



PHARMACOPHORE MODELING STUDIES ON KNOWN MMP-9 ENZYME INHIBITORS TO IDENTIFY THE IMPORTANT COMMON FEATURES

ÖNEMLİ ORTAK ÖZELLİKLERİ TANIMLAMAK AMACIYLA, BİLİNEN MMP-9 ENZİM
İNİHİTÖRLERİ ÜZERİNDE YAPILAN FARMAKOFOR MODELLEME ÇALIŞMALARI

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ABSTRACT

Objective: *In this study, pharmacophore models were generated to explain the structure–activity relationships by using the known MMP-9 inhibitors.*

Material and Method: *Pharmacophore models were generated to explain the specification of the structure–activity relationships of common pharmacophoric sites of the known MMP-9 inhibitors. For this study Discovery Studio 3.5 software was used. A set of known MMP-9 inhibitors (NFH, Batimastat, Marimastat, Prinomastat, CGS-27023A, and Ro32-3555) were used for common feature pharmacophore generation method. Selected hypothesis included two hydrogen bond acceptor, one hydrogen bond donor, and one hydrophobic feature.*

Result and Discussion: *All of the tested inhibitors except CGS-27023A and Ro32-3555 fitted the selected pharmacophore model perfectly. These two inhibitors did not fit the A2 feature. It can be concluded that A1, D1, and H1 features at the given positions could be necessary for the activity. Additionally, we compared the pharmacophore model with NFH and MMP-9 enzyme complex to identify the important interactions. At the given positions of all of the pharmacophoric features, there is an interaction with the protein. This is also supported our pharmacophore hypothesis. As a result, this pharmacophore model could be useful to design new small molecule inhibitors of MMP-9 enzyme.*

Keywords: *cancer, inflammatory diseases, MMP-9, pharmacophore modeling, structure–activity relationships*

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ÖZ

Amaç: Bu çalışmada, yapı-aktivite ilişkilerini açıklamak amacıyla bilinen MMP-9 inhibitörleri üzerinden farmakofor modeller oluşturuldu.

Gereç ve Yöntem: Bilinen MMP-9 inhibitörlerinin ortak farmakoforik özellikleri belirlenerek, yapı-aktivite ilişkilerini açıklamak üzere farmakofor modeller oluşturuldu. Bu çalışma için Discovery Studio 3.5 programı kullanıldı. Bilinen MMP-9 inhibitörleri (NFH, Batimastat, Marimastat, Prinomastat, CGS-27023A ve Ro32-3555), ortak farmakofor özelliklerin belirlenmesinde kullanıldı. İki hidrojen bağı alıcısı, bir hidrojen bağı donörü ve bir hidrofobik özellik içeren hipotez seçildi.

Sonuç ve Tartışma: CGS-27023A ve Ro32-3555 dışındaki test edilen tüm inhibitörler, seçilen farmakofor modele mükemmel bir şekilde uydu. Bu iki inhibitör A2 özelliğine uymadı. Buna göre, etki için verilen konumlardaki A1, D1 ve H1 özelliklerinin gerekli olabileceği sonucuna varıldı. Ayrıca, önemli etkileşimleri tanımlamak için farmakofor model, NFH ve MMP-9 enzim kompleksi ile karşılaştırıldı. Tüm farmakoforik özelliklerin verilen pozisyonlarda, protein ile bir etkileşim gözlemlendi. Bu durum farmakofor hipotezimizi de desteklemektedir. Sonuç olarak, bu farmakofor model, MMP-9 enziminin yeni küçük molekül inhibitörlerini tasarlamak için yararlı olabilir.

Anahtar Kelimeler: enflamatuar hastalıklar, farmakofor modelleme, kanser, MMP-9, yapı-etki ilişkileri

INTRODUCTION

The matrix metalloproteinases (MMPs) are a family of zinc-containing enzymes, which are structurally related to endopeptidases that mediate the impairment of connective tissue macromolecules. Because of their central role in re-modelling connective tissue, both as part of normal physiological growth and repair, and as part of disease processes, there is quite a lot interest in these proteins as many targets, in a wide range of inflammatory and degenerative diseases, such as atherosclerosis and arthritis, and also in cancer [1-4]. Over many years in the pathogenesis of cancer and arthritis the importance of the MMP family has been determined. But it is only relatively recently that MMPs and their expressed by lipid-laden macrophages. The proofs from histological investigations and molecular genetic studies implicates that over-expression of MMP9 in the vascular re-modelling events preceding plaque rupture, acute myocardial infarction are the most common causes [5]. More recently, animal studies have shown that a reduction of MMP9 activity, either by through pharmacological intervention or genetic manipulation, has an impact on ventricular re-modelling following infarction. Thus, in the pathogenesis of heart, MMP9 activity could be a key mechanism failure [6].

Many small molecule inhibitors of MMP-9 which effectively treat cancer and arthritis have been studied in human. Figure 1 shows five MMP-9 inhibitors that have reached clinical trials (Batimastat, Marimastat, Prinomastat, Ro32-3555 and CG-S27023A) and another known inhibitor (NFH) which has a crystal structure, complex with the MMP-9 enzyme [7-12].

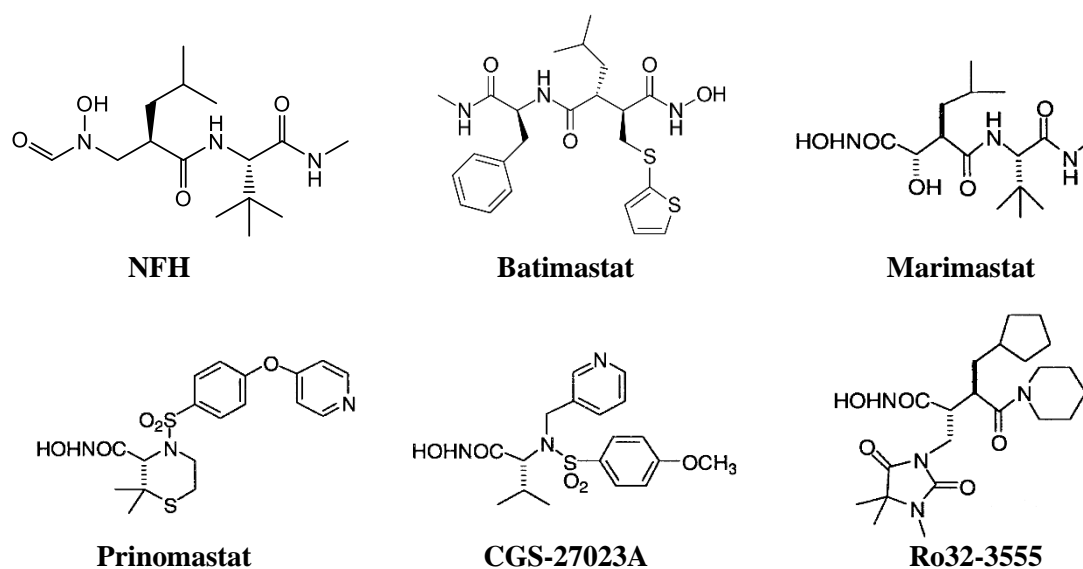


Figure 1. Structures of known MMP-9 inhibitors

In an earlier study, the crystal structure of the catalytic domain of human MMP-9 enzyme with peptidic reverse hydroxamate inhibitor (NFH) complex was determined. In the centre of catalytic there is an active-site zinc ion which co-ordinated by an essential glutamic acid residue (Glu402) and three histidine residues (His401, His405 and His411) (Figure 2) [7].

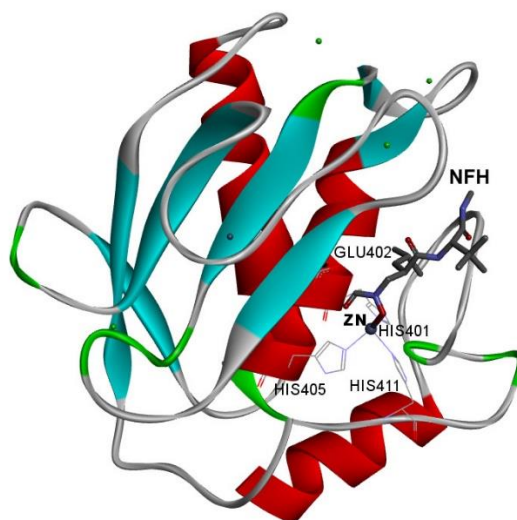


Figure 2. Crystal structure of the catalytic domain of human MMP-9 enzyme with peptidic reverse hydroxamate inhibitor (NFH) complex (PDB ID: 1GKC).

Herein, pharmacophore models were generated to explain the structure–activity relationships by using the known MMP-9 inhibitors. Then we compared the pharmacophore model with NFH and MMP-9 enzyme complex (Figure 2) to identify the important interactions.

MATERIAL AND METHOD

In this study we used common feature pharmacophore generation method to explain the specification of the structure–activity relationships of pharmacophoric sites of the known MMP-9 inhibitors. We used Discovery Studio 3.5 software for built the compounds and generated the standard 3D structures. The geometry of the inhibitors was optimized by using ABNR Minimization Method and for each inhibitor, conformational models were automatically generated. The “best conformer generation” procedure was applied to provide the best conformational coverage for a maximum number of conformers generated, defaulted to 255 in a 0–20 kcal/mol range from the global minimum. The generated conformations were used to align common molecular features and generate the pharmacophore hypotheses [13-15].

A set of known MMP-9 inhibitors (NFH, Batimastat, Marimastat, Prinomastat, CGS-27023A, and Ro32-3555) shown in Figure 1 were selected as the training set for use common feature pharmacophore generation method. Then 10 pharmacophoric hypotheses were generated from these aligned inhibitors. We selected the hypothesis which have two hydrogen bond acceptor (A1 and A2), one hydrogen bond donor (D1), and one hydrophobic (H1) features (Figure 3a).

RESULT AND DISCUSSION

Selected pharmacophore hypothesis included two hydrogen bond acceptor (A1 and A2), one hydrogen bond donor (D1), and one hydrophobic (H1) features. The hypothesis and mapping of all of the inhibitors are shown in Figure 3. All of the tested inhibitors except CGS-27023A and Ro32-3555 fitted the pharmacophore model perfectly (Figure 3 and Figure 4). According to the mapping of CGS-27023A, and Ro32-3555, these compounds fitted the three features of the model (A1, D1, and H1) but did not fit the A2 feature. Fit values of the tested inhibitors are shown in Table 3. It can be concluded that A1, D1, and H1 features at the given positions could be necessary for the activity.

Table 1. Fit values of the tested inhibitors

Inhibitor Name	Fit value
Batimastat	3,99931
NFH	3,78265
Marimastat	3,56112
Prinomastat	3,44325
CGS-27023A	2,98623
Ro32-3555	2,95924

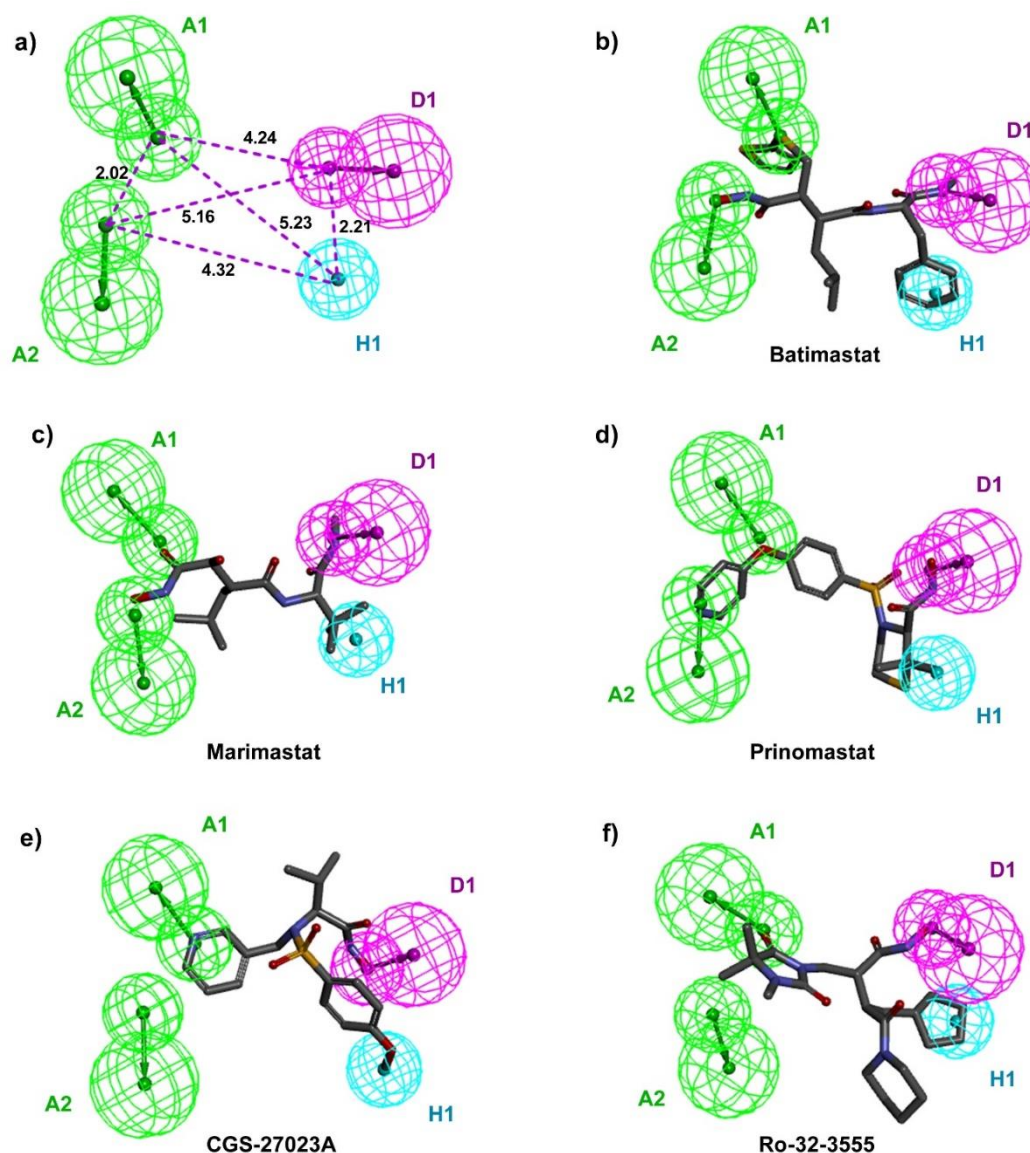


Figure 3. a) The selected pharmacophore model and distances between the features. b) Mapping of Batimastat to pharmacophore model. c) Mapping of Marimastat to pharmacophore model. d) Mapping of Prinomastat to pharmacophore model. e) Mapping of CGS-27023A to pharmacophore model. f) Mapping of Ro32-3555 to pharmacophore model.

Additionally we compared this pharmacophore model with the X-ray crystal structure of MMP-9 and NFH complex (PDB ID: 1GKC) (Figure 4). At the given positions of all of the pharmacophoric features, there is an interaction with the protein. At the A1 position there is metal interaction with Zn ion, at the A2 position there is a metal interaction with Zn ion and H bond with Glu402, at the D1 position there is a H bond with Gly186 and at the H1 position there is hydrophobic interactions with His401 and Tyr423. All of these are also supported our pharmacophore hypothesis. It is reported that interaction with the Zn ion is necessary for the activity. And according to our hypothesis at the given

positions of A1 and A2 features, there can be seen interactions with Zn ion. So, it can be concluded that for interaction with Zn ion, the A1 and A2 features, especially A1 could be very important for the activity. As a result, this pharmacophore model could be useful to design new small molecule inhibitors of MMP-9 enzyme.

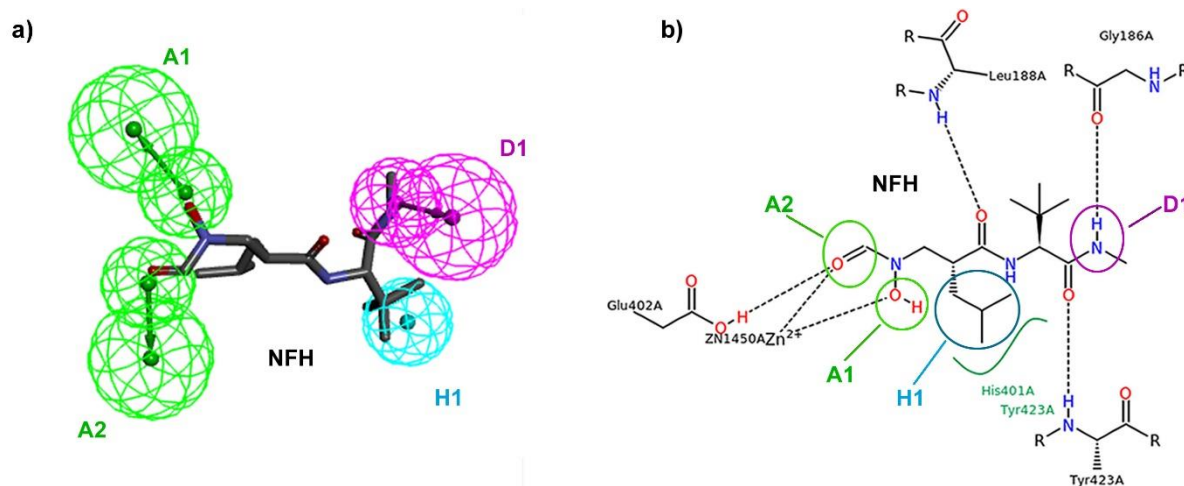


Figure 4. a) Pharmacophore mapping to the NFH b) Interactions between NFH and MMP-9 enzyme from X-ray crystal complex (PDB ID 1GKC).

CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

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