Original Article

DESIGN AND SYNTHESIS OF SOME NEWER IMIDAZOLYL HETEROCYCLES AS POTENT *BTK* INHIBITORS FOR THE TREATMENT OF RHEUMATOID ARTHRITIS

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ABSTRACT

Objective: The rheumatoid arthritis as a global health problem over the past few decades, Emphasizes the need for discovery of new therapeutic disease modifying anti-rheumatoid Arthritis drugs (DMARD's). *Bruton's tyrosine kinase (BTK)* is a cytoplasmic, non-receptor, tyrosine kinase which is expressed in most of the hematopoietic cells and plays an important role in the development, differentiation and proliferation of B-lineage cells, thus making *BTK* an efficient therapeutic target for the treatment of rheumatoid arthritis. This prompted us to synthesise a novel series of Imidazolyl Heterocycles as potent *BTK (Bruton's Tyrosine Kinase)* inhibitors with alleged Anti-Rheumatoid Arthritis properties.

Methods: Newer *BTK* inhibitors containing one hydrogen bond acceptor (HBA), one hydrogen bond donor (HBD) and three hydrophobic features based on that pharmacophore model for *BTK* were designed. The designed compounds were sorted by applying ADMET properties, Lipinski rule of five, molecular docking and Novelty prediction to refine the designed ligands. Finally, different five compounds containing Imidazole as the heterocyclic nucleus have been synthesized and characterized by different analytical methods like Chromatographic data, Elemental analysis and Spectral studies by IR, ^{1}H NMR, ^{13}C NMR, GC-MS. Molecular docking studies were performed against *BTK* using GLIDE 10.2.

Results: Several important hydrogen bonds with *BTK* were revealed, which include the gatekeeper residue Glu475 and Met477 at the hinge region.

Conclusion: Overall, this study suggests that the proposed ligands are found to be more effective *BTK* inhibitor as Anti-Rheumatoid arthritis agents.

Keywords: Rheumatoid arthritis, Molecular docking, ADMET properties, *BTK* inhibitors, Pharmacophore model, Bruton's tyrosine kinase

INTRODUCTION

Rheumatoid arthritis (RA) is an autoimmune disease caused chronic inflammation of the joints, tissues that surround joints, and other organs in the body. The major characteristic of the disease is a symmetric polyarthritis involving the small joints of the hands and feet, although other joints are often involved. Approximately 1% of the general population is afflicted by RA, in which the occurrence is two to three times more predominant in women than men [1]. It has been projected that 55 to 70% of patients with RA have progressive disease, resulting in joint destruction and disability. RA is also associated with a reduced life expectancy primarily caused by cardiovascular disease and connective tissue diseases [2].

Bruton's tyrosine kinase (BTK) is a member of the Tec family of non-receptor tyrosine kinases, which is expressed in all cells of hematopoietic lineage exclude plasma cells, natural killer cells, and T lymphocytes [3]. *BTK* is activated by phosphatidylinositol 3-kinase-dependent plasma membrane recruitment and phosphorylation on tyrosine 551 by the Src-family kinase Lyn. Once activated, *BTK* induces phospholipase C and Ca^{2+}dependent signalling leading to the activation of nuclear factor-B and nuclear factor of activated T-cell-dependent pathways [4]. B-cell expansion and production of Auto Antibodies by polyclonal B cell activation is a characteristic of RA [5]. Thus selective inhibition of *BTK* may be a promising therapeutic target for B cell inhibition in RA as well as for B cell lymphoma. Ibrutinib (PCI-32765), Dasatinib, LFM-A13, CC-292 and ONO-WG_307 are well known *BTK* Inhibitors, with varying specificities [6]. Though many inhibitors are reported and some are in clinical trials, none are FDA approved and are selective to *BTK*. Hence, designing potent and specific *BTK* inhibitors becomes vital.

Here, we are using computer-aided drug design approaches to identify potent and novel *BTK* inhibitors which can cause inhibition of *BTK*. By reviewing the literature, the best model, Hypo 1(HBD, HBAL, HYP), was validated and used for database screening. Based on the 3D pharmacophore model Hypo 1 nearly 30-40 leads were designed and docked against *BTK* using Glide 10.2. Lead 7 with score-7.070 kcal/Mol were selected and the potential leads were optimised by checking their drug-like properties such as ADMET properties, Lipinski rule of five, Molecular docking and Novelty prediction. Binding confirmation of the selected compounds was predicted by Molecular docking studies using Glide 10.2. Finally, the appropriate binding modes of final selected compounds were synthesised based on the synthetic feasibility and characterised by Chromatographic and Spectral studies. The overall experimental chart was depicted in fig. 1.

MATERIALS AND METHODS

Selection of target

Protein data bank (PDB) is a crystallographic database for three-dimensional structural data of large biological molecules, such as proteins, nucleic acid and complex assemblies. PDB is a key resource in areas of structural biology such as structural genomics. The targets creating the greatest enthusiasm at this time for the treatment of rheumatoid arthritis and inflammatory diseases include Janus-associated kinase (*JAK*), spleen tyrosine kinase (*SYK*), phosphodiesterase-4, *Bruton's tyrosine kinase* (*BTK*) and phosphatidylinositol-3 kinase. Ultimately human trials will help to understand the potential risks and benefits of these novel approaches across a number of diseases [7]. The target for the treatment of rheumatoid arthritis was listed in table 1. Recent researchers suggested *BTK* as a therapeutic potential target to treat rheumatoid arthritis and cancers [8]. Which made us select the *BTK* as the protein target in this study.

Crystal structure: *BTK*

PDB code: 5FBN

Method: X-RAY DIFFRACTION

Resolution: 1.8 Å

R-Value Free: 0.226

R-Value Work: 0.179

The 3D crystal structure of *BTK* was depicted in fig 2.

Active site of selected pdb

The active site of protein was identified by using molegro molecular viewer software. In that ligand mapping is used to show the active site of the existed co-crystal ligand were listed below,

5WE 702 (C): Gln 459, Ser 453, Leu 460, Tyr 461, Asp 426, Gly 462, Ile 397, Trp 421, Tyr 425, Val 427.

5WF 701 (C): Asn 484, Cys 481, Gly 409, Gly 480, Leu 408, Thr 410, Gly 411, Thr 476, Ala 478, Val 458, Glu 475, Met 477, Leu 528, Ala 428, Val 416, Leu 460, Met 450, Asp 539, Leu 542, Phe 442, Phe 540, Met 449, Val 463, Ile 472, Lys 430.

5WE 703 (D): Gln 459, Ser 453, Trp 421, Leu 460, Ile 397, Ser 394, Gly 462, Val 427, Tyr 461, Met 450, Asp 426, Trp 421, Tyr 425.

5WE 704 (D): Tyr 631, Arg 618, Glu 624.

5WE 705 (D): Glu 624, Arg 618, Tyr 627, Thr 628, Tyr 631.

5WF 702 (D): Asn 484, Cys 481, Gly 480, Leu 408, Thr 410, Thr 474, Gly 411, Leu 528, Glu 475, Val 416, Ala 428, Asp 539, Ala 446, Leu 542, Lys 430, Ser 538, Leu 460, Leu 542, Ser 538, Ala 446, Leu 542, Met 449, Phe 540, Val 463.

Pharmacophore identification

When reviewing the efficient journals and research articles, the best pharmacophore model was identified. For designing *BTK* Inhibitors (HBD, HBAL, HYP). The *BTK* inhibitors also found to contain acidic head attached to an aromatic scaffold, a linker and a hydrophobic tail. Based on these features a library has been generated. The library consisting of nearly 30-40 leads as potent *BTK* inhibitors. Common structural features of *BTK* Inhibitor were depicted fig. 3.

Lead optimization

The newly generated ligands were subjected into Molecular docking, ADME properties, Lipinski's rule of five, Novelty prediction and Toxicity prediction were used as a filter for refinement of newly generated ligands. From the molecular docking studies, the ligand which has the best docking score was selected. During ADME investigation the compounds were checked for the low blood-brain barrier (BBB), optimal solubility, good absorption non-inhibition to CYP2D6, Non-Hepatotoxicity. Lipinski's rule of five estimates the absorption and intestinal permeability of the compound. Lipinski's rule of five states that, the compounds that are well absorbed have a logP value less than 5, Hydrogen bond donor less than 5, Hydrogen bond acceptor less than 10, Molecular weight of less than 500 Da and fewer than ten rotatable bonds. Then the designed ligands were subjected into toxicity prediction with the "OSIRIS" online software. That shows toxicological properties of the newly designed ligands. The properties like Teratogenicity, Mutagenicity, irritant and Reproductive effect. Then the novelty of the compounds was checked by using online software "Zink Data Base". The compounds having better-estimated activity value and filtered by drug-like properties was considered for further molecular docking.

Molecular docking

In the drug designing process, the Molecular Docking is used as filtering techniques, it is used to find the most appropriate conformation and interaction of each ligand at the active site of the target. Docking studies were performed using Glide 10.2 Schrodinger. In this studies calculation a high resolution 1.8 Å Crystal structure PDB ID: 5FBN of *BTK* bound with an inhibitor was selected as a target. Force field was used to add Hydrogen to the protein molecule. The binding site was identified based on the volume occupied by co-crystal ligand in the protein. The optimized ligands were docked into the active sites of the target. To predict the binding affinity of the ligand to the target, standard precision was used as the default scoring function. Based on the scoring function, Molecular interaction and the formation of Hydrogen bond interaction, Pi-Pi static interaction between the ligand and the active site residue of the target protein and the best-Docked poses were selected.

Chemical synthesis

Best docking score leads which were exhibiting the drug likeliness properties were selected for the synthesis. Based on the synthetic feasibility some of the ligands were selected and synthesized as follows,

Step I: Synthesis of 2-substituted 4, 5-diphenyl imidazole2-(chloromethyl)-4, 5-diphenyl-1*h*-imidazol (radiswiski synthesis from benzil) [9]

Benzil (25 mmol, 5.25g), aldehyde (25 mmol) and ammonium acetate (10g) were dissolved in glacial acetic acid and then refluxed for 3-5 h. After refluxing, the reaction mixture was left overnight and filtered to remove any that may be present. Water (300 ml) was then added to the filtrate and the precipitate formed was collected. The filtrate was neutralized with ammonium hydroxide and then the second crop of the solid was collected. The two crops of the solid were combined, dried and recrystallized from ethanol. The purity of the product was established by a single spot on TLC. The percentage yield was found to be 80 % w/w. Melting point 68-70 °C.

Step II: Synthesis of 2-(chloromethyl)-4, 5-diphenyl-1*h*-imidazole from step I product [10]

2-(chloromethyl)-4, 5-diphenyl-1*H*-imidazol prepared by reported method (0.2 mol), ammonia (0.1 mol) in acetone-water mixture was added. K_2CO_3 (0.1 mol, 14.0g) was added as an acid acceptor. The resulting mixture was refluxed for 3 h with occasional shaking. The resulting suspension was poured into ice cold water (500 ml) and then filtered. The solid product was collected and dried to give 2-(chloromethyl)-4, 5-diphenyl-1*H*-imidazol. The purity of the product was established by a single spot on TLC. The percentage yield was found to be 80 % w/w. Melting point 81-82 °C.

Step III: synthesis of *n*-[(4,5-diphenyl-1*h*-imidazol-2-yl)methyl]benzamide from step II product [11]

Aromatic acid (0.029 mol) and2-(chloromethyl)-4, 5-diphenyl-1*H*-imidazol (0.026 mol), were dissolved in dry pyridine (0.25 mol). The solution was refluxed for 8 h. The solution was cooled and poured into water. The separated mass was filtered, washed with water and dried. The products were recrystallized with ethanol. The purity of the product was established by a single spot on TLC. The percentage yield was found to be 80 % w/w.

Similarly, the different compounds were synthesized with different substituted Aromatic acids such as Para amino benzoic acid, Anthranilic acid, Hippuric acid, Phenoxy acetic acid, Salicylic acid. The synthesis reactions were depicted in fig. 4.

RESULTS AND DISCUSSION

Pharmacophore identification

By reviewing the literature, Pharmacophore model consisting of one hydrogen bond acceptor (HBA), one hydrogen bond donor (HBD) and three hydrophobic features (HYP) was found to be best Pharmacophore features for designing *BTK* inhibitor. Based on the above Model we have generated nearly 30-40 new leads as a *BTK* Inhibitor. Chemical features of the best pharmacophore 'Hypo 1' with its distance constraints were depicted in fig. 5 and the generated leads were listed fig. 6.

Molecular docking

All the newly designed leads were subjected into the docking studies against *BTK* as the target at the active site of gatekeeper residue Glu475 and Met477 at the hinge region. The docking tool Glide 10.2 Schrodinger was used. The list of docking score of new leads was tabulated in table 2.

Lead optimization

The newly generated leads were further subjected into ADME properties and Lipinski's rule of five, Novelty prediction used as a filter for refinement of newly generated ligands. The leads which are exhibiting the ADMET properties and also Lipinski rule of five were selected further study. The some of the selected lead and its derivatives were synthesized based on the synthetic feasibility.

Chemical synthesis

Based on the synthetic feasibility, the best lead with docking score of 7 has been selected and its analogues were synthesized. Certain compounds containing imidazolyl heterocycles were condensed with different aromatic acids such as Para amino benzoic acid, anthranilic acid, salicyclic acid, Phenoxy acetic acid, Hippuric acid resulting in five different newly synthesized compound like IPABA, ISA, IPAA, IHA, IAA. The chemical structure of synthesized ligands were given in table 3.

Characterization

The newly synthesized ligands were characterized and identified by analytical techniques such as chromatographic technique such as (TLC, GC-MS) Spectroscopic analysis (1H NMR, ^{13}C NMR), melting point, molar refractivity. GC-MS spectra shows the percentage purity and exact molecular mass of the separated compounds. ^{1}H NMR spectra predict the Number of proton and Type of proton, ^{13}C NMR shows the Number of carbon present in the synthesized compounds respectively. Melting point that may predict the purity of the synthesized compounds with the sharp melting point and TLC is used to identify the reaction completion between the reactants and the result were shown in below,

IAA

IR (KBr) cm^{-1}: Amide NH str (3733 cm^{-1}); Hetero Ar NH (3494.76 cm^{-1}); Ar CH (2923.87 cm^{-1}); Alkyl CH (2854.44 cm^{-1}); Amide C=O (1674.09 cm^{-1}); C=N (1604.66 cm^{-1}); C-N bending (1481.22 cm^{-1}); Ar NH bending (1373.22 cm^{-1}); C-C bending (1072.34 cm^{-1}); **^{1}HNMR:** imidazole NH (13.4); Alkyl CH (4.46); Amide NH (8.00); Aromatic CH (7.48); Aromatic NH (4.0); **^{13}CNMR:** Imidazole C (129.4); Aromatic C (133.1); Alkyl C (38.4); Amide C (167.9); **GC-MS:** percentage purity-95.021 %; Mass-364.2523 g/Mol. **Melting point:** 50-80 °C; **Yield**-78.3284 %; **Rf**-0.54;

IPABA

IR (KBr) cm^{-1}: Amide NH str (3757 cm^{-1}); Hetero Ar NH (3448.47 cm^{-1}); Ar CH (2923.87 cm^{-1}); Alkyl CH (2854.44 cm^{-1}); Amide C=O (1658.66 cm^{-1}); C=N (1596.94 cm^{-1}); C-N bending (1481.22 cm^{-1}); Ar NH bending (1365.50 cm^{-1}); C-C bending (1072.34 cm^{-1}); **^{1}HNMR:** imidazole NH (13.4); Alkyl CH (4.46); Amide NH (8.00); Aromatic CH (7.48); Aromatic NH (4.0); **^{13}CNMR:** Imidazole C (129.4); Aromatic C (133.1); Alkyl C (38.4); Amide C (167.9); **GC-MS:** percentage purity-93.979 %; Mass-364.2236 g/Mol. **Melting point:** 60-85 °C; **Yield**-57.36 %; **Rf**-0.77;

ISA

IR (KBr) cm^{-1}: Amide NH str (3795.63 cm^{-1}); Hetero Ar NH (3733.91 cm^{-1}); Phenolic OH (3440.76 cm^{-1}); Ar CH (2923.87 cm^{-1}); Alkyl CH (2854.44 cm^{-1}); Amide C=O (1674.09 cm^{-1}); C=N (1643.23 cm^{-1}); C-N bending (1481.22 cm^{-1}); Phenolic C-O and O-H coupled bending (1481.22 and 1450.36 cm^{-1}) C-C bending (1072.34 cm^{-1}); Alkyl CH (694.32 cm^{-1}); Ar CH (763.76 cm^{-1}); **^{1}HNMR:** imidazole NH (13.4); Alkyl CH (4.46); Amide NH (8.00); Aromatic CH (7.48); Aromatic OH (5.0); **^{13}CNMR:** Imidazole C (129.4); Aromatic C (133.1); Alkyl C (38.4); Amide C (167.9); **GC-MS:** percentage purity-93.421 %; Mass-365.2668 g/Mol. **Melting point:** 55-75 °C; **Yield**-59.24%; **Rf**-0.83;

IPAA

IR (KBr) cm^{-1}: Amide NH str (3733.91 cm^{-1}); Hetero Ar NH (3409.90 cm^{-1}); Ar CH (3055.02 cm^{-1}); Alkyl CH (2923.44 cm^{-1}); Amide C=O (1674.09 cm^{-1}); C=N (1596.94 cm^{-1}); C-N bending (1481.22 cm^{-1}); C-O str (1442.65 cm^{-1}); C-N str (1481.22 cm^{-1}); Ar CH bending (771.47 cm^{-1}); Alkyl CH bending (694.22 cm^{-1}); **^{1}HNMR:** imidazole NH (13.4); Alkyl CH (4.46); Amide NH (8.00); Aromatic CH (7.48); **^{13}CNMR:** Imidazole C (129.4); Aromatic C (133.1); Alkyl C (37.9); Amide C (169.0); **GC-MS:** percentage purity-94.169 %; Mass-379.1965 g/Mol. **Melting point:** 45-70 °C; **Yield**-58.43 %; **Rf**-0.83;

IHA

IR (KBr) cm^{-1}: Amide NH str (3749.34 cm^{-1}); Hetero Ar NH (3487.04 cm^{-1}); Ar CH (2923.87 cm^{-1}); Alkyl CH (2854.44 cm^{-1}); Amide C=O (1650.95 cm^{-1}); C=N (1550.65 cm^{-1}); C-N bending (1458.08 cm^{-1}); Ar CH bending (771.47 cm^{-1}); C-C str (1164.92 cm^{-1}); Alkyl CH bending (694.32 cm^{-1}); **^{1}HNMR:** imidazole NH (13.4); Alkyl CH (4.46); Amide NH (8.00); Aromatic amide NH (8.0); Aromatic CH (7.48); **^{13}CNMR:** Imidazole C (129.4); Aromatic C (133.1); Alkyl C (38.4); Amide C (167.9); Aromatic amide C (171.1); **GC-MS:** percentage purity-98.308 %; Mass-404.3175 g/Mol. **Melting point:** 70-85 °C; **Yield**–57.31 %; **Rf**-0.80;

Molecular docking

A better understanding of the interaction is obtained by viewing the molecule in the active site. The synthesized ligands were docked. The interaction diagram and binding mode of the docked ligands in the active sites of *BTK* were shown in fig. 7 and fig. 8 respectively. The docking score were tabulated in table 4.

CONCLUSION

Inhibition of *BTK* as developed as a new promising target in the field of Rheumatoid Arthritis and Malignancy, allergy or hypersensitivity as it is involved in several signalling pathways. Thus as an attempt to ligand-based pharmacophore modelling was done to find the important chemical features which can inhibit the *BTK* activity. The five feature pharmacophore models, Hypo1, were developed consisting of 1 HBAL, 1 HBD, 3HYP features. The best hypothesis Hypo1was used as a 3D structural query to screen the chemical database for retrieving new potent inhibitors of *BTK*. Lipinski's rule of five, ADMET properties screening assisted us to discard the non-drug-like leads. Furthermore, the screened drug-like lead was identified and subjected to molecular docking study against *BTK* as a target using Glide 10.2. Finally, the selected lead and its analogues were synthesized with different aromatic acids and also characterized by spectral studies. The purity was also checked by TLC chromatographic technique. The potency of the synthesized compounds to inhibit BTK will be evaluated by performing Enzyme inhibition studies. Further evaluation studies like *In vitro and In vivo* arthritis activity will also be carried out to prove the efficacy of all the newly synthesized compounds as potent anti-rheumatoid arthritic agents

CONFLICT OF INTERESTS

Declare none

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