合物探索が達成される。顕著な新規性と独創性があり，また，本人自身が分子設計，有機合成，受容体結合実験までのすべてを手がけており，申請者を博士学位に値する能力をもつものと判断する。したがって，本学位論文審査委員会は、当該論文の審査ならびに最終試験の結果に基づき、申請者に対して博士（理学）の学位を授与することが適当であると結論した。