Chapter

3

Rationale, Objective & Plan of Work

3. RATIONALE, OBJECTIVE & PLAN OF WORK

3.1. RATIONALE & OBJECTIVE

Nitrogen and sulphur containing five membered heterocyclic compounds have occupied enormous significance in the field of drug discovery process. A bicyclic ring system with diverse chemical reactivity and broad spectrum of biological activity has been selected as novel subject for this study. Benzothiazole derivatives and their biological screening have acquired greater importance in recent decades. Exhaustive study on Benzothiazole ring suggests that, it is a privileged scaffold in modern medicinal chemistry particularly in discovering new anticancer and potent antimicrobial agents. Many fold changes can be done in its structural aspects for developing a second generation lead molecule, which allows achieving new pharmacological profile, with significant cytotoxicity against the cancer cell lines and selective potency against the microbial strains. Therefore, in the present study, an effort has been made to develop novel benzothiazole analogues.

Earlier, Bradshaw *et al.* reported that compounds within the substituted 2-arylbenzothiazole class represent extremely potent and selective antitumor agents [Bradshaw *et al.,* 1998]. Further, Vicini *et al.* have reported some Schiff bases of benzothiazole showing antimicrobial and anticancer activity [Vicini *et al.,* 2003]. Jagessar and his co-worker reported the presence of hydrogen bonding domain amide (–CONH–) seems to be valuable in the structures of antimicrobials [Jagessar and Rampersaud, 2007]. In 2010, Bhuva *et al.* reported substituted benzothiazole derivatives as tyrosine kinase inhibitors and Kamal et al. showed the DNA binding ability of substituted benzothiazole conjugates, which is crucial for the cytotoxicity of these compounds [Bhuva and Kini, 2010; Kamal *et al.,* 2010]. Taking observations from these evidences it was thought worthwhile to design templates of benzothiazole derivatives.

Modifications of the benzothiazole ring with respect to the oxygen-nitrogen donor atoms have pronounced effects on their coordination propensities and related pharmacological activity. Hence addition of semicarbazone/thiosemicarbazone/hydrazone or oxime group was made to study the effect of these pharmacophores on biological activity. These groups impart hydrophilicity and electron donating ability to the proposed compounds while substitution of arylidene and cycloalkylidine groups impart lipophilicity, which are essential for the structural model of benzothiazole containing antimicrobial and anticancer drugs. Thus, these observations prompted us to synthesize proposed benzothiazole compounds having different substitutions on the aromatic ring possessing

different linkages so as to produce novel, potent and selective drug molecules and being evaluated for the biological activity.

The involvement of the EGFR family of tyrosine kinases in cancer proliferation suggests that an inhibitor which blocks the tyrosine kinase activity of the entire EGFR family could have significant therapeutic potential. So we selected EGFR as a biological target for carrying out the enzymatic study of our synthesized compounds and learn the mechanism of activity. Glucosamine-6-phosphate synthase catalyses the formation of UDP-GlcNAc. Since N-acetylglucosamine is an essential buildings block of both bacterial cell wall and fungal cell wall chitin, and are potential target for antibacterial and antifungal agents. Therefore, both the enzymes were selected as targets of interest to simulate their binding preference with synthesized molecules using molecular modelling docking tool and predict the *in-silico* pharmacokinetic properties to assess the drug-likeness of synthesized compounds.

Based on above rationale, in our present investigation, we attempted to design five series of 2-aryl benzothiazole analogues by various structural modifications to study the effect of substituents on antimicrobial and antiproliferative activity (Fig. 3.1).

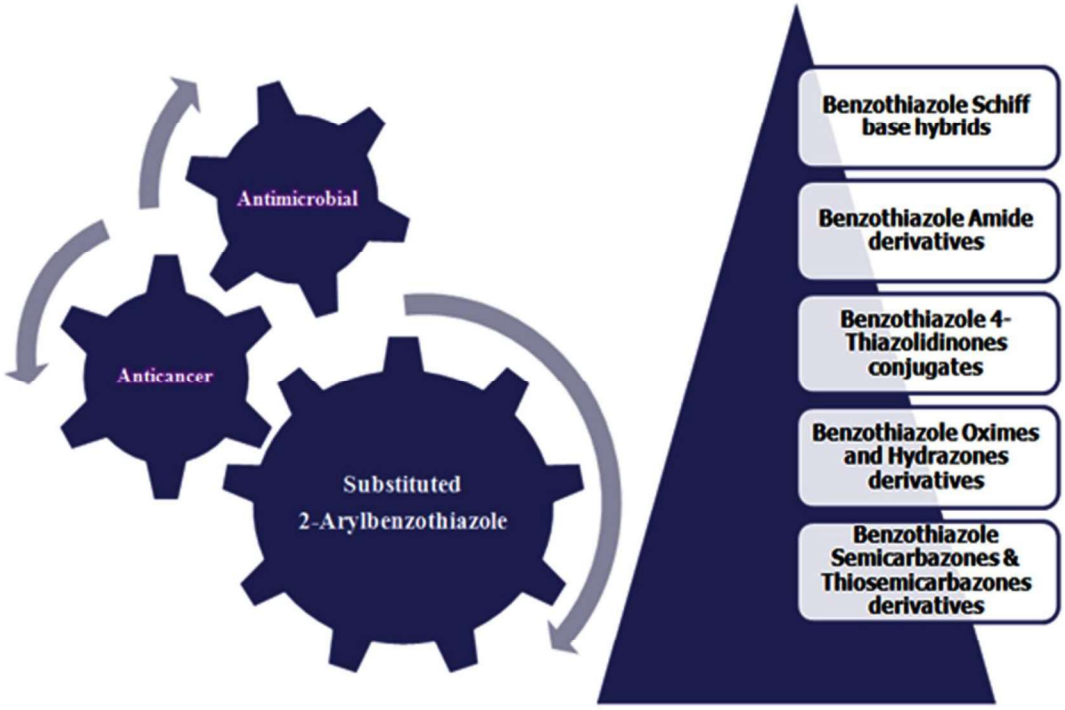

Fig. 3.1 Rationale of approach

3.2. DESIGN OF 2-(4-AMINOPHENYL)BENZOTHIAZOLE DERIVATIVES

Antimicrobial and Antitumor potential of benzothiazole compounds prompted us to continue our study towards design and synthesis of novel benzothiazole derivatives by various structural modifications (Fig. 3.2).

- Benzothiazole Schiff base hybrids were designed and synthesized to study the effect of substituted arylidine-benzenamine group and cycloalkylidine-benzenamine group on biological activity.

- The medicinal potential of oxazolidinones impelled us to extend the schiff base series, by synthesizing new 4-thiazolidinone derivatives.

- Hybridization of a bioactive moiety amide as hydrogen bonding domain with benzothiazole pharmacophore may result in synergistic antimicrobial and potential cytotoxic effect (due to increased lipophilicity).

- As reported in literature, a combination of heterocyclic compounds like benzothiazole, benzoxazole, benzimidazole *etc.* with potent cytotoxic thiosemicarbazone and semicarbazone pharmacophore has been shown to produce synergetic effects on the antimicrobial and anti-proliferative activity of the parent ligands so we planned to synthesize thiosemicarbazone and semicarbazone derivatives of benzothiazole bearing amides.

- Apart from thiosemicarbazones and semicarbazones, numerous hydrazones and oxime derivatives were are also found to be cytotoxic, so derivatives of benzothiazole bearing amide moiety were synthesized to investigate the effect of these pharmacophores on biological activity.

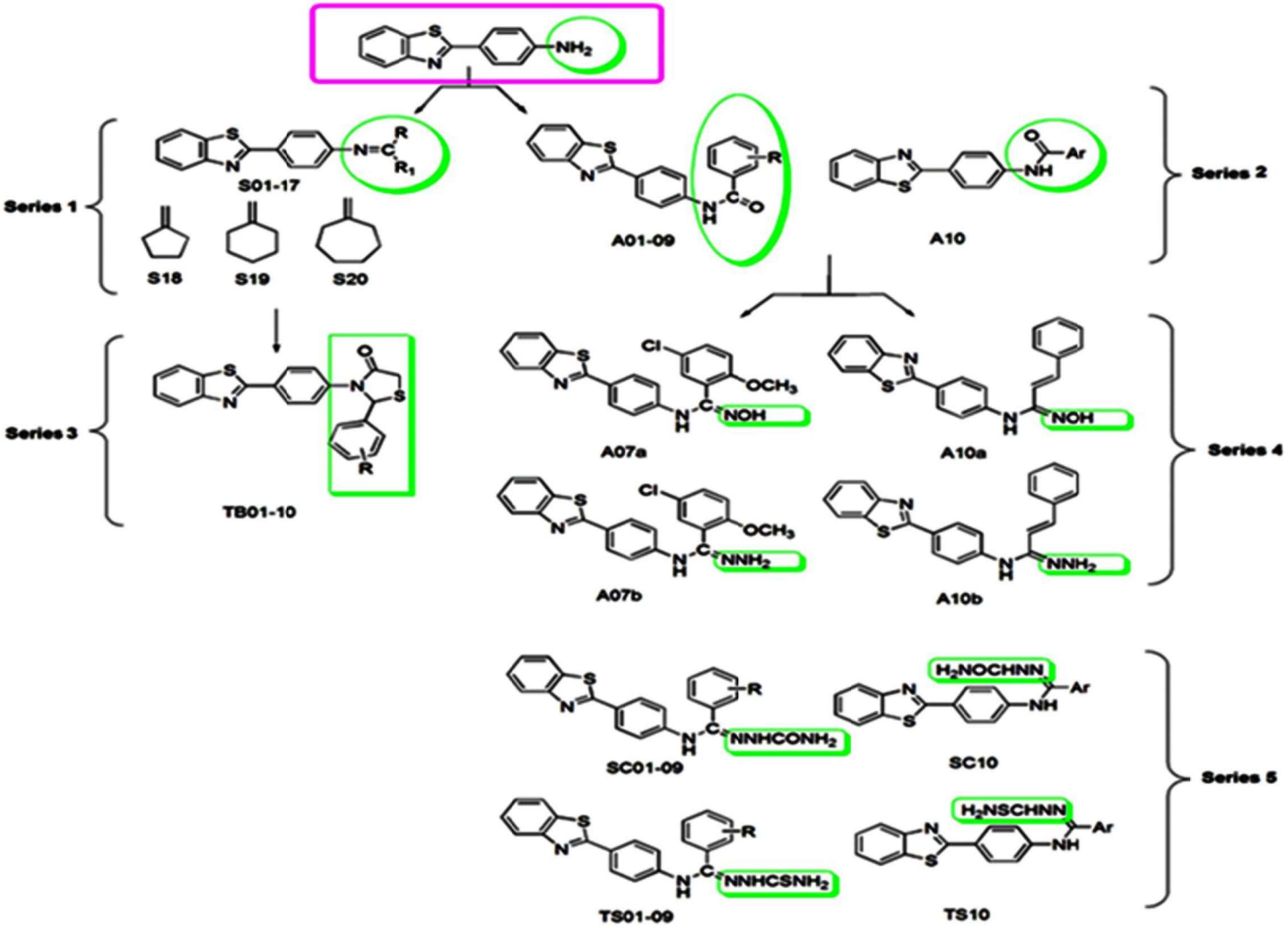

Fig. 3.2 Design of benzothiazole derivatives

3.3. PLAN OF WORK

A brief outline of the research work carried out is summarized as under.

3.3.1. Design and synthesis

- Schiff Bases of 2-(4-aminophenyl)benzothiazole i.e. 4-arylidine-benzenamine /cycloalkylidine-benzenamine benzothiazole.
- 4-Thiazolidinone derivatives of 4-arylidine-benzenamine/ cycloalkylidine-benzenamine benzothiazole.
- Benzothiazoles bearing amide moiety of 2-(4-aminophenyl)benzothiazole.
- Oxime and hydrazone derivatives of Benzothiazoles bearing amide moiety
- Semicarbazone and thiosemicarbazone derivatives of Benzothiazoles bearing amide moiety

3.3.2. Characterization of synthesized compounds

- Physicochemical characterization including solubility testing, melting point and TLC analysis (R_f value) etc.
- Structural confirmation by FT-IR, ^{1}H-NMR, ^{13}C-NMR, Mass and Elemental (CHN) analysis.

3.3.3. Biological evaluation

- *In-vitro* antimicrobial activity by agar disc diffusion method.
- Membrane permeabilization studies and DNA binding/cleavage activity.
- *In-vitro* anticancer activity by MTT assay.
- Flow cytometry (FACS), Apoptosis assay and DNA fragmentation assay.

3.3.4. In-Silico study

- Docking analysis using Sybyl-X1.2 and Autodock 4.0 molecular modeling software package
- Pharmacokinetic prediction by preADMET server