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Robust design of some selective matrix metalloproteinase-2 inhibitors over matrix metalloproteinase-9 through in silico/ fragment-based lead identification and *de novo* lead modification: Syntheses and biological assays

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ABSTRACT

Broad range of selectivity possesses serious limitation for the development of matrix metalloproteinase-2 (MMP-2) inhibitors for clinical purposes. To develop potent and selective MMP-2 inhibitors, initially multiple molecular modeling techniques were adopted for robust design. Predictive and validated regression models (2D and 3D QSAR and ligand-based pharmacophore mapping studies) were utilized for estimating the potency whereas classification models (Bayesian and recursive partitioning analyses) were used for determining the selectivity of MMP-2 inhibitors over MMP-9. Bayesian model fingerprints were used to design selective lead molecule which was modified using structure-based *de novo* technique. A series of designed molecules were prepared and screened initially for inhibitions of MMP-2 and MMP-9, respectively, as these are designed followed by other MMPs to observe the broader selectivity. The best active MMP-2 inhibitor had IC_{50} value of 24 nM whereas the best selective inhibitor (IC_{50} = 51 nM) showed at least 4 times selectivity to MMP-2 against all tested MMPs. Active derivatives were non-cytotoxic against human lung carcinoma cell line—A549. At non-cytotoxic concentrations, these inhibitors reduced intracellular MMP-2 expression up to 78% and also exhibited satisfactory anti-migration and anti-invasive properties against A549 cells. Some of these active compounds may be used as adjuvant therapeutic agents in lung cancer after detailed study.

1. Introduction

Remodeling of extracellular matrix (ECM) is important in many physiological and pathological events. Matrix metalloproteinases (MMPs) are zinc-dependent endopeptidases which are involved in remodeling of ECM. The MMPs are implicated in numerous disease conditions such as cardiovascular disorders (atherosclerosis, restenosis, hypertension, heart failure, aortic aneurysm, etc), pulmonary disorders [chronic obstructive pulmonary diseases (COPD), bronchial asthma, pulmonary fibrosis, etc], rheumatic diseases (rheumatoid arthritis, lupus erythematosus, systemic sclerosis, etc) and diabetes mellitus as well as cancer. Owing to these broad ranges of applications, MMPs are still considered as potential targets for drug development.[1]

Although several MMP inhibitors (MMPIs) entered clinical trials, none of these has been established as an anticancer drug due to the adverse effects that mainly stem from the broad spectrum of MMP inhibition. Poor pharmacokinetic profiles are well-known obstruction for the discovery of MMPIs.[2] Most of the trialed compounds are hydroxamate derivatives that show poor aqueous solubility as

Abbreviations: CoMFA, comparative molecular field analysis; CoMSIA, comparative molecular similarity analysis; DS, discovery studio software; FT-IR, Fourier transform infrared spectroscopy; HDAC, histone deacetylase; HMBC, heteronuclear multiple bond correlation; MMP, matrix metalloproteinase; MM-GBSA, molecular mechanics combined with generalized Born and surface-area salvation; Mp, melting point; MS, mass spectroscopy; MLR, multiple linear regression; NMR, nuclear magnetic resonance; QPLD, quantum polarized ligand docking; QSAR, quantitative structure activity relationship; RMSD, root mean square deviation; ROC, receiver operating characteristic; RMSF, root mean square fluctuation.

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well as non-specificity towards MMPs and other zinc containing enzymes.[2,3] So far, 28 subtypes of MMPs have been identified and are classified into six subfamilies. One of the subfamilies, gelatinase is having two enzymes—MMP-2 (gelatinase A) and MMP-9 (gelatinase B).[4–6] The MMP-2 has been characterized as the most validated target for cancer whereas MMP-9 is reported as an anti-target in advanced state of the disease due to its anti-angiogenic and anti-tumorigenic functions.[7–12] It was earlier reported that MMP-9 is responsible for the formation of tumstatin that suppress angiogenesis via αVβ3 integrin.[13] The MMP-9 deficient mice were found to have increased invasiveness in neuroendocrine tumorigenesis.[14] Apart from the risks of angiogenesis and metastasis, increased hemorrhage and brain edema were also reported for MMP-9 suppression.[6] In addition, long time suppression of MMP-9 disrupts recovery in cardiac ischemic patients and may lead to cardiac failure.[15] Therefore, it is necessary to design MMPIs that would be active as well as selective against MMP-2 but non-selective towards other MMPs particularly MMP-9.

Computational chemistry gained significance in drug design and development in last few decades. In the current study, robust in silico fragment-based technique was adapted for the development as well as lead modification of the potent and the selective MMP-2 inhibitors. The 'fragments' are smaller and less complicated molecular residues that may be efficiently optimized into the lead compound series if the structural insight is obtained at the outset for the binding interaction between the fragment hit and the target protein of interest. This hypothesis is supported by the reports of clinical trials of drug molecules developed from weakly acting fragment.[16] As MMP-2 and MMP-9 enzymes belong to same MMP subgroup (gelatinase), these have highly homologous catalytic sites[12] for the enzyme actions. Therefore, initially in silico studies were performed considering the higher differences in affinities between MMP-2 and MMP-9. The designed compounds, obtained through the molecular modeling study were synthesized and their enzymatic activities were measured initially against these two MMPs followed by other MMPs to observe the broader selectivity. In addition, some of these active compounds were also tested against nuclear extract of histone deacetylase (HDAC) enzymes consists mainly of histone deacetylase 1 (HDAC1) and histone deacetylase 2 (HDAC2) to justify the selectivity of these compounds against other Zn dependent metalloenzymes. Some highly active designed MMP-2 inhibitors were further investigated for cytotoxicity, apoptosis, cellular expression, anti-migratory and anti-invasive properties against A549 cell line- a highly invasive as well as MMP-2 and -9 over-expressing human lung carcinoma cell line.[17] The initial work of these studies is reported here. The work may help to get some useful leads as adjuvant therapeutic agents in lung cancer.

2. Materials and methods

2.1. Molecular modeling study

Two different types of molecular modeling techniques were performed. The regression analyses (2D and 3D QSAR studies as well as pharmacophore mapping technique) were considered with an aim to understand the mechanistic activity of the designed molecules. The classification analyses (Bayesian modeling and recursive partitioning techniques), on the other hand, were performed to ensure the selectivity of the designed molecules towards MMP-2 comparing with that of MMP-9 which is of the same class.

2.1.1. Dataset

Two hundred fourteen (214) structurally diverse compounds were collected from the published work.[18–37] The biological activity value [IC_{50} (nM)] was converted to the negative logarithmic scale [$pIC_{50} = \log 10^9/IC_{50}$] and was used as the dependent variable in different regression analyses except the pharmacophore mapping method where IC_{50} value was used. Another one hundred fortynine (149) compounds with both MMP-2 and MMP-9 inhibitory activities were collected from the published work[38–43] of Johnson and Johnson Pharmaceutical Research and Development. Around 20% of the data (33 compounds) with the higher MMP-2 selectivity [i.e., the higher MMP-9 IC_{50} (nM)/MMP-2 IC_{50} (nM)] were denoted as the 'selective' and other compounds as the 'non-selective'. These compounds were used for the classification model development.

2.1.2. Division into training and test sets

For regression analyses, initially 25% of the dataset was selected as the test set by diversity based data splitting technique in Accelrys Discovery Studio 3.0 (DS)[44] following the method described earlier.[45] Fifty three (53) compounds were selected as the test set and remaining one hundred sixtyone (161) compounds were considered as the training set. To understand whether the current splitting maintains uniformity in both sets[46], these compounds were analyzed by the principal component analyses (PCA) method.[45] The PCA plot is provided in Supporting information (Fig. S1), which demonstrates that the test set compounds are uniformly distributed in three dimensional PCA plot. Therefore, similarity and uniformity of both of these sets are justified. The classification analyses data were divided randomly into one hundred twenty (120) [21 selective and 99 nonselective] training set and twenty nine (29) [12 selective and 17 non selective] test set compounds.

2.1.3. 2D QSAR model

Different molecular 2D and 3D descriptors were calculated for the dataset compounds through Dragon 2.1[47], DS[44] and Canvas[48] tools. The multiple linear regression (MLR) models were developed on the training set by the forward stepwise regression method following the procedure described earlier.[49] Statistical qualities of the training set MLR equations were justified by the square of correlation coefficient (R^2), adjusted R^2 (R^2_A), variance ratio (F), probability factor related to F-ratio (p) and standard error of estimate (s). To check the predictability of these 2D-QSAR models, leave-one-out (LOO) cross-validation method[50] was used as an internal validation tool. The LOO cross-validated regression coefficient (Q^2) was used for justifying internal predictabilities of the model along with parameters like $r^2_{m(LOO)}$ and $\Delta r^2_{m(LOO)}$.[51] The R^2_{Pred}[52] was used as an external validation parameter to verify the model predictability on the test set. Moreover, $r^2_{m(Test)}$ and $\Delta r^2_{m(Test)}$[53] were also used for justifying external predictabilities of the 2D QSAR model. To predict the overall predictability of models, $r^2_{m(Overall)}$ and $\Delta r^2_{m(Overall)}$[53] parameters were calculated. In order to verify the null hypothesis, Y-based randomization technique[54] was performed for the stepwise multiple linear regression (sMLR) model. Two parameters—R^2_P and cR^2_P[55] were taken into consideration as the validation parameters for the Y-based randomization test. For the MLR models, variance inflation factors (VIF) values[56,57] and applicability domain[58] were also determined to justify significance, robustness and reliability of these 2D-QSAR models.

2.1.4. Pharmacophore mapping method

Hypogen algorithm[59] was used to develop 3D-QSAR pharmacophore models using DS.[44] Thirty (30) structurally diverse compounds were selected as the training set from the current database with the help of *Find diverse molecules* tool of DS.[44] Remaining one hundred eighty four (184) compounds were used as the test set. The BEST conformation generation method with an energy threshold of 20 kcal/mol was used for conformation generation. All parameters were set to default value except uncertainty value and excluded volume. These were set to 1.5 and 8,