STRUCTURE BASED DESIGN AND DEVELOPMENT OF KINASE INHIBITORS AND DENDRIMERIC NANOPROBE BASED ON 2-AMINOTHIAZOLE TEMPLATE

A Thesis submitted

in partial fulfillment for the Degree of

Doctor of Philosophy

by

SARAH TITUS

Department of Chemistry

INDIAN INSTITUTE OF SPACE SCIENCE AND TECHNOLOGY

THIRUVANANTHAPURAM

JUNE, 2016

ABSTRACT

Small-ring heterocycles are considered to be '*privileged medicinal scaffolds*' which offer ideal properties for quality lead compounds or fragments for drug discovery due to their low molecular weight, desirable physical properties as well as their ability to accommodate substituents around the core in defined three dimensional orientations. The interactions of these designer small molecular systems with the target can often be tailored which offers a high degree of diversity and has proven to be useful for the search of new therapeutic agents. The understanding of molecular reasons for the activity of a drug or drug candidate is one of the priorities of modern medicinal chemistry. In this regard rational drug discovery or *omics*–guided drug design could be used to achieve the molecular design of new structures where the selection of a specific and druggable biological target, which is well identified as associated with a disease and whose activity could be modulated by a small molecule is considered to be inevitable. In the present work we decided to explore this target based approach which include the exploration of the medicinally relevant chemical as well as biological space.

Among various heterocyclic systems, thiazole ring, especially aminothiazole is bestowed with wide spectrum of biological activities and has attracted considerable attention as a scaffold for drug discovery. Following clear insights on the role of kinase proteins in cell cycle and tumorigenesis, Aurora kinases emerged as a major class of therapeutic targets for anticancer drug development. The different kinase inhibitory activities of aminothiazole derivatives have been explored by many research groups including their potential as Aurora kinase inhibitors. The results of *in vitro* studies also indicated very good cytotoxicity of such derivatives against many of the cancer cell lines. Thus aminothiazoles store the potential to be used as an effective scaffold for the design of more potent drug candidates and can also be explored in the development of materials for biomedical applications.

Driven by the anticancer activity of reported 2-aminothiazole derivatives, in part A of the present study, we felt it worth examining the possibilities of developing unexplored members from this family by expanding the chemical space around the template. Well-supported by the reported bioactivity of different hydrazone containing heterocyclic compounds, especially that of 2-hydrazinothiazoles, we have selected hydrazone moiety as a suitable fragment for the modification of 2-aminothiazole core. Thus combining the hydrazone fragment with the 2-aminothiazole ring we have designed novel class of 2-aminothiazoles bearing hydrazono unit at the C*4* of thiazole ring to afford 4-hydrazinothiazoles with four sites for diversity multiplication. The plausible routes for the synthesis of the designed core were investigated and [4+1] ring synthesis route using aminoamidinothiourea as the C-N-C-S fragment and α-haloketone as the *C5* contributor was designed and formulated for the target compounds. The required aminoamidinothiourea was generated in two successive condensations of binucleophilic aminoguanidine with a carbonyl compound and an isothiocyanate. The synthetic protocol offered a huge advantage of accommodating a large number of commercially available carbonyl compounds, isothiocyanates and α-haloketones as fragments for the construction of our proposed 4-hydrazinothiazoles and thus found to be well suited for drug design.

Utilizing the possibility of diversity multiplication in the designed scaffold a virtual library of 120 molecules was designed by varying the carbonyl component, isothiocyanate and α-haloketone (4×6×5=120) to generate four families of compounds belonging to isopropylidene, isobutylidene, cyclohexylidene and benzylidene classes of 4-hydrazinothiazoles. The potential of the designed scaffold as Aurora kinase inhibitor was identified by the preliminary molecular docking studies on three different class of anticancer drug targets *viz;* estrogen receptor, cyclin dependent kinase and Aurora kinase. Encouraged by the promising results from *in silico* screening, the feasibility of the chemical synthesis was investigated and two-step as well as *one-pot* sequential multicomponent synthesis for the desired compounds were developed. The versatility of the synthetic protocol was proven by the synthesis of 54 derivatives of 4-hydrazinothiazoles (yield >80%) among which structural elucidation was carried out for 26 compounds. Single crystal X-ray diffraction studies on selected members of 4-hydrazinothiazole family revealed interesting solvation patterns which were guided by the hydrazone capping unit on the *C4* position of the thiazole ring. Detailed investigations on the crystal landscapes further suggested the potential of these molecules to be tested in materials science applications for the development of synthetic water/ion channels.

In vitro screening of 36 derivatives in six human cancer cell lines (MCF-7, A549, HL-60, OVCAR-3, SK-MEL-2, SW620) using Sulphorhodamine B assay with Adriamycin as the positive control had identified three hits, one compound in the cyclohexylidene family against A549 cell line and two compounds-one each from isobutylidene and benzylidene family - were found to be active against MCF-7 with GI_{50} values in micromolar concentrations. With a view of generating novel lead like molecules, presently, we focused on one of the families, 4-benzylidenehydrazinothiazoles, for the hit to lead generation. Accordingly, the virtual library was expanded to 22500 molecules of 4-benzylidenehydrazino thiazoles by utilizing diversity multiplication available in the core scaffold and by considering the commercial availability of reagents for synthesis. The *in silico* screening of the expanded library in the active sites of 7 different Aurora proteins (six from Aurora kinase A and one from Aurora kinase B) showed promising results comparable with well-known Aurora inhibitors in the clinical and preclinical studies and also with anticancer drugs already available in the market. Detailed investigation of the interactions of the hit molecule in the active sites of target proteins suggested it to be an ATP competitive Type I inhibitor. The effect of ring substitution on the binding pattern of the molecule was studied in detail as a preliminary step towards the hit to lead generation. The computation of pharmaceutically relevant descriptors showed that most of the ligands from the virtual library were within the recommended range of that for 95% of drugs with little or no violation from Lipinski's *'rule of five'* which proved the 'drug-likeness' of the designed derivatives.

As a first step towards our long term goal of developing a multifunctional dendrimer system for drug delivery, along with the small molecule drug discovery process we have carried out preliminary steps toward the development of suitable carrier system for the designed scaffold. To contribute to that, development of a multifunctional carrier system based on polyamidoamine dendrimer was initiated by investigating the possibility of constructing heterocyclic ring systems on to the periphery of the dendrimers. Owing to the specific interest in 2-aminothiazole core,

in part B of the present work, we have designed polyamidoamine-2-aminothiazole conjugate as a tunable template.

A potential precursor platform for building different heterocycles on to the periphery of polyamidoamine dendrimer was obtained through a divergent covalent amidine transfer strategy. The applicability of the synthon in heterocyclic nanovectorization was demonstrated by the design and formulation of synthetic routes for anchoring of 2-aminothiazole template through [4+1] ring closure. With an intension of developing the conjugate as a potential nanoprobe for imaging applications, we attempted to impart useful photophysical properties to the conjugate. To this end, we used coumarin moiety as a photounit, which was introduced through the α-halo component during the thiazole ring synthesis. Following the optimized reaction procedure, we have synthesized generation zero of polyamidoamine-coumarinoyl-2-aminothiazole conjugate and was characterized using spectroscopic techniques.

The molecular dynamics simulations (5ns) on the dendrimer conjugate in DMSO and water as well in different pH conditions predicted the detailed internal structure of the dendrimer including the free-space and distribution of solvent molecules. The change in the structural features with change in solvent environment was noticed whereby the conjugate showed a larger radius of gyration in DMSO than in water. The pH dependence studies provided useful insights on the use of such systems as carriers. The larger internal cavity radius observed for the conjugate at pH 4 has given a preliminary idea about the encapsulation efficiency and thereby the release mechanism of suitable guest molecules.

The polyamidoamine-coumarinoyl-2-aminothiazole conjugate was highly soluble in most of the organic solvents and the fluorescence emission studies of the conjugate in different solvents revealed it as an excellent solvent polarity indicator which suggested its potential to be developed as labelling agents for cell membranes, protein binding sites and liposomes. The conjugate was identified with positive solvatochromism with emission maxima ranging from 467 to 557nm depending upon the polarity of solvent with exceptionally large Stokes shift values ranging from 98 to 175nm along with moderate to good quantum yield (0.05-0.77).

The present study has contributed to the development of synthetic routes to a highly privileged scaffold for drug discovery based on 2-aminothiazole template which is discussed in part A of the thesis. The chemical library based on the scaffold was identified with three hits in the anticancer drug screening against human cancer cell lines. The expanded library, during *in silico* screening, indicated promising results comparable with well-known Aurora inhibitors and also with marketed anticancer drugs. Detailed investigations suggested the hit molecule to be an ATP competitive Type I inhibitor. In the second part of the thesis, development of a divergent strategy for the covalent immobilization of various heterocycles onto polyamidoamine dendrimer was proposed following the accomplishment of a universal amidinothiourea platform on the dendrimer periphery through amidine transfer. The utility of amidinothiourea anchored dendrimer in heterocycle synthesis was demonstrated by the construction of 2-aminothiazole unit following a [4+1] ring construction strategy and a conjugate with a coumarin unit was identified with excellent photophysical properties. The study is first of its kind to develop a versatile platform for heterocycles on polyamidoamine dendrimer with tunable properties and hence potential applications in biomedical field.