

**COMPUTATION ASSISTED *DE NOVO* DESIGN AND
DEVELOPMENT OF COMBINATORIAL FLUOROPHORE
LIBRARY FOR THERANOSTICS**

*A Thesis submitted
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by

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ABSTRACT

The synergism between therapy and diagnostics is considered as a new innovation to the treatment techniques. Thus theranostics, the combination of therapeutics and imaging capabilities into a single package, has emerged as a powerful tool towards personalized medicine and is anticipated to revolutionize modern treatment modalities. Transformation of the idea of theranostics from the lab to the clinic is possible only by the advancements in individual components. Moreover, rather than depending on the strength of individual components, a single molecule possessing the desired attributes may contribute significantly to the realization of the concept. The development of trackable therapeutics will allow the real-time monitoring of drug release and its pharmacokinetics and thereby increase the treatment efficacy. The advancements in the field of single molecule based theranostics are possible only by accelerating the development of novel core scaffolds. The symbiosis of computational and synthetic chemistries may open new vistas in the theranostic field, which is rarely explored. In this regard, we formulated our research problem to design novel core molecules by exploring the hidden potential of computational and classical chemistry aimed towards contributing significantly to the growing field of theranostics.

In our search for novel organic functional molecules, we hypothesized the combination of diverse heterocycle fragments through molecular hybridisation, a widely adopted technique in drug discovery for the design of core skeleton for the envisaged theranostic platform. Our longstanding interest in 1,3-thiazole, coupled with its extensive pharmaceutical relevance and untapped potential as fluorophore core prompted us to choose this member of azole family as the anchoring unit. A computer aided fluorophore design strategy was adopted by placing donor-acceptor fragments as end groups, utilizing the intramolecular charge transfer phenomena resulting in a novel 5-(hetero-2-yl)-1,3-thiazole core. Molecular engineering around the thiazole core utilizing its *C2*, *C4* and *C5* positions afforded a library of multi-directional charge transfer molecules. The preliminary structure property study carried out with the aid of DFT and TD-DFT methods revealed that *C5* position of thiazole was critical in imparting colour tunability. The calculations also helped to identify the potential of *C4* position to emerge as an orthogonal handle.

Motivated by the rational design of novel molecules based on bi(heteroyl) thiazole-het core, we further proceeded with the retrosynthetic design and development of facile routes to generate the combinatorial library of fluorophores. Using the untapped potential of classical chemistry, we identified a classical [4+1] thiazole ring route where carbonyl compounds, secondary amines and halo methyl heterocycles served as building blocks for the modular synthesis of multi-heterocyclic D-A systems. The versatility of the developed method was validated by the synthesis of a 70 member library built on 5-(thiophene-2-yl)-1,3-thiazole and 5-(furan-2-yl)-1,3-thiazole cores, and out of which 35 members were fully

characterized. We also attempted to adapt our synthetic strategy to suit green chemistry protocols and successfully developed a one-pot multi-component mechanochemical method to synthesize these thiazoles. Compared to the existing literature methods, utilizing highly expensive transition metal catalysts for constructing bi(hetero)aryl core, our method is simple, highly versatile, economical, having a high atom economy and synthesised using readily available reagents.

In order to validate the theranostic potential of the synthetically achieved systems, we next proceeded with the systematic exploration of their therapeutic and diagnostic properties. For the validation of the therapeutic potential, both *in vitro* and *in silico* methods were employed. The preliminary *in vitro* studies using selected members of the synthesized library confirmed that one of the molecules of the 5-(thiophene-2-yl)-1,3-thiazole family was active against HL-60 (leukemia) cell line whereas the same molecule exhibited promising results in MCF-7 (breast cancer) and HT-29 (colon cancer). Inspired by these findings, and considering the synthetic feasibility and availability of reagents, we generated a 43200 member virtual library by diversity amplification around the core. The computation of pharmaceutically relevant descriptors using ADME predicting tool indicated that 97.5% molecules in the designed library were within the range of properties recommended for 95% of drugs in the market and hence the druggable nature of the core scaffold was confirmed. Further *in silico* analysis were carried out in three different families of cancer biomarker proteins viz- human estrogen receptor, aurora kinase, and cyclin dependent kinase using the in-house virtual library. The results from docking studies were compared with those of respective classes of protein inhibitors and known anticancer drugs and it was found that the molecules retained most of the crucial binding interactions with the proteins. Among the studied cores, molecules built on 5-(furan-2-yl)-1,3-thiazole core was found to have a better binding affinity for the active site of proteins. Specifically, N-containing heterocycles viz; 3-pyridyl, 2-substituted quinoxalines and pyrazines played vital roles in ATP competitive binding in aurora kinase proteins. Thus the designed scaffold holds the promise for developing potent drug molecules.

Excited by the vast potential of these cores in therapeutics, we next attempted to evaluate their photophysical properties for the development of druggable fluorophore molecules for applications in the field of theranostics. The photophysical properties of developed fluorophores were studied in six different solvents of varying polarity. The study of structure photophysical relationship shed light on the importance and influence of different fragments on the photophysical properties of 5-(hetero-2-yl)-1,3-thiazoles. The significance of C5 position in developing colour tunable fluorophore was further confirmed by experiments. The C4 position can be used a gateway for a second charge transfer channel by choosing appropriate donor and acceptor fragments. All the molecules exhibited solvent dependant photophysical properties and showed positive solvatochromism with large Stokes shift values which are desirable attributes for imaging applications. Additionally, the molecules displayed very high quantum yield values up to 87%, especially in non polar solvents. These molecules are further capable of exhibiting colour tunable solid state emission and are thus among one of the smallest family

of organic molecules capable of exhibiting solid state red emission. The crystal structure analysis revealed the molecular rigidity obtained by the multiple short interactions to be responsible for the solid state emission. The preliminary evaluation of theranostic property in HeLa and L929 cell lines identified that the molecules were potential candidates for the development of theranostic platforms.

We further performed computational calculations to understand the fundamental nature of the core molecules. A benchmark study using twelve different functionals identified the hybrid functional PBE0 as the best functional to describe the vertical absorption energy with a mean absolute error less than 0.3 eV. This result would help in designing molecules with tailored wavelength of interest in future research. The solvent effect on the photophysical properties was also verified by computational calculations using polarizable continuum model. The intramolecular charge transfer was verified by the partial density of states calculation by identifying the percentage contribution of various fragments to HOMO and LUMO and the information was used to design the multidirectional charge transfer compounds. The computational calculations assisted in identifying the existence of charge separated quinoid state in polar solvents and gave insights on the conformational preference of the molecules.

After achieving the main objectives of the thesis, we also investigated the potential of the multi-heterocyclic 1,3-thiazole core in multi-functional material development by expanding the prospects of thiazole chemistry from drug discovery to advanced functional materials. The molecules with a nitro substituent at C5 of thiophene behaved as static functional molecules whereas the aldehyde derivatives hold promise as dynamic functional systems. The study also revealed the potential of the core to exhibit the aggregation induced emission phenomena and the molecular dynamics study confirmed the time dependent formation of aggregates. The sensitivity of the molecules towards the HCl vapours were detected and a naked eye sensor for acid vapours was developed. The sensor behaviour was then rationalised by computational studies. Further, the observed mechanoresponsive fluorescence behaviour of the aldehyde substituted molecules widens their scope as advanced functional materials.

It is noteworthy that the present study has successfully and significantly contributed to the emerging area of theranostics through the development of trackable therapeutics. The salient features of the work include the combined approach by utilizing the prospectives of computational chemistry and classical chemistry to design and develop novel single small organic molecule based fluorescent therapeutic agents. Further, the concept of molecular hybridisation of heterocycles was used for the development of a novel 5-(hetero-2-yl)-1,3-thiazole core scaffold capable of accommodating panoply of substituents around the core. A simple, economical and highly versatile synthetic route was developed by using commercially available building blocks. *In vitro* and *in silico* methods were used to reveal the therapeutic potential of the systems and detailed photophysical studies unveiled the probable imaging capabilities. Further computational calculations helped us to identify the fundamental nature of the core scaffold. Finally, the

potential of the bi(hetero)aryl core from medicinal chemistry to materials chemistry transformations for multi-functional material development was illustrated. The research has opened new avenues in heterocyclic chemistry research, especially in thiazole chemistry, by identifying novel molecular systems with a broad spectrum of tunable properties and is expected to realise its goal of single molecule based trackable therapeutics for applications in personal medicine in the future.