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# Discovery of potent inhibitor for matrix metalloproteinase-9 by pharmacophore based modeling and dynamics simulation studies

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ABSTRACT

Matrix metalloproteinase-9 (MMP-9) is an attractive target for anticancer therapy. In the present study ligand based pharmacophore modeling was performed to elucidate the structural elements for a diverse class of MMP-9 inhibitors. The pharmacophore model was validated through Güner-Henry (GH) scoring method. The final pharmacophore model consisted of three hydrogen bond acceptors (HBA), and two ring aromatic regions (RA). This model was utilized to screen the natural compound database to seek novel compounds as MMP-9 inhibitors. The identified hits were validated using molecular docking and molecular dynamics simulation studies. Finally, one compound named Hinokiflavone from Juniperus communis had high binding free energy of −26.54 kJ/mol compared with the known inhibitors of MMP-9. Cytotoxicity for hinokiflavone was evaluated by MTT assay. Inhibition of MMP-9 in the presence of hinokiflavone was detected by gelatin zymography and gelatinolytic inhibition assay. Results revealed that the natural compounds derived based on the developed pharmacophore model would be useful for further design and development of MMP-9 inhibitors.



## 1. Introduction

Matrix metalloproteinases (MMPs) are the family of zinc-dependent neutral endopeptidases that are collectively capable of degrading essentially all the components of the extracellular matrix [1–4]. They are classified as collagenases, gelatinases, stromelysins and matrilysin according to their substrate specificity [5,6]. They constitute the main group of proteolytic enzymes that are involved in the tumor invasion, metastasis, and angiogenesis [7,8]. Among all, matrix metalloproteinase-9 (MMP-9) is particularly involved in inflammatory processes, bone remodeling and wound healing. It is also implicated in pathological processes such as rheumatoid arthritis, atherosclerosis, tumor growth, and metastasis [9–11]. Difference in production levels of regulatory mechanisms of MMP-9 subsequently results in restricted, extensive, or improperly timed degradation of extracellular matrices [12]. The role of MMP-9 in many pathological diseases has laid a foundation for the identification of selective inhibitors.

The structure of the MMPs includes a signal peptide, a propeptide, a catalytic domain with a highly conserved zinc-binding site, and a haemopexin-like domain, which is linked by a hinge region [13]. The structure of the catalytic domain of human MMP-9 (without the fibronectin repeats) consists of five-stranded β-sheet and three α-helices, which is similar to other MMPs. The catalytic center is composed of the active-site zinc ion, which is co-ordinated by three histidine residues (401, 405 and 411) and an essential water molecule [14]. MMP-9 is illustrious among the other MMPs by the incidence of three head to tail cysteine rich repeats which resemble fibronectin type II repeats. This insert is mandatory for their interaction with substrates like gelatin, laminin and collagen [15]. In most of the MMPs, the hydrophobic specificity (S1) pocket is the major determinant for their substrate specificity [16]. The specificity loop greatly differs in length among MMPs and encompasses from nine (in MMP-1, -9, -11, and -23) to 13 residues (in MMP-17 and -25) [17]. In MMP-1 and MMP-7, the size of the S1 subsite is reduced by the presence of arginine and tyrosine respectively. In other MMPs, the S1 cavity has a long open channel with different amino acid residues [18]. For example in MMP-2, the S1 loop is mainly delimited by the presence of Pro417, Gly418, Ala422, Ile424, and Thr426, and showed a large aperture at the end of the pocket [16]. In contrast to MMP-2, the S1 pocket of MMP-3 is delimited by different residues (Thr232, Glu233, Tyr237, Leu239, and His241) and exhibits a smaller aperture at the end of the cavity. Another interesting difference is the role played by specific S1 loop residues which can influence the access of a long side chain residue of the substrate into the S1 cavity of MMPs [18].

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Most MMPs inhibitors are classified according to their ZBG. The most common ZBG used in MMPI is the hydroxamic acid group which tightly binds with the catalytic zinc ion using two identical bonds and extends the binding in the P1 and S1′ selectivity pockets of MMPs. These hydroxamic acid inhibitors produced nanomolar inhibitors with high potency against MMPs. The failure of hydroxamic acid in the clinical trials is due to lack of selectivity toward zinc ion and poor pharmacokinetics and oral bioavailability. A number of efforts have been made to identify alternatives to the hydroxamic acid group. Later hydroxamic acid group was replaced by the carboxylic acid groups which results loss in potency. This is due to the changes in binding mode of carboxylate ligand in the active site of MMPs. However, Castelhano and co-workers reported the selectivity and potency of MMPI with several different ZBGs (for example, hydroxamates, 'reverse' hydroxamates, carboxylates, thiols, phosphinates) on a common indolactam/isobutyl backbone moiety. In contrast, the unique findings reported strongly that high affinity ZBGs can be used as the basis for new patterns of selective inhibition against MMPs. This indicates that by linking the different zinc binding groups with the common backbone can improve the selectivity against deep S1′ pocket of MMPs [19].

Several generations of synthetic MMP inhibitors (MMPIs) have been tested in phase III trials on humans such as hydroxamate, non hydroxamate, synthetic collagen peptido-mimetic, non-peptidic, synthetic tetracycline derivatives and biphosphonate inhibitors, and have been demonstrated to have no therapeutic efficacy [20]. Several grounds for the failure of MMPIs in human clinical studies include high toxicity, which causes severe musculoskeletal side-effects and the lack of specificity of the inhibitors, which can lead to unexpected and redundant effects [21,22]. Regardless of the clinical failures, the recent development of highly selective inhibitors for MMP-12 and MMP-13 suggests that specific targeting of metzincins may not be difficult [23,24]. The negative results obtained from the clinical trials for the synthetic compounds stirred up an interest to work on the natural compounds. There are prior reports on the inhibitory activity of natural compounds against MMPs [25]. For example myricetin, a flavonoid found in berries, fruits, vegetables, herbs and tea greatly decreased the activity of MMP-2 in human colorectal carcinoma cell lines. Neovastat (shark cartilage) was analyzed in regard to anti-angiogenic and anti-metastatic effects on the activity of several MMPs. Neovastat inhibits the enzymatic activity of MMP-2 with minor inhibition of MMP-1, 9, 7 and 13 [26]. Hence natural compounds could be a better choice for selective inhibition of MMP-9.

In modern computational biology, pharmacophores based approach is used to delineate the essential features of one or more molecules with the same biological activity. The pharmacophore based modeling of ligands is a well-established approach to quantitatively discover common chemical features among a considerable number of structures. Pharmacophore mapping can be used in designing the inhibitors in several ways, including justification of activity trends in molecules, searching of databases to find new chemical entities and to identify important features for activity [27].

In this study, the pharmacophoric features of the inhibitors of MMP-9 have been developed. The pharmacophore based modeling has been carried out to identify the best features, such as hydrogen bond acceptor, donor, aromatic ring, and aliphatic chain for MMP-9, from the existing drug which facilitates the drug activity for the identification of novel inhibitors from the natural compound database. Furthermore, docking and molecular dynamics (MD) simulation were performed to analyze the binding affinity of the identified natural compounds. The high scored hit from MD studies was taken for in vitro studies to check the biological activity against MMP-9. The overall workflow for ligand-based pharmacophore in screening the novel compounds for MMP-9 is given in Fig. 1.

## 2. Materials and methods

### 2.1. Ligand preparation

A data set of ligand molecules having MMP-9 inhibitory activity were collected from the literature (Supporting information Table S1) [28–36]. Quantitative pharmacophore was generated for the molecules based on the diversity of their chemical structure and biological activity against human MMP-9 inhibitors. The MMP-9 inhibitors used in this study were further energy minimized using Ligprep module of Schrödinger software [37]. The conformations of the above structures were generated using the MMFFs force field, with an implicit GB/SA solvent model. A maximum of 1000 conformers were generated per structure by a preprocess minimization of 1000 steps using ConfGen algorithm. During the search, hydrogen-bonding interactions were suppressed to facilitate conformations in which the ligand bonds to the receptor, and not just conformations with internal hydrogen bonding, as this is essential for the model.

### 2.2. Pharmacophore model generation

The quantitative pharmacophore model was built using the Phase software [38]. The diverse dataset were used to generate

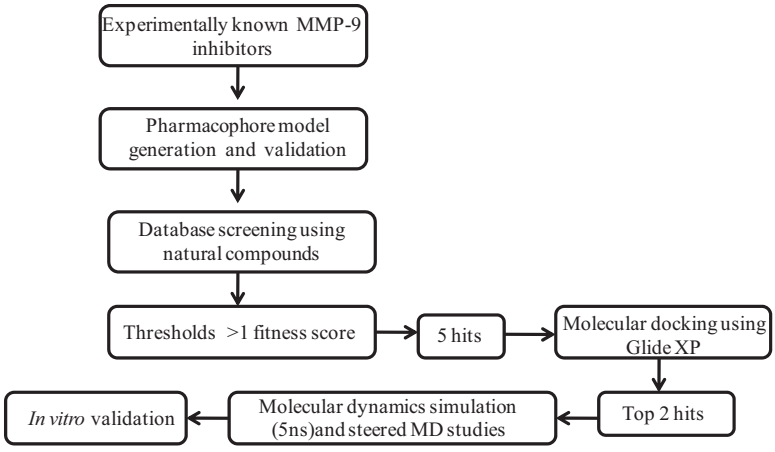


**Fig. 1.** Workflow for identifying novel inhibitors using ligand-based pharmacophore modeling.