Chapter

6

Summary and Conclusions

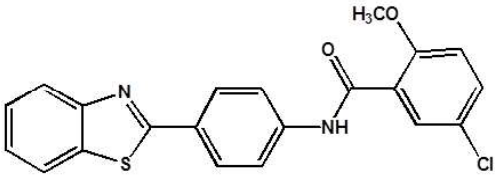
SUMMARY

Research and development of new antimicrobial and anticancer therapeutic agents are of paramount importance because of the innate ability of pathogens and tumor cells to develop resistance to existing therapies. Modern advances in cellular and molecular biology have enhanced our understanding of the various mechanisms of these diseases. In the recent years, benzothiazole analogues have attracted considerable attention in antimicrobial and anticancer research. Multiple analogues of these compounds enabled medicinal chemists to modify them and yield better antimicrobial and antineoplastic agents with improved therapeutic profiles but still the search is going on to obtain ideal drug candidates for microbial and cancer chemotherapy. So with the objective of developing potent antimicrobial and anticancer agents, we attempted to design and synthesized five different series of 2-(4'-aminophenyl)benzothiazole analogues by various rationally based structural modifications. Purity of synthesized compounds was ascertained by their melting points and R_f values (TLC analysis). The chemical structures of 2-(4'-aminophenyl)benzothiazole analogues were established by spectroscopic (UV-VIS, FT-IR, ^{1}H NMR, ^{13}C NMR, Mass) and elemental analysis.

Synthesized compounds were evaluated against various pathogenic bacterial species (Gram-negative and Gram-positive) viz., *Escherichia coli, Staphylococcus aureus, Salmonella typhi, Enterococcus faecalis, Pseudomonas aeruginosa and Klebsiella pneumoniae*. Antifungal activity of the compounds was evaluated against fungal species viz. *Candida albicans, Candida tropicalis* and *Candida krusei*. Further, potent analogues were screened for the mechanism of action in microbial strains. We studied the ability of antimicrobial inhibitors to bind with Lipopolysaccharides (LPS), to depolarize both the outer and cytoplasmic membranes, to interact with lipid monolayers, and to kill different bacterial strains. To explore the hypothesis that permeabilization of the cytoplasmic membrane is responsible for killing, we monitored cell viability via FACS (fluorescence assisted cell cytometer) analysis and cytoplasmic membrane permeabilization at the same time. Finally, we evaluated the interaction of lead molecules with plasmid DNA to probe into the molecular mode of action. Some of the compounds were found to be superior over the reference antimicrobial drugs used. All the used techniques completely described the mechanism of action and hence showed a good correlation between their calculated MIC's and its lethality. Docking results were carried out in order to rationalize the results. Further, on the basis of *in-silico* ADME studies, potent compounds could be further exploited for the development of more effective antimicrobial agents.

Similarly, synthesized compounds were evaluated for *in-vitro* anticancer activity by standard MTT assay, against two human ovarian cancer cell lines i.e. SKOV3 (endometroid adenocarcinoma) & A2780-S (undifferentiated EOC cell) and also on its cellular chemoresistant model (cisplatin resistant model, A2780-CR and paclitaxel resistant model, A2780-PR). The synthesized compounds were also evaluated on two human cervical cancer cell lines SiHa (HPV+ve) and C-33A (HPV−ve). Some of the potent compounds were further evaluated for cytotoxicity against normal ovarian surface cell line IOSE 364 and normal human embryonic kidney cell line HEK-293 to check their safety profile. Compounds having significant anticancer activity and good safety profile were evaluated for their ability to induce apoptosis triggered pathway in cancer cells and was determined by staining the cells with the AnnexinV-AlexaFlor488 and PI. Further, Cell cycle distribution was analyzed using FACS Calibur flow cytometer in conjunction with PI staining. It was performed to determine the nature of phase arrest in cell cycle. And finally, the DNA fragmentation of synthesized compounds was observed to examine the molecular mechanisms. As per literature, the mechanism involved in the benzothiazole cytotoxicity is the inhibition of EGFR. Hence, to determine the extent of ligand binding interaction, western blot analysis was carried out to determine the extent of inhibition of phosphorylation pattern in EGFR receptor. Further, to get an insight about binding preference of synthesized compounds within the active site of target enzyme (ATPase domain of EGFR, a hypothetical binding model has been proposed by docking studies using the Autodock 4.0 molecular modelling software and *in-silico* pharmacokinetic properties were also predicted to check whether compounds are able to move in higher phases of drug development or not.

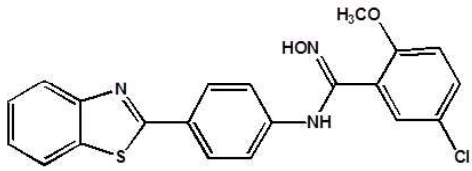
The study delineates the development of a total of **64** new potentially active antimicrobial and anticancer agents based on the benzothiazole pharmacophore. Out of 64 benzothiazole derivatives **7** of them are considered as lead molecules towards the development of antimicrobial and antitumor agents. It can be inferred that the desirable improvement in antimicrobial and anticancer activity of synthesized compounds requires electron releasing groups such as methoxy, hydroxy and electronegative groups such as chloro, fluoro for achieving the best biological spectrum. These studies are expected to provide useful insights into the roles of various substitution patterns on the benzothiazole derivative and also help to design more potent compounds in near future.



A07

N-(4-(benzo[d]thiazol-2-yl)phenyl)
-2-methoxy-5-chloro-benzamides

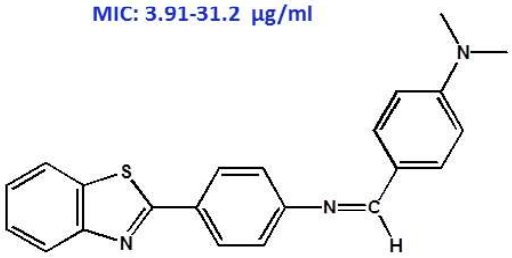
MIC: 3.91-31.2 µg/ml



A07a

N-(4-(benzo[d]thiazol-2-yl)phenyl)-(1′-oxime)-
2-methoxy-5-chloro-benzamides

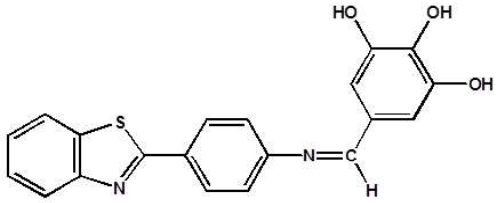
MIC: 3.91-31.2 µg/ml



S08

4-((4-(benzo[d]thiazol-2-
yl)phenylimino)methyl)-N,N-dimethyl
benzenamine

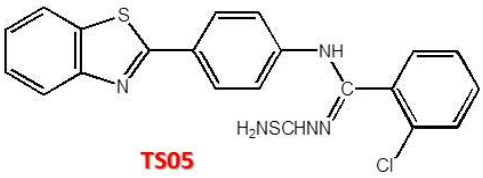
MIC: 3.91-31.2 µg/ml



S15

N-(3,4,5-trimethoxybenzylidene)-4-
(benzo[d]thiazol-2-yl)benzenamine

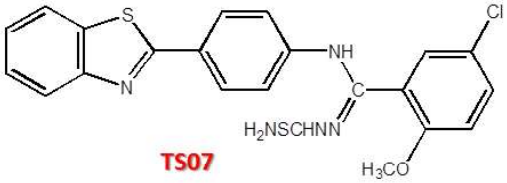
MIC: 3.91-31.2 µg/ml



TS05

N-(4-(benzo[d]thiazol-2-yl)phenyl)-
(1′-thiosemicarbazone)-2-chloro-benzamides

MIC: 1.56-15.6 µg/ml

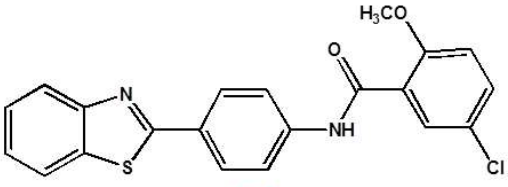


TS07

N-(4-(benzo[d]thiazol-2-yl)phenyl)-
(1′-thiosemicarbazone)-2-methoxy-5-chloro-
benzamides

MIC: 7.81-15.6 µg/ml

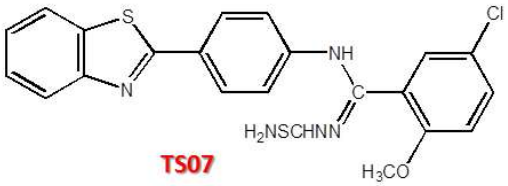
Potent Antibacterial Agents



A07

N-(4-(benzo[d]thiazol-2-yl)phenyl)
-2-methoxy-5-chloro-benzamides

MIC: 3.91-7.81 µg/ml

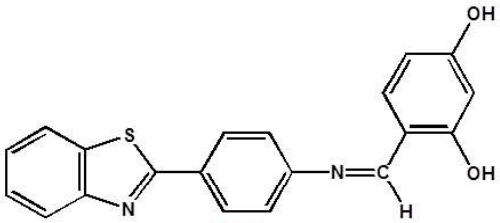


TS07

N-(4-(benzo[d]thiazol-2-yl)phenyl)-
(1′-thiosemicarbazone)-2-methoxy-5-chloro-
benzamides

MIC: 3.91-7.81 µg/ml

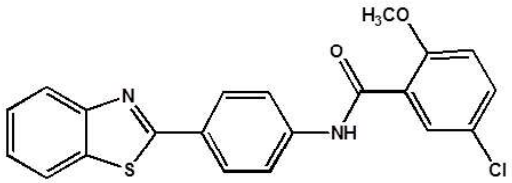
Potent Antifungal Agents



S05

N-(2, 4-dihydroxybenzylidene)
-4-(benzo[d]thiazol-2-yl)benzenamine

IC_{50}: 34 µg/ml; (SKOV3, A2780-S, A2780-CR cell lines)



A07

N-(4-(benzo[d]thiazol-2-yl)phenyl)
-2-methoxy-5-chloro-benzamides

IC_{50}: 6.13 µM; (SiHa cell line)

Potent Anticancer Agents

CONCLUSIONS

The study delineates the development of a total of **64** new potentially active antimicrobial and anticancer agents based on the benzothiazole pharmacophore. Our findings of biological screenings hold promise for excellent framework towards the search for potent antimicrobial and antitumor agents. By analysing the experimental results, it has been concluded that a total of five series have been designed, synthesized and screened for their pharmacological activity.

The Major findings of present research work concluded as follows.

The results of the *in vitro* pharmacological activity revealed compounds **S02**, **S08**, **S13**, **S15** and **S19** exhibited good antimicrobial activity with MIC value in the range of 3.91–31.2 µg/ml against *S.aureus, S.typhi, P.aeruginosa* and *E.coli.* Structure activity relationship suggests compounds having electron donating substituents such as methoxy, hydroxyl on phenyl ring as well as schiff bases of cyclic ketones have pronounced activity. Compounds **S08** and **S15** showed potent activity with membrane perturbing mode of action, explicated by membrane depolarization, fluorescent assisted cell cytometry (FACS) and intracellular mode of action revealed by plasmid DNA cleavage. Molecular docking studies also revealed compounds **S08, S13** and **S15** has minimum docking energy and may be considered as good inhibitor of GlcN-6-P.

Compound **A07** displayed most potent inhibitory activity with minimum inhibitory concentration (MIC) values of 15.6, 7.81, 15.6, 3.91, 7.81, 3.91 µg/ml against *S.aureus*, *E.coli*, *S.typhi*, *K.pneumoniae, C. albicans and C. tropicalis,* respectively. Structure–activity relationship (SAR) studies revealed that electronic and lipophillic factors of phenyl ring had a significant effect on the antimicrobial activity of the designed compounds. Compounds **A07** and **A10** showed excellent activity and membrane perturbing mode of action at concentration higher than MIC. These compounds were also able to alter the electrophoretic mobility of DNA. Molecular docking studies revealed compound **A07** and **A10** showed minimum docking energy on GlcN-6-P.

Compounds **TB01** and **TB06** were found to be the most active against *E.coli* and *C.albicans* with MIC values in the range of 15.6–125 µg/ml. Compounds **TB03** and **TB07** showed good activity against *E.coli* & *S.aureus* in the MIC range 31.2–62.5 µg/ml. SAR revealed electron donating groups had a great effect on the antimicrobial activity of these compounds. All the used techniques completely described the mechanism of action and hence showed a good correlation between their calculated MIC's and its lethality. Docking

studies revealed compound **TB06** showed maximum docking score on GlcN-6-P, thus dry lab findings are in agreement with wet lab.

Compounds **A07a** and **A07b** turned out to be the most potent analogues having MIC values in the range of 3.91-31.2 µg/ml against *S.aureus, S.typhi, P.aeruginosa* and *E.coli.* The new benzothiazole derivatives displayed different mode of action as elucidated by their studies on intact bacterial cells and plasmid DNA. Structure activity relationship studies revealed compound **A07a** containing oxime moiety along with less bulky electron releasing and lipophillic group (methoxy and chloro) had significant activity. Molecular docking studies revealed compound **A07a** showed maximum docking score (5.7 and 6.12) with GlcN-6-P.

Compound **TS05** displayed most potent activity with MIC values of 3.91, 7.81, 1.56 µg/ml against *S. aureus*, *E. coli* and *P. aeruginosa* respectively. Compound **TS07** shows excellent MIC in *C. krusei* and *C. albicans* in range 3.91 – 7.81 µg/ml. SAR study revealed variation in activity of the compounds can be correlated to their lipophilic and electronic behavior. These derivatives showed excellent cell selectivity and killed the bacteria by membrane disruption and also able to alter the electrophoretic mobility of DNA. Compounds **SC02, SC08, SC09, TS07** and **TS08** were nicely accommodated in the GlcN-6-P receptor with **SC02** exhibited maximum docking score (8.12).

Thus, it can be inferred that the desirable improvement in antimicrobial activity of synthesized compounds requires electron releasing groups such as methoxy, hydroxy and electronegative groups such as chloro, fluoro for achieving the best antimicrobial spectrum. Some of the compounds were found to be superior over the reference drugs used. All the used techniques completely described the mechanism of action and hence showed a good correlation between their calculated MIC's and its lethality. Docking results were carried out in order to rationalize the results.

The results of antiproliferative screening indicated that compound **S05** containing dihydroxy substituents had potent inhibitory activity with IC_{50} value 34µg/ml against SKOV3, A2780-S and A2780-CR cell lines. It showed more potent cytotoxicity in combination with cisplatin and paclitaxel than alone in the selected cell lines (SKOV3, A2780 and A2780-CR models). The *in vitro* cytotoxicity of the compounds on IOSE 364 cell line was evaluated to establish the selectivity. Molecular docking study exhibited good binding against epidermal growth factor receptor, which was further ascertained by immunoblot assay using specific antibody against phosphorylated EGFR, and thus unravelling the targeted anticancer mechanism. Benzothiazole derivatives also exhibit pronounced cytotoxicity in the HPV16-positive SiHa cells as compared to HPV-negative

C-33A cells. Compound **A07** showed significant cytotoxicity with IC_{50} value of 6.13 µM against HPV +ve SiHa cell line with potential apoptosis inducing behaviour. The *in-vitro* cytotoxicity of the compounds on HEK-293 cell line was evaluated to establish the selectivity. Cells treated with benzothiazole derivatives showed prominent morphological features as evidenced by cell shrinkage, membrane blebbing, apoptotic nuclei and DNA fragmentation.