Abstract

The dissertation entitled "**Stimuli-responsive Turn-on Fluorogenic Donors of Hydrogen Sulfide (H_2S) and Prodrugs of Anti-Cancer Compounds**" consists of four chapters based on the results of experimental works performed during the complete course of Ph.D. research tenure.

The **first chapter** discusses the general introduction to H_2S and the different types of donors being developed in the literature. The introduction section is divided into several sub-sections. Firstly, a brief introduction about the different sources (plant, dietary) of H_2S was discussed. Secondly, the detailed endogenous production of H_2S and its metabolism in the biological system have been discussed. Thirdly, owing to the importance of H_2S in the biological system, different tools for the quantitative detection of H_2S have been discussed. Fourthly, a brief discussion about the different types of inhibitors that are used for the quenching of endogenous enzymes present in the biological system. Finally, a brief discussion about the role of H_2S in different disease conditions is highlighted. Moreover, the rate of release of H_2S (fast or sustained) from the H_2S releasing compounds (inorganic and organic donors) has been associated with either beneficial or toxic effects in the cellular system. The synthetic donors of H_2S are widely developed to mimic the slow and sustained endogenous production of H_2S in the biological system. A thorough discussion has been made on the development of different stimuli-responsive organic donors of H_2S such as- (i) hydrolysis-based H_2S donors, (ii) biothiol-triggered H_2S donors, (iii) light-triggered H_2S donors, (iv) enzyme-triggered H_2S donors etc. To quantify the release of H_2S from these donors, several detection techniques has been developed and improved over the years. The most widely used technique is the methylene blue (MB) assay wherein the sulfide species react in the presence of acidic solutions of *N*,*N*-dimethyl-*p*-phenylene diamine sulfate, ferric chloride ($FeCl_3$), and zinc acetate dihydrate $Zn(OAc)_2{\cdot}2H_2O$ to generate methylene blue (MB) with a characteristic absorbance maxima at 670 nm in the UV-Vis spectrum. The concentrations of H_2S can be determined by using a calibration curve obtained using samples of known concentrations of Na_2S. However, the limit of detection of this method is relatively lower (about 1 μM) due to the formation of dimer and trimer species in solutions. Additionally, the quantification of intracellular H_2S release is not possible due to the highly acidic conditions used in the measurement of H_2S. Notably, other detection techniques such as lead acetate has also been used to detect H_2S by measuring the formation of insoluble compound lead sulfide, which showed turbidity at 390 nm in the UV-

Vis spectrum. Electrochemical methods such as the ion-selective electrode method and polarographic sensors have also been used for the quantification of H_2S. The ion-selective electrodes use a silver sulfide membrane that specifically interacts with S^{2-} creating a change in potential across the membrane. The electrodes are not expensive, easy to operate and showed high selectivity towards H_2S. However, the main drawback of the electrochemical method is that frequent reconditioning of the electrode is necessary to remove the impurities of the previous experiments. Furthermore, these electrodes operate under highly basic conditions that are not suitable for monitoring intracellular H_2S release. Another method for the quantification of H_2S release is the monobromobimane method (MBB) that reacts with H_2S at basic pH (9.5) to generate a highly fluorescent sulfide dibimane (SDB) product. The formation of SDB can be detected using HPLC equipped with a fluorescence detector as a high fluorescence intensity band is generated that can be easily observed. The detection limit for the MBB method is 2 nM. However, the major drawback of this technique is that MBB showed side reactions with thiols. Although, several turn-on fluorogenic sensors of H_2S such as azide-based, nitro-based, disulfide-based and metal-based sensors have been developed for monitoring the intracellular level of H_2S, some of these sensors are also reactive towards the cellular abundant thiols. Moreover, these sensors also consume some amount of the intracellular H_2S for its sensing process and thereby reduces the intracellular level of H_2S further. Therefore, with the state-of-art knowledge on this topic, the real-time and convenient monitoring of the exogenous H_2S donation process is not feasible.

In the **second chapter**, a biothiol-triggered organotrisulfide-linked fluorogenic donors of H_2S such as **UTS-1** and **UTS-2** were developed that are compatible in both aqueous and cellular environments. The release of H_2S and fluorophore enables proper monitoring of the intracellular H_2S release and its further trafficking towards a specific intracellular organelle such as lysosome. The release of fluorophore from **UTS-1** and **UTS-2** was confirmed by spectroscopic studies. The H_2S releasing ability of **UTS-1** and **UTS-2** was measured by methylene blue assay. Both the probes release H_2S in the presence of cysteine and glutathione. Both the synthesized trisulfide donors are non-toxic in cancer (HeLa) and normal cell (HEK-293). In the **third chapter**, TrxR-responsive organopolysulfide-based fluorogenic donor of H_2S (**DCI-PS**) with concomitant release of NIR fluorophore are described. All the spectroscopic and kinetic studies with **DCI-PS**/**DCI-DS** revealed its much higher reactivity towards DTT (for TrxR activity) as compared to the cellular abundant biothiol GSH. The turn-on fluorogenic H_2S donation process from the cellular non-toxic **DCI-PS** was studied in a representative breast

cancer cell line (MDA-MB-231) for the sustained donation of H_2S with concomitant release of red-emitting NIR fluorophore. The participation of TrxR was supported by significant inhibition of the fluorogenic processes in the presence of TrxR selective small-molecule inhibitors. In the **fourth chapter**, cysteine-responsive isothiocyanate-based fluorogenic donors of H_2S **AM-ITC** and **NB-ITC** were developed. The spectroscopic studies for **AM-ITC** reveal that the probe is only reactive towards cysteine over other thiols such as GSH, DTT and Hcy. Mechanistic investigations reveal that isothiocyanates react with cysteine to release H_2S and the naphthalimide amine-based fluorogenic anti-cancer compounds. The higher anti-cancer activity of the released anti-cancer compound amonafide as compared to the prodrug **AM-ITC** was evidenced by the MTT assay in representative breast cancer cells as well as HeLa cells.