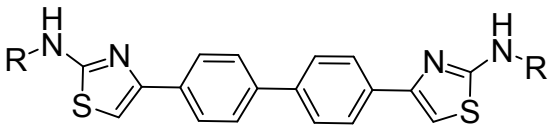
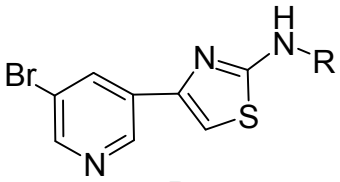
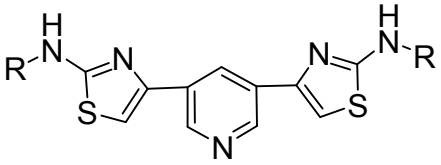
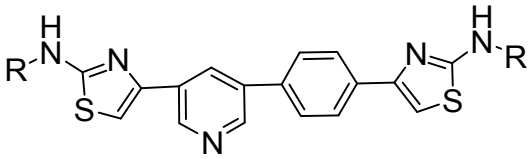
6. SUMMARY AND CONCLUSION:

In the present research work, a series of thiazole derivatives were designed, synthesized and characterized by ^{1}H-NMR, ^{13}C-NMR and LC-MS. These compounds were designed structurally based on the marine natural products cystothiazole A and myxothiazole, which possess bithiazole skeletal as well as displayed potent antifungal activity and cytotoxicity. Hence the schemes were developed to synthesize three series of bithiazole derivatives as mimics of cystothiazole A and myxothiazole with some structural changes, the two thiazole rings were elongated by the insertion of the aromatic rings and the side chains were modified with effective aromatic and aliphatic substitutions.

In Scheme-1, one of the major intermediate substituted thioureas (7a-u) was synthesized as displayed in Table 3.1.In Scheme-2, the two thiazole rings were elongated by two phenyl rings which looked like biphenyl skeletal which is represented as Precursor A in the Table 6.1 and the synthesized thiazole derivatives (8a-u) are displayed in Table 3.2. In Scheme-3, the design contained the 5-bromo-monothiazole ring which is represented as Precursor B in the Table 6.1 and the synthesized thiazole derivatives (15a-o) are displayed in Table 4.1. In Scheme-4, the distance between the two thiazole rings was reduced by one aromatic ring by a pyridine skeletal. This is represented as Precursor C in the Table 6.1 and the synthesized bithiazole derivatives (19a-u) are displayed in Table 4.2. In Scheme-5, again the two thiazole rings were elongated with two aromatic rings of phenyl and pyridine ring skeletal which is represented as Precursor D in the Table 6.1 and the synthesized bithiazole derivatives (25a-u) are displayed in Table 4.3.

Table 6.1 Table of precursors of (8a-u), (15a-o), (19a-u) and (25a-u)

 A	 B
 C	 D

The elongation of the thiazole rings in the bithiazole derivatives has given a major contribution in the biological evaluation because the free NH_2 group attached in the thiazole ring at second position is also extended. Hence, the potential of the bithiazole derivatives to bind the biological target keeps on changing by the H-bonding.

The newly synthesized four series of thiazole derivatives (8a-u), (15a-o), (19a-u) and (25a-u) were evaluated for antimicrobial and cytotoxicity activities. The antibacterial screening was performed against *Staphyllococcus aureus (ATTC-25923), Escherichia coli (ATTC-25922), Pseudomonus aeruginosa (ATTC-27853), Klebsiella pneumonia (recultered).*

The antifungal screening was performed against *Penicillium marneffei (recultered), Trichophyton mentagrophytes (recultered), Aspergillus flavus (NCIM No.524), Aspergilus Fumicatus (NCIM No.902).*

The *in vitro* antimicrobial study was performed and *minimum inhibitory concentration* (MIC) and was recorded in 6.25–100 µg/ml by agar streak method using *ciprofloxacin* and *ketoconazole* as a standard of antibacterial and antifungal respectively.

The *in vitro* cytotoxicity study was performed for the newly synthesized thiazole derivatives (8a-u), (15a-o), (19a-u) and (25a-u) against *He-La carcinoma cell line* performed using MTT assay.

6.1. ANTIBACTERIAL ACTIVITY:

The investigation of antibacterial activities of the mono thiazole derivatives (15a-o) has shown good to moderate activities in 12.5-100 µg/ml compared to the bithiazole derivatives against all strains. Among the bithiazole derivatives of (8a-u), (19a-u) and (25a-u), the more potent active compounds are given below in Table 6.2.

Table 6.2 Table of antibacterial activity of potent and good active compounds

Antibacterial activity (in all strains)	**Precursor A (8a-u)**	**Precursor C (19a-u)**	**Precursor D (25a-u)**
Potent active	8c, 8i, 8j, 8t and 8u	19a, 19c, 19i, 19j, 19t and 19u	25c, 25f, 25i,25l,25t and 25u
Good active	8a, 8b and 8k	19d, 19e, 19f, 19g, 19h, 19k, 19l, 19s and 9o	25b, 25d, and 25k

From the table 6.2, all the three series of bithiazole derivatives show potent activity in all the strains. In compounds 8t, 19t and 25t the substitution R = H was mentioned that the precursor A, C and D (Table-6.1) itself possess potent activity. The elongation of thiazole rings in the bithiazole skeletal has reduced the antibacterial activity even though the polar group of pyridine ring is present in the precursor D. The substitutions on the NH- group also contributes a major role in the antibacterial activity, because the compounds 8t, 19t, 25t, 8u, 19u and 25u have shown potent activity due to the presence of $-CF_3$ and free NH_2 group. The other potent compounds shown in the table also have same type of substitutions. So the changing of substitution on the NH group is contributing more towards the bacteria.

Among the three precursors, the precursor C shows potent active for more substitutions of MIC in the range of 6.25-50 µg/ml, because elongation of thiazole rings in the bithiazole skeletal is less and the thiazole rings are attached in the nonlinear manner. Precursor D also possess nonlinear manner but shows less potent activity compared to Precursor C and D.

6.2. ANTIFUNGAL ACTIVITY:

The investigation of antifungal activities of the mono thiazole derivatives have shown good to moderate activities compared to the bithiazole derivatives of more potent activity against all strains. Among the bithiazole derivatives of (8a-u), (19a-u) and (25a-u) the more potent active compounds are given below in Table 6.3. From the Table 6.3, all the three series of bithiazole derivatives show good activity in all the strains. In compound 25t, the substitution R= H was mentioned that the precursor D (Table-6.1) itself possess potent activity. The other precursor A

and C were not shown potent antifungal activity and the different substituted derivatives (8a-u and19a-u) were also shown good to moderate activity.

Table 6.3 Table of antifungal activity of potent and good active compounds

Antifungal activity (in all strains)	**Precursor A (8a-u)**	**Precursor C (19a-u)**	**Precursor D (25a-u)**
Potent active	-	-	25b, 25i, 25k, 25t and 25u
Good active	8c, 8i and 8t	19a, 19c, 19l and 19u	25a, 25c, 25f and 25j

The elongation of thiazole rings in the bithiazole skeletal has reduced the antifungal activity in the precursor A. The substitutions on the NH- group also contributes a major role in the antifungal activity, due to the presence of $-CF_3$ and free NH_2 group in precursor D and other derivatives (25b, 25i, 25k and 25u) have also shown potent to good antifungal activity. So the changing of substitutions has contributed more in the antifungal activity of precursor D.

Among the three precursors, the precursor C has shown potent active for more substitutions of MIC in the range of 6.25-100 µg/ml, because elongation of thiazole rings in the bithiazole skeletal is more and the thiazole rings are attached in the nonlinear manner. Precursor C also possesses nonlinear manner but shown less potent activity compared to Precursor D and shown better activity than precursor D.

6.3. CYTOTOXICITY ACTIVITY:

On the cytotoxicity screening, all the bithiazole derivatives have displayed good to potent activity in the *He-La cell line* medium.

Among them, the precursor A compounds (8a-u) have displayed potent activity due to the distance between the free NH atom at the 2nd position of the thiazole ring is more and the presence of the -CF_3 groups at 3,5-position of phenyl rings are increasing the lipophilicity and polarity of the compound. So cleavage of the mitochondrial is possible and the survival of the cell will be reduced which may lead to the cell death.

Compound 8k shows IC_{50} value of 159.5 ng/ml at 24 hrs and 70.3 ng/ml at 48 hrs respectively, due to the presence of electro negative fluoro atom at 3rd position of phenyl group. Compound 8t shows IC_{50} value of 73.88 ng/ml in 24 hrs and 58.58 ng/ml in 48 hrs respectively, due to presence of free NH_2 group. Compound 8u shows optimum inhibition with IC_{50} value of 65.14 ng/ml in 24 hrs and 41.64 ng/ml in 48 hrs respectively, due to the presence of electro negative –CF_3 group at 3, 5-position of phenyl group.

In the pyridine containing bithiazole derivatives, 19i showed potent inhibition of IC_{50} value of 83.03 ng/ml in 24 hrs and 56.85 ng/ml in 48 hrs respectively, due to presence of electro negative –CF_3 at 3rd position of the phenyl group.

In the pyridine and phenyl containing bithiazole derivatives, compounds 25i, 25k, 25q and 25u showed good inhibition. Among them 25q showed potent inhibition of IC_{50} value of 94.9 ng/ml in 24 hrs and 65.14 ng/ml in 48 hrs respectively, due to presence of electro negative chloro

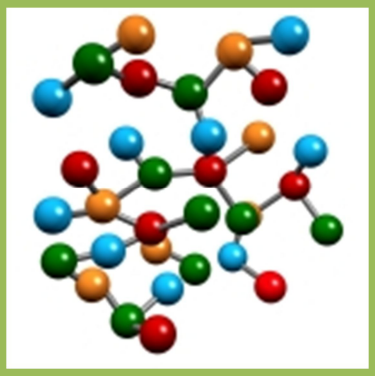
atom at 3, 5- positions of the phenyl group. Compound 25u showed very good changes of IC_{50} value of 343.1 ng/ml in 24 hrs and 73.88 ng/ml in 48 hrs respectively, due to the presence of electro negative -CF_3 group at 3, 5- positions of the phenyl group. So the Compound 25u is found to be time dependent cytotoxic against the *He-La carcinoma cell line*.

To conclude, the newly synthesized four series of thiazole derivatives (8a-u), (15a-o), (19a-u) and (25a-u), were screened for the *in vitro* antimicrobial study performed with *minimum inhibitory concentration (*MIC) and recorded in 6.25-100 µg/ml. The *in vitro* cytotoxicity study was performed with IC_{50} value of 41.64 ng/ml against the *He-La carcinoma cell* by the MTT assay method.

The potent thiazole derivatives 8c, 8i, 8j, 8t, 8u, 19a, 19c, 19i, 19j, 19t, 19u, 25c, 25f, 25i, 25l, 25t and 25u are recommended for further antibacterial study.

The potent thiazole derivatives 25b, 25i, 25k, 25t and 25u are recommended for further antifungal study.

The potent thiazole derivative 8a is strongly recommended for further *in vivo* cytotoxicity study on several cell lines and clinical research.



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