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Research Article

DEVELOPMENT OF THE PHARMACEUTIC CRYSTALLIZATION PROCESS USING *PLASMODIUM FALCIPARUM*, A DERIVATIVE OF THIOSEMICARBAZONE

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ABSTRACT

Objective: The current study was designed with the goal of analyzing novel derivatives of organic and semi-organic NLO molecules that are just entering this field as the foundation for future therapeutic development.

Methods: Thiosemicarbazones (TSC) are semicarbazide analogs that have sulfur atoms in place of oxygen atoms. The greatest number of therapeutic treatments for various disorders are also being used carbonyl compounds. These two types of organic compounds were combined, and their replacement with crystal growth procedures using solution growth was tested as an antibiotic for a few fastidious and nonfastidious species. The preparation of meta-substituted benzaldehydes using TSC. Tests were conducted with this single component. The following experimental technique was used to determine the antibacterial activity of *Plasmodium falciparum.* Half-inhibitory maximum (IC_{50}) by calculation technique, cell viability % calculation method, and MTT assay by colorimetric method.

Results: Method using agar discs. Using the agar disc diffusion method to measure the inhibition zone width and comparing the minimal inhibitory concentration (MIC) and inhibition zone width of available antibiotics against the aforementioned organism using data from the EUCAST and NCCLS databases, The absolute, relative, and mound slope values of an antibiotic that is currently on the market may be determined using the GraphPad Prism software; the epidemiological cutoff value can be determined using the ECOF Finder software; and the nature of the antibiotic can be determined using the WHONET 5.6 software. The newly developed antibiotics' size and structure are contrasted with the structures of commercially available antibiotics and living organisms.

Conclusion: The design of new drugs using novel derivatives of organic and semiorganic NLO molecules is based on the in vitro method of analysis, which has recently been introduced to this field. *P. falciparum* has an extremely low MIC value (0.625 µg/mL) against TSCMNB. The MIC break points are always larger than, not equal to, the MIC values and fall within the range of 110 µg/mL–130 µg/mL. We concluded that all of the novel compounds exhibit a susceptible form of inhibition.

Keywords: MTT Assay, Cell viability, IC_{50}, Agar dis diffusion, Graph pad, ECOF finder, EUCAST, NCCLS.

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INTRODUCTION

Due to the rapid emergence of antibacterial and antifungal treatment resistance, treating microbiological diseases brought on by bacteria and fungus has become a significant global issue. The resistance of numerous targets to the current medications and the mutations of bacteria and viruses have emerged as major issues in the medical industry. To address these dangers, new treatments and medicines are therefore required. It has been observed that metal complexes with thiosemicarbazone (TSC) ligands and their derivatives exhibit good medicinal properties and seem advantageous in terms of generating less toxic and more potent medications. A review of the literature reveals that metals are selective for target cells and that their coordination affects how well ligands bind to proteins [1].

Metals have proven to be quite useful in the fields of medical chemistry and drug creation. The bonding of metal atoms or ions with ligands forms coordination complexes [2]. The prevalence of fungus infections has increased globally in recent decades. One of the main objectives of medicinal chemistry is the creation of novel therapeutic agents. Due to their extensive range of biological activities, which include antimycobacterial, antibacterial, antifungal, antiviral, and antimalarial effects, as well as their adaptability as nitrogen and sulfur donors, which enables them to bring on a great variety of coordination modes, TSCs have long been studied for medicinal studies.

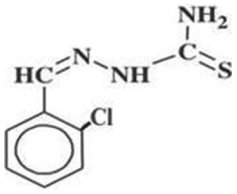
Due to their tendency to react with a variety of metals, TSCs are a crucial class of N and S donor ligands [3]. Schiff bases have biochemical and pharmacological applications because they may be effective antibacterial and anticancer agents [4,5]. The efficacy of second harmonic generation in free TSC ligands and their metal complexes has been hypothesized to be aided by significant electron delocalization in the TSC moiety [6,7]. We describe the crystal structure of a novel Schiff base compound generated from thiosemicarbazide and 3-nitrobenzaldehyde here as part of research on non-linear optical materials, notably TSCs and their metal complexes.

TSC organic crystals exhibit strong heat stability and non-linear optical characteristics. TSC compounds that have an asymmetrized electron conjugation system between the electron donor and acceptor groups are also extremely polarizable substances that can be used in NLO applications. The production, growth, and anti-microbial effectiveness of TSC derivatives of 2 chlorobenzaldehyde crystals are thus reported in the current work.

METHODS

Chemicals used for the study

The solution-growth technique Crystals of TSC of 2-chlorobenzaldehyde have been grown in the supersaturated solution at room temperature. The structures of these crystals are crystallized below.



Antimicrobial study

Disc diffusion test-preparation of Mueller-Hinton agar

The manufacturer's instructions must be followed for making Mueller-Hinton agar from a dehydrated base that is readily available in the marketplace. Allow it to cool in a water bath at 45–50°C right after autoclaving. To achieve a consistent depth of around 4 mm, pour the freshly prepared and cooled medium into glass or plastic petri dishes with flat bottoms. For plates with a diameter of 150 mm, this equates to 60–70 cc of medium. The agar medium needs to reach room temperature before being stored in a refrigerator (2–8°C) until it is time to utilize the plate. After cooling, plates must be used within 7 days by incubation at 30–35°C for 24 h or longer [8].

Disc diffusion strategies

Antimicrobial susceptibility scrutiny commonly uses the Kirby-Bauer and Stokes' [9] techniques, with the Kirby-Bauer method being approved by the NCCLS. On an agar plate subculture, at least 3–5 carefully isolated colonies with the same morphological type are chosen. Each colony's pinnacle is touched with a loop, and the boom is then put into a tube with 4–5 mL of an appropriate broth medium and tryptic soy broth. The broth subculture is incubated at 35°C for typically 2–6 h, or until it reaches or exceeds the turbidity of the 0.5 McFarland wide spread.

Analyzing plates and deciphering results

Each plate is proved 16–18 h after incubation if the inoculum is authentic and the plate was properly marked. The diameter of the disc is measured along with the sizes of the zones of total inhibition as determined by unassisted vision. The bacteria are classified as sensitized, intermediate, or resistant to the antibiotic drugs that have been tested based on the diameters of the zones of inhibition.

Minimal inhibitory concentration (MIC)

MIC break point

The term "MIC breaking point" refers to the antibiotic concentration at which bacteria are most effectively inhibited. The break point of the given organism is the sensitive (S) MIC. More than 90% are likely to be effective. (I) Intermediate If antibacterial concentrations are used, they might be effective at higher doses. Resistant (R) MIC>organism break point. You won't be able to take safe amounts of the medicine at high concentrations.

IC_{50} and IC_{90} values

After studying the MIC values, additional investigation of the inhibitory concentrations (IC) at 50% and 90% of the bacterial lines is finished. In order to assess each drug's efficacy, the IC_{50} or IC_{90} is essentially used to calculate the dosage of an antimicrobial agent to treat the disease in the area. Utilizing software from Graph Pad Prism, the IC_{50} and IC_{90} values are determined.

MTT assays study

Cell culture

Bacteria were cultivated in liquid medium (DMEM) containing 10% fetal bovine serum, 100 ug/mL antibiotics, and a 5% CO_2 atmosphere at 37°C.

MTT assays

Any test involving different strains or clinical samples of *ex vivo* cells requires assays that allow for the quantitative measurement of cellular death during cell subculture. Place 1,000–100,000 cells in a 96-well plate at the suitable spacing and incubate for the required amount of time (often 6–48 h) while using the proper stimulant. Remove the media and give the cells a PBS wash. MTT prepared in medium should be uploaded at a final concentration of 0.5 mg/mL.

When intracellular crimson formazan crystals can be observed under a microscope, incubate for 30–4 h at 37°C. Tritrate and solubilizing solutions should be uploaded instead of MTT. For 30–2 h, incubate at ambient temperature or at 37°C until the cells have listed and the red crystals have disappeared. The 570 nm absorbance measurement.

Application of computers in antibacterial susceptibility testing

WHONET 5.6 Software

The instructions at http://www.who.Int/emc/WHONET/instructions.htm were discovered using the Whonet 5.6 software. This program is helpful in identifying clusters of resistant isolates and emerging outbreaks, as well as providing local laboratories with the most recent recommendations and techniques.

GraphPad Prism 8 Software

GraphPad Prism 8 software was utilized to find out the IC_{50} and EC50 values by nonlinear regression methods.

Test ECOF finder

The Test ECOF Finder was utilized to find out the epidemiological cutoff value (ECV) for the drug against bacteria's and to find out the drug wild types are not non wild types.

RESULTS AND DISCUSSION

Antimicrobial activites

Agar disk diffusion study

To determine the *Plasmodium falciparum* inhibitory zone, petridish plates are employed. 1.25 µg/mL is the ideal antibiotic concentration for this TSC3NB. At a dosage of 1.25 µg/ml of TSC3NB, the maximal inhibition zone for this *P. falciparum* organism is 27 mm (Fig. 1). The zone with <20 mm is susceptible, according to the EUCAST and NCCLS databases (Fig. 2).

As a result of the foregoing discussions, our new antibiotic, TSC3NB, is susceptible to the bacteria *P. falciparum*. An *in-vitro* study showed that it is more effective against *P. falciparum* bacteria than existing antibiotics, and it is also resistant to human malaria, blood cancer, kidney failure, and liver fever.

Agar Disk Diffusion Methods(Inhibition Zone with) *P. falciparum*-concentration of antibiotic 1.25 (µg/l).

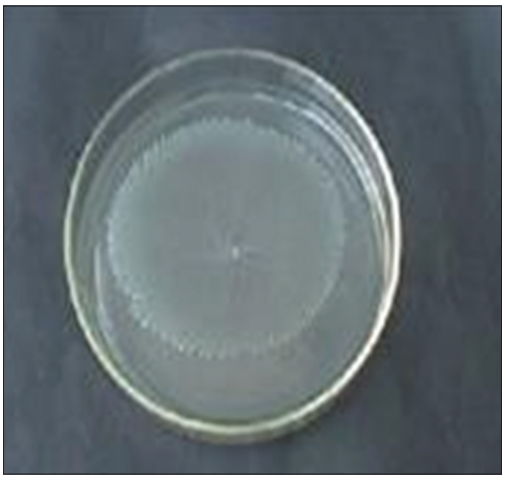


Fig. 1: TSC3NB (27 mm)

***P. falciparum* versus TSC of M-Nitrobenzaldehyde**

A little gram-negative parasite called TSC3NB is evaluated for compatibility. Through the use of the EUCAST and NCCLS databases, the antimicrobial activity was evaluated using the MTT assay, disc diffusion, MIC, graph pad Prism 8.3, Ecoffinder, and WHONET 5.6 analysis methods and compared to that of the currently used antibiotics against the *P. falciparum* organism. In the lab, slow evaporation solution growth procedures are used to create the antibiotic TSC3NB, and the recrystallization method is used to purify it. A common way to determine the degree of *P. falciparum* organism inhibition by laboratory-made TSC3NB is the MTT assay method.

The procedure is explained in the experimental part. The results are tabulated, and the optical density values for cell viability with and without the TSC3NB were colorimetrically determined at a 570 nm filter (Table 1 and Fig. 3).

The blank is preserved as the control (100%), and the percentage of inhibition is computed using the formula for TSC3NB values ranging from 0.625 µg/mL to 160 g/mL. The relation of TSC4NB concentration to cell viability is inverse. The MIC is the lowest TSC4NB concentration at which the highest proportion of inhibition occurs. The MIC break point is the antibiotic concentration at which inhibition starts to occur. The MIC break point for this organism against TSC4NB is determined to be 1.25 µg/mL. Because the MIC point in this instance is higher than the MIC, the antibiotic TSC3NB is susceptible to this meticulous bacterium (Table 2 and Fig. 3).

The existing antibiotics for this organism, *P. falciparum*, are imipenem, azithromycin, roxithromycin, and telithromycin. Their MIC break point is obtained from the EUCAST and NCCLS databases. Their susceptible MIC break points are <2 mg/l, <1 mg/l, <0.5 mg/l, and <0.25 mg/l, respectively, as shown in Table 3 and Fig. 4. TSC3NB has an optimum MIC break point of 11 mg/l or 110 µg/mL. IC_{50} values are the amount of drug that leads to inhibiting half of the targeted bacteria; they are manually calculated by using the formula. It was found to be 1.08 µg/mL. Graph Pad Prism 8 software is utilized to find the IC_{50} value and to draw the curve between cell viability percentage and log c (µg/mL). The IC50 absolute value was 0.86 µg/mL, and the relative IC_{50} range is between 0.79 and 0.94 µg/mL. This is around 1 µg/mL. This IC_{50} is optimal for good drugs. The hill slope value of the curve is –ve, which indicates the +ve inhibition of *Escherichia coli* by the TSC3NB derivative. The IC_{50} values of existing antibiotics against this bacteria are also less than or equal to one µg/mL.

For this parasite, *P. falciparum*, there are now four medications available: imipenem, azithromycin, roxithromycin, and telithromycin. Their MIC break point is found in the databases of EUCAST and NCCLS. According to Table 3 and Fig. 4, their susceptible MIC break points are 2 mg/l, 1 mg/l, 0.5 mg/l, and 0.25 mg/l, respectively. The ideal MIC breakpoint

Fig. 2: TSC2CB (14 mm)

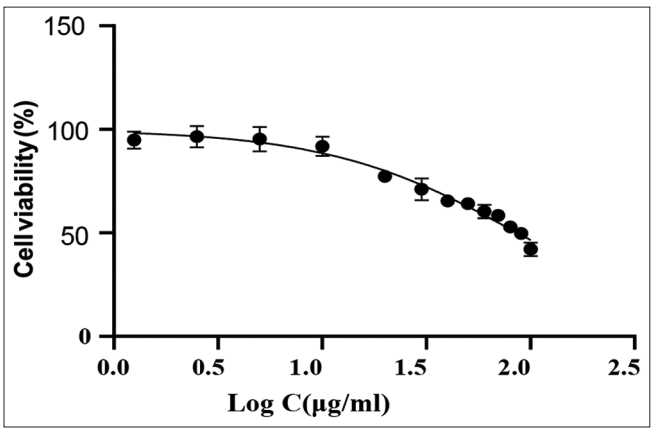


Fig. 3: Hill slope curve

Table 1: The percentage of cell viability of metabolic activity used for MTT assay test

S. No	Tested sample concentration (µg/ml)	Cell viability (%) (In Triplicates)			Mean value (%)
1.	Control	100	100	100	100
2.	100	39.48	40.98	45.70	42.06
3.	90	50.00	51.28	47.85	49.71
4.	80	51.28	52.14	55.15	52.86
5.	70	59.22	56.43	59.65	58.44
6.	60	63.09	56.65	61.37	60.37
7.	50	65.66	62.44	63.94	64.02
8.	40	65.87	64.37	65.87	65.37
9.	30	77.25	68.45	67.59	71.10
10.	20	77.46	79.61	74.67	77.25
11.	10	93.77	95.06	86.48	91.77
12	5	98.28	99.14	88.41	95.27
13	2.5	96.56	97.85	90.12	94.84
14	1.25	99.14	99.78	90.55	96.49
15	0.625	91.63	96.56	88.84	92.34

Table 2: Analysis of log (inhibitor) versus normalized response variable slope

log (inhibitor) versus normalized response--variable slope	
Best-fit values	
Log IC_{50}	1.937
Hill Slope	−0.9465
IC_{50}	0.8642
95% CI (Profile likelihood)	
Log IC_{50}	1.901–1.977
Hill Slope	−1.068–−0.8370
IC_{50}	0.7963–0. 9495
Goodness of Fit	
Degrees of Freedom	37
R squared	0.9557
Sum of Squares	579.2
Sy.x	3.957
Replicates test for lack of fit	
SD replicates	3.622
SD lack of fit	4.653
Discrepancy (F)	1.651
p-value	0.1424
Evidence of inadequate model?	No
Number of points	
# of X values	39
# Y values analyzed	39

for TSC3NB is 11 µg/mL or 110 µg/mL. The IC_{50} values are manually derived using the algorithm and represent the amount of drug leads needed to block 50% of the desired bacterium. The measurement was 1.08 g/mL. The IC_{50} value and the curve between the cell viability % and log c (µg/mL) are determined using the graph pad prism 8 software. The relative IC_{50} range is between 0.79 and 0.94 µg/mL, whereas the absolute IC_{50} value was 0.86 µg/mL. approximately 1 µg/mL.

Ecoffinder study

For this medicine against organisms, the ECV is determined using the ECOF FINDER program. The ECV value is 0.08 µg/mL, and the MIC for this *P. falciparum* organism is 1.25 µg/mL at a 95% population. Since the MIC in this instance is higher than the ECV, the organism is not the bacterium's wild-type distribution for the given medication. The clinical treatment failure rates of TSC3NB against *P. falciparum* are, therefore, null and void, and it is possible that this strain of TSC3NB has an acquired form of resistance to this microorganism. As can be seen in (Figs. 5 and 6) for the density curve and cumulative curve, this ECV value of 0.08 µg/mL is much below the suggested CLSI and EUCAST break points.

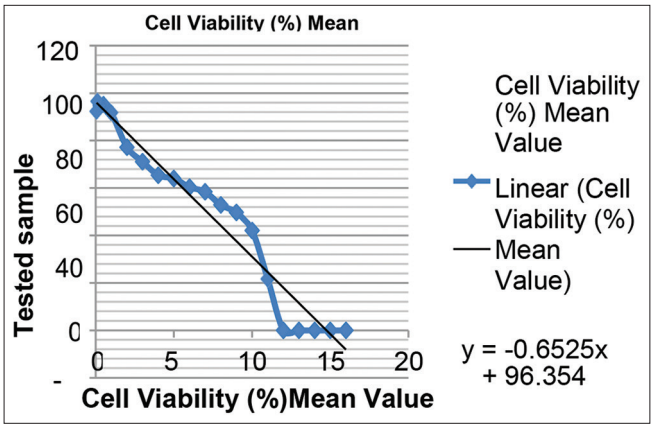


Fig. 4: MIC and MIC break point curve

Table 3: MIC and MIC break point

S. No	Tested sample concentration (µg/mL)	Cell viability (%) Mean value
1	160	0
2	150	0
3	140	0
4	130	0
5	120	0
6	110	21.65
7	100	42.06
8	90	49.71
9	80	52.86
10	70	58.44
11	60	60.37
12	50	64.02
13	40	65.37
14	30	71.1
15	20	77.25
16	10	91.77
17	5	95.27
18	2.5	94.84
19	1.25	96.49
20	0.625	92.34

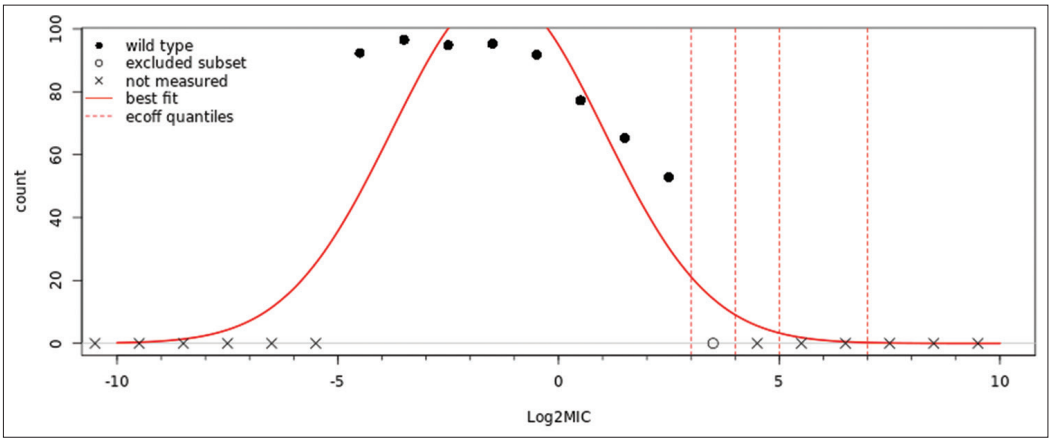


Fig. 5: Density curve

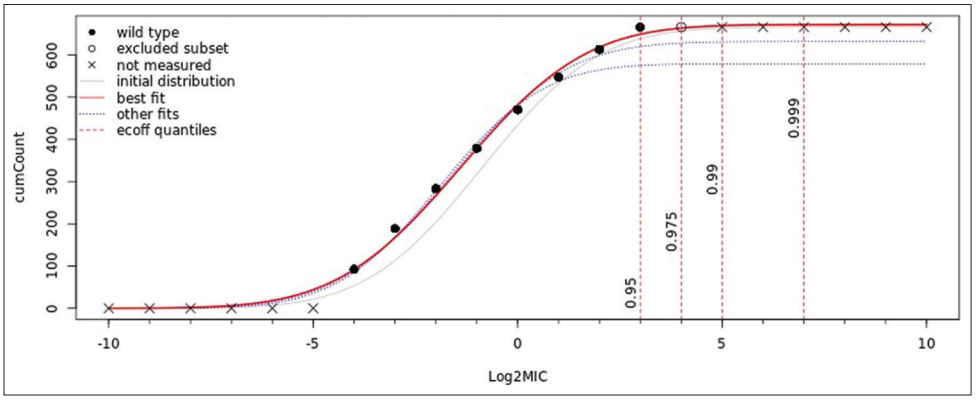


Fig. 6: Cumulative curve

Table 4: Whonet output data

Code	Antibiotic name	Antibiotic class	Antibiotic subclass	Code	BreakPoints	Number	%R	%I	%S	%?	%R 95%C.I.
RXT_ND30	Roxithromicin	Macrolides		RXT		1	0	0	0	100	0.0–94.5
TLT_ND15	Telithromycin	Macrolides	Ketolide	TLT	19–21	1	0	100	0		0.0–94.5
TCY_ND10	Tetracycline	Tetracyclines		TCY		1	0	0	0	100	0.0–94.5
NAL_ND30	Nalidixic acid	Quinolones	Quinolone	NAL	14–18	1	0	100	0		0.0–94.5
BETA_LACT	Beta-lactamase			BETA		1	0		100		
ESBL	ESBL			ESBL		1	0		100		

Whonet 5.6 Software analysis

Utilizing the Whonet 5.6 software. The effect of current antibiotics on the *P. falciparum* bacterium has been examined. Blood samples are the only kind used, and just one isolate was used. The following antibiotics, which were examined against *P. falciparum*, are listed in Table 1 along with their class, subclass, code, method, break point, range of susceptibility, isolate number, percentage of resistance, and percentage of intermediate antibiotics. In this instance, all antibiotics are resistant because their MIC break point is lower than the MIC values depicted in Fig. 3. However, the TSC3NB has a lower MIC than the MIC break point, making it susceptible. They are all ineffective against this organism. WHONET Study results reveal that all the Existing antibiotics in medicals against the above mentioned bacteria (Organisms) are having comparatively poor performance than the TSC derivatives. Structures and Size of all Existing antibiotics in medicals against the above mentioned bacteria (Organisms) are having comparatively larger shape and size than the TSC derivatives. There fore They Lost their Surface area and enter in to and Exit from bacteria is difficult for existing antibiotics. The epidemiology cut off value (ECV) for all the three TSC derivatives against all the organisms are found around 0.08 (95%) Dg/ml (Table 4) in all the eleven cases the MIC values are higher than there ECV value therefore organisms tested in this study are not belonging to wild type distribution against all the three TSC derivatives. Hence there is no chances for failure in the clinical treatment is confirmed.

Plasmodium falciparum
Number of isolates=1
Use expert interpretation rules
Specimen type-Blood

Genuine and occasionally fatal allergic reactions, or anaphylactoid reactions, have occurred in individuals taking anti-infection medications. People who have a history of affect ability to numerous allergens are prone to these reactions. Penicillins and cephalosporins have been known to interact. If a reaction occurs, stop taking the affected drug unless the condition is dangerous and can only be treated with that anti-infection. Genuine anaphylactoid reactions necessitate prompt epinephrine crisis management. As demonstrated, oxygen, intravenous steroids, and aircraft route executives, including intubation, should also be used. Nearly all antibacterial agents have been used to account for *pseudomembranous colitis*, which has escalated in severity from mild to dangerous. Thus, patients who present with looseness of the bowels should take this analysis into consideration.

CONCLUSION

The design of new drugs using novel derivatives of organic and semiorganic NLO molecules is based on the in vitro method of analysis, which has recently been introduced to this field. The MIC values are quite low for the parasite *P. falciparum* (0.625 μg/mL) against TSCMNB, according to the conclusions drawn from the aforementioned antimicrobial. The MIC break points are always larger than, not equal to, the MIC values and fall within the range of 110 μg/mL–130 μg/mL. We came to the conclusion that all of the novel compounds exhibit a susceptible form of inhibition. The graph pad prism software also determined the half inhibitory maximum value (IC_{50}), and all of the TSC derivatives have an IC_{50} value that is <1 (0.7–0.9 μg/mL).

According to the results of the WHONET investigation, all currently utilized antibiotics in medicine were not as effective against the aforementioned microbes (organisms) as TSC compounds. All currently prescribed antibiotics for treating the aforementioned bacteria (organisms) have shapes and sizes that are disproportionately larger than TSC derivatives; as a result, they lost surface area and found it challenging to enter and exit bacteria. In all instances where the MIC values are higher than the ECV value, the ECV for all of the TSC derivatives evaluated in this study against all of the organisms is found to be approximately 0.08 (95%) μg/mL, indicating that the organisms do not belong to the wild-type distribution against these TSC derivatives. Therefore, it is established that there are no chance for the clinical treatment to fail. Low physician knowledge of RUM and high drug costs prevent patients from taking medications as prescribed.

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CONFLICTS OF INTEREST

The authors declare that there were no conflicts of interest in this research.

AUTHORS FUNDING

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