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Research paper

Design, synthesis and anti-HIV activity of novel quinoxaline derivatives

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ABSTRACT

In order to design novel anti-HIV agents, pharmacophore modelling, virtual screening, 3D-QSAR and molecular docking studies were performed. Pharmacophore model was generated using 17 structurally diverse molecules using DISCOtech followed by refinement with GASP module of Sybyl X. The best model containing four features; two donor sites, one acceptor atom and one hydrophobic region; was used as a query for virtual screening in NCI database and 6 compounds with Q_{fit} value $\geq$98 were retrieved. The quinoxaline ring which is the bio-isostere of pteridine ring, retrieved as a hit in virtual screening, was selected as a core moiety. 3D-QSAR was carried on thirty five 5-hydroxy-6-oxo-1,6-dihydropyrimidine-4-carboxamide derivatives. Contour map analysis of best CoMFA and CoMSIA model suggested incorporation of hydrophobic, bulky and electronegative groups to increase potency of the designed compounds. 50 quinoxaline derivatives with different substitutions were designed on basis of both ligand based drug design approaches and were mapped on the best pharmacophore model. From this, best 32 quinoxaline derivatives were docked onto the active site of integrase enzyme and *in-silico* ADMET properties were also predicted. From this data, synthesis of top 7 quinoxaline derivatives was carried out and were characterized using Mass, ^{1}H-NMR and ^{13}C-NMR spectroscopy. Purity of compounds were checked using HPLC. These derivatives were evaluated for anti-HIV activity on III-B strain of HIV-1 and cytotoxicity studies were performed on VERO cell line. Two quinoxaline derivatives (**7d** and **7e**) showed good results, which can be further explored to develop novel anti-HIV agents.

1. Introduction

Acquired immune-deficiency syndrome (AIDS) is a fatal condition developed due to infection of human immunodeficiency virus (HIV). The infection with HIV virus weakens body's defense system and hence, person becomes susceptible to various infections [1]. Integrase enzyme, unique to the human immunodeficiency virus (HIV), lacks its equivalent in the human body. The adverse effects like rash and neuropsychiatric disorders associated with non-nucleoside reverse transcriptase inhibitors (NNRTIs) and cardio toxicity associated with protease inhibitors are of major concerns of current drug therapy. Thereby, replacement with integrase inhibitors which are expected to have least side effects in drug regime, can be beneficial [2,3]. Drug resistance due to development of resistance virus is another major drawback of available anti-HIV drugs. The combination of other anti-HIV drugs with integrase inhibitors has shown prominent effects on drug resistant virus [4]. HIV integrase with high therapeutic index is a rational target for treating HIV infection and preventing AIDS. Raltegravir [5], Elvitegravir and Dolutegravir are three integrase inhibitors available in market. Dolutegravir came into the market in 2013 and no resistance has been reported till date. Resistance to Raltegravir and Elvitegravir is already reported, but no side effects are noted [6,7]. Hence, there is a urgent need to develop more efficient anti-HIV agents.

To design novel anti-HIV agents, two ligand based drug design approaches viz. pharmacophore modelling and 3D-QSAR were used. The results of both methods were combined to design novel quinoxaline derivatives, followed by synthesis and characterization. These derivatives were further evaluated for anti-HIV and cytotoxicity studies.

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2. Results and discussion

2.1. Pharmacophore model generation and validation

Ligand based pharmacophore model was generated to gain information about necessary features to be considered for designing of anti-HIV agents. All seventeen inhibitors of training set (Structures and IC_{50} values of all molecules are given in Table 1) were aligned using DISCOtech module of Sybyl X; molecular modelling software by Tripos Inc., St. Louis, MO. Top five generated pharmacophore models along with their size, hits, score, tolerance and D_{mean} are shown in Table 2. The model 36 with highest score was further used for refinement using GASP. Results of pharmacophore hypothesis refined by GASP are shown in Table 3. The four refined pharmacophore models were generated. The fitness score is calculated as sum of three terms. These terms are the number and similarity of overlaid features, the common volume of all aligned molecules, and the internal *van-der Waals* energy of each molecule. Thus, the model represented by well aligned common features of compounds had high fitness score.

Further, top three models with size 4 were validated using receiver operating curve (ROC) analysis and Guner-Henry (GH) studies. In receiver operating curve (ROC) analysis, the validity of a particular pharmacophore is indicated by the area under the curve (AUC) of the corresponding ROC curve, the overall accuracy, specificity, true positive rate and false negative rate of the pharmacophore. The ROC curves of all three pharmacophore models (1–3) are shown as Supporting information Fig. SF-1 and ROC performances are summarized in Supporting information Table ST-1. The AUC of ROC curve of model-2 was 0.83 and overall accuracy (ACC) was 0.96 against the randomly selection value of 0.5. Hence, after comparing all parameters of three models, model 2 was found to be more reliable in identifying diverse HIV-1 integrase inhibitors. The results of GH score study, carried out simultaneously, are shown in Supporting information Table ST-2. In GH analysis, the percent active (%A), percent yield (%Y); enrichment ratio (E) and Guner-Henry (GH) score variables were calculated. Model-2 succeeded in retrieving 92% of the active compounds. An enrichment factor of 5.13 and a GH score of 0.83 indicated the good quality of the model.

Model 2 with the highest fitness score was found as the best model with four features: one acceptor atom, two donor sites and one hydrophobic region. Three dimensional pharmacophore model 2 is represented as Fig. 1 and was used further for virtual screening.

2.2. Virtual screening

The best 3D-pharmacophore model 2 was considered for substructure search using NCI database. All parameters were kept unchanged except priority which was kept high. Substructure search retrieved 1,76,009 compounds from NCI database containing more than 2,65,000 compounds. These compounds were filtered to remove bad fragments, duplicate structures, and counter ions. The remaining 82,395 compounds were filtered using Lipinski's rule of five and finally 68,704 compounds were obtained which were used to design novel anti-HIV agents. 6 compounds exhibited Qfit value $\geq$ 98%. The flow chart of virtual screening process is shown in Fig. 2.

2.3. 3D quantitative structure activity relationship study (3D-QSAR)

3D-QSAR study was carried out on a series of thirty five 5-hydroxy-6-oxo-1,6-dihydropyrimidine-4-carboxamide derivatives reported as HIV-1 integrase inhibitors and results of the same is already published [23]. The contour maps generated in 3D-QSAR studies were used for designing of novel molecules shown in current study.

2.4. Designing of novel anti-HIV agents

Various processes and steps used in designing of novel quinoxaline derivatives are shown in Fig. 3. Designing of the molecules was carried out using pharmacophore features of the best validated model 2, structural features of hits obtained through virtual screening of pharmacophore model and contour maps analysis of 3D-QSAR model. Pteridine ring containing molecules were retrieved as hits in virtual screening with Q_{fit} value more than 98. Quinoxaline ring behaves as a bio-isostere of pteridine ring and hence, was selected as a core moiety to design novel anti-HIV agents. Earlier also, quinoxaline derivatives were reported as anti-HIV agent [23–25]. The decision for various substitutions on quinoxaline ring was carried out from the information obtained through contour maps analysis [26] and features of the best pharmacophore model. The contour map analysis showed that electronegative, bulky and hydrophobic groups substituted at proper positions would improve inhibitory activity. The incorporation of electronegative substitutions like halogens (−F, −Cl, −Br), carboxylic acid and sulfonyl groups were incorporated at 6th and 7th position of quinoxaline ring. The unsubstituted quinoxaline derivatives as well as substituted at 2nd and 3rd positions, with groups like methyl, amino, phenyl and substituted phenyl were designed. These substitutions were varying from non-bulky group like hydrogen, to less bulky groups like methyl and amine, to the bulky groups like phenyl and substituted phenyl. Wide range of substitutions was used to design novel molecules, so that SAR can be studied on the series. The substituted phenyl groups included fluorophenyl, methylphenyl and methoxyphenyl. Total 50 quinoxaline derivatives were designed and were mapped on the best pharmacophore model 2. From this, 32 quinoxaline derivatives were retrieved with Q_{fit} value more than 80. These derivatives were further used for molecular docking studies along with three standards.

2.5. Molecular docking

The 32 designed quinoxaline derivatives, which were mapped well with pharmacophore model, along with standards, were docked into a protein's binding site using software GOLD (version 5.2) [27]. The top 7 derivatives based on GOLD-score and standard drugs along with number of hydrogen bonds formed and the amino acid residues, with which hydrogen bonds are formed, are shown in Table 4. The entire series of designed compounds showed key interactions similar to standard drugs: Raltegravir, Elvitegravir and Dolutegravir. The crucial metal chelation was also observed in all designed quinoxaline derivatives.

The docking pose of standard drugs and designed compounds are shown in Supporting information Fig. SF-2. The nitrogen atoms of quinoxaline ring in the designed derivatives were found to form interacting bonds with Asp64, Asp116 or Glu152 amino acid residues of DDE loop present in catalytic core domain (CCD) of integrase enzyme (These amino acids are highlighted with bold in Table 4). Compound **7g** scored highest amongst designed compounds and showed total 8 H-bonds (including 5 H-bonds with Glu152 and Asp64). The oxygen atom of *p*-methoxyphenyl substitution formed one hydrogen bond with Glu152 (at a distance of 2.58 Å). N-4 of quinoxaline ring formed two hydrogen bonds, C-3 formed one hydrogen bond and C-5 formed one hydrogen bond with Asp64 (at distances of 2.66 Å, 2.60 Å, 2.68 Å and 2.06 Å, respectively). This DDE loop is responsible for binding of 3′ end of viral DNA to 5′ end of host DNA in cell nucleus. Hence by binding to this loop, drug can