Section A-Research Paper
ISSN 2063-5346

Novel Schiff Bases Derived from Substituted 1,3,4-Thiadiazole (TDA): Synthesis, Biological Evaluation and In Vitro Anti–Microbial Studies

Sajid Ajit Malak
Assistant Professor, Department of Chemistry, H.J. Thim College of Arts and Science, Jalgaon, Maharashtra
Email ID: sajidmalik20025@gmail.com

Madhuri Satish Patil
Associate Professor, Department of Chemistry, JDMVPASC College of Arts and Science, Jalgaon, Maharashtra
Email ID: madhurisatishpatil@gmail.com

Mohd. Mustakim Sahrif
Assistant Professor, Department of Chemistry, H.J. Thim College of Arts and Science, Jalgaon, Maharashtra
Email ID: bagwanms@rediffmail.com

Abstract

The 1, 3, 4-thiadiazole heterocyclic nucleus is widely recognized in the scientific community due to its presence in numerous natural products and medicinal agents. The present study focuses on Schiff bases on substituted 1,3,4-thiadiazole (TDA): synthesis, biological evaluation, and in vitro antimicrobial activity. In this work, the synthesis of Schiff bases (S1-S8) was carried out by reacting 5-(napthalen-4-yl)-1,3,4-thiadiazole-2-amine with substituted benzaldehyde. The reaction was conducted at room temperature using a grinding method and catalytic amounts of ethanol. This procedure successfully yielded the desired products S1-S8. The antibacterial and antifungal activities of all synthesized compounds were tested against several pathogens. In our study, we found that substances S2 and S3 exhibited broad-spectrum antibacterial activity, meaning that they were effective against every kind of microorganism we used. In addition, substances S8, S6, and S5 showed no antimicrobial activity against the whole range of bacteria and fungi examined. New compounds and targets in microbial cells or clinically developed antimicrobials should be studied to combat opportunistic infections.

Keywords: ***Substituted 1,3,4-Thiadiazole (TDA); synthesis; Schiff bases; antimicrobial activity; antifungal activity.***

Introduction

Antimicrobial drugs represent a significant advancement in therapeutics, making them one of the most notable contributions of the 20th century. The commercialization of drugs significantly altered the perspective of physicians regarding the potential impact they can have on diseases (Basher et al., 2020). The thiadiazole moiety is known for its versatility and ability to display a diverse range of biological activities. The component functions as a "hydrogen binding domain" and a "two electron donor system" within a restricted pharmacophore (Senthil Kumar Raju et al., 2022). Thiadiazole has the potential to serve as a bio-isosteric substitute for the thiazole moiety. It exhibits similar characteristics to third and fourth-generation cephalosporins. Therefore, it can be utilized in the formulation of antibiotic preparations. Thiadiazole is a five-membered ring system that consists of two nitrogen atoms and one

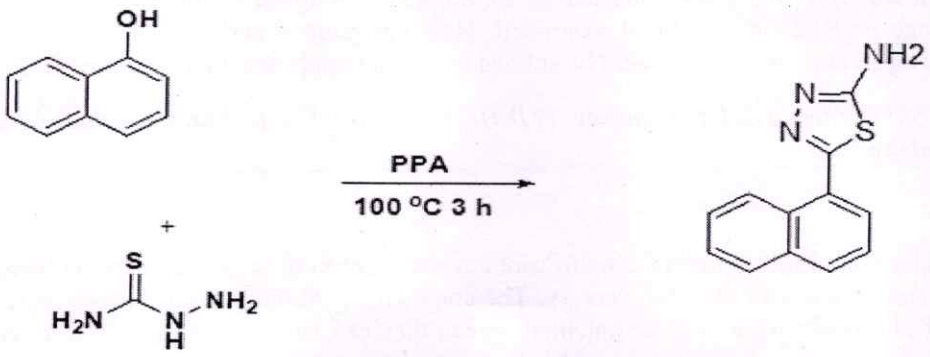
sulfur atom. The isomer of 1, 3, 4-thiadiazole in the thiadiazole series, along with its dihydro-derivatives, has been extensively documented in the existing literature on thiadiazole compounds. The literature review revealed that the thiadiazole nuclei exhibit antimicrobial properties. The compound exhibits antitubercular, anticancer, anti-inflammatory, antidepressant, anticonvulsant, antioxidant, anti-leishmanial, and radioprotective activities (Hameed et al., 2019).

There are several drugs available in the market that contain thiadiazole nuclei, including acetazolamide, methazolamide, and sulfamethazole, among others. Subsequent research efforts have resulted in notable advancements in the understanding of chemistry and biology. Schiff's bases or imine compounds are chemical products that contain an azomethine moiety (-CH=N-). These compounds are significant due to their extensive range of biological and pharmacological activities. These compounds possess a widely recognized antimicrobial activity. A significant quantity of molecules containing the imine moiety has been synthesized and evaluated for their antimicrobial properties (Malik & Nema, 2016). There are numerous biological effects that Schiff bases and derivatives of thiadiazoles can have. As antibacterial, antitubercular, and anticancer medicines, they are employed. 1,3,4-thiadiazole and imine are both molecules with well-established antibacterial properties. As a result, drugs containing the two groups may have increased antibacterial action. Therefore, creating novel products with moieties and testing their biological and antibacterial activity is a wise investment (Basher et al., 2020). The present study focuses on the Schiff Bases Derived from Substituted 1,3,4-Thiadiazole (TDA) its synthesis, biological evaluation and in Vitro anti–microbial activity.

Materials and Method

Synthesis of Thaidiazoles

A 100 mL round bottom flask containing polyphosphoric acid was charged with 0.011 moles of thiosemicarbazide and 0.011 moles of 1-napthoic acid. The reaction mixture was subjected to stirring for 30 minutes on a hot plate. The reaction mixture underwent reflux for three hours, during which it was periodically monitored using thin-layer chromatography (TLC). After reaction completion, approximately half (50%) of the solvent was obtained through vacuum distillation within the temperature range of 35-40 °C. Following 30 minutes of stirring the reaction mass, it was subjected to filtration and subsequently rinsed with water. The resulting mixture was then introduced into a container containing 20 grams of crushed ice. The purity of the isolated solid was enhanced through the process of recrystallization using a hexane-ethyl acetate solvent mixture (**Scheme 1).**

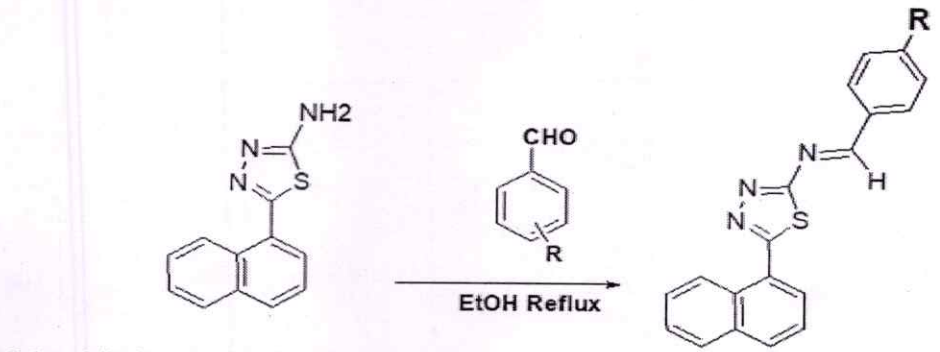


Scheme 1. Synthesis of Thaidiazoles

Synthesis of Schiff Bases

The Schiff bases (S1-S8) were synthesized by reacting equimolar quantities of 5-(napthalen-4-yl)-1,3,4-thiadiazole-2-amine and substituted benzaldehyde (**Scheme 2**). Prior to combining, each component was

dissolved in a small quantity of ethanol. Also, the reaction mixture was stirred for two hours at room temperature. The reaction mass was introduced into the cold water. Also, the solid product obtained was collected through the process of filtration and subsequently dried at a temperature of 80 °C using a drying oven. The product was subjected to re-crystallization using ethanol as the solvent, followed by a drying process. This resulted in the production of a pure product with a yield of 85%. Further, the obtained compounds were characterized using ^{1}HNMR spectroscopy, FTIR spectroscopy and Mass spectrometry.

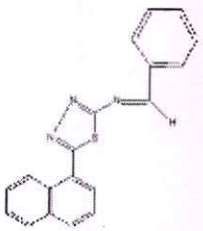
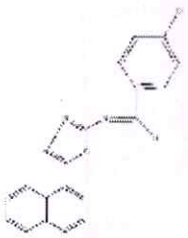


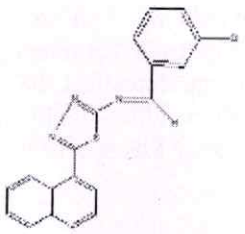
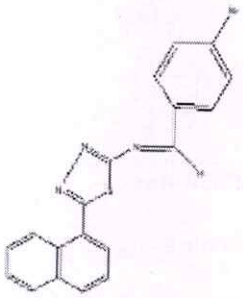
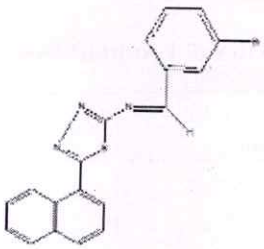
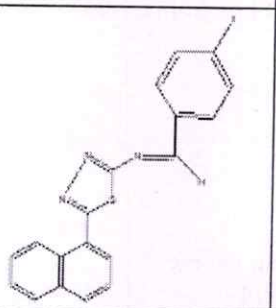
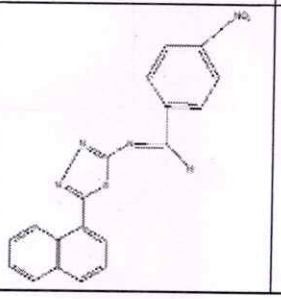
5-(naphthalen-4-yl)-1,3,4-thiadiazol-2-amine

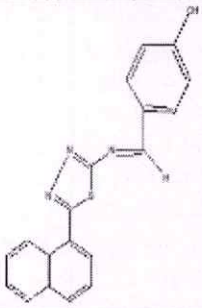
S-1 to S-8

Scheme 2: Synthesis of Schiff Bases

Table 1: Physical properties of compounds (S1 – S8)

Sr. No.	Name of derivative	Structure	Color and State	Yeild (in %)	MP (°C)
1	S-1		Yellow Solid	90	120
2	S-2		Pale yellow Solid	92	110

3	S-3		Yellow Solid	90	114
4	S-4		White Solid	90	108
5	S-5		Yellow Solid	90	116
6	S-6		Orange Solid	90	114
7	S-7		Pale yellow Solid	90	98

8	S-8		White Solid	88	120

Antimicrobial activity

Utilizing various bacteria, the compounds' in vitro antimicrobial properties were assessed using a micro-broth dilution experiment. The following microbial strains were acquired from the National Chemical Laboratory, Pune, India: Pseudomonas aeruginosa NCIM 5031, Escherichia coli NCIM 2065, Bacillus subtilis NCIM 2699, Aspergillus Niger NCIM 620, Aspergillus fumigatus NCIM 902, and Aspergillus flavus NCIM 549. At 37 °C, bacterial strains were kept alive in nutritional broth (NB), while fungi were kept alive in Sabouraud dextrose broth.

Preparation of inoculums

For Bacteria - The inoculum strains of bacteria were grown to an optical density of 0.6 at 600 nm at 37 degrees Celsius. For susceptibility testing, the number of viable bacteria in a sample was determined by serial plate dilution and then adjusted to between 105 and 106 CFU/mL.

For Fungus - The inoculums of the fungi were cultivated in cultures on potato dextrose agar for 10 days. After scraping the conidia with a sterile spatula, 8-10 mL of distilled water was added to the Petri plates and the culture was allowed to grow. Each fungal spore concentration was fine-tuned using a spectrophotometer (A595 nm) to the final value - 105 spores/mL, approximately.

Micro broth dilution assay

Micro broth dilution technique 20 was used to determine the MIC as per NCCLS recommendations. The experiment was conducted using Hi-media 96-well culture plates. Eight different concentrations of the compounds were prepared by dissolving them in DMSO and placing the resulting solutions (20, 10, 5, 2.5, 1.25, 0.625, 0.3125, and 0.15625 mg/mL) in the wells. Dimethyl sulfoxide was utilized to make the negative control, while Tetracycline and Amphotericin B in the same quantities were employed for the positive control. The bacterial and fungal cultures in the 96 well plates were then incubated at 37°C for 24 and 48 hours, respectively. The minimal inhibitory concentration (MIC) of a substance is the lowest concentration at which its effects on plant development are detectable. These tests were conducted three times.

Results and Discussion

Characterization of the Synthesized Compounds: The synthesis of Schiff bases (S1-S8) was carried out by reacting 5-(napthalen-4-yl)-1,3,4-thiadiazole-2-amine with substituted benzaldehyde. The reaction was conducted at room temperature using a grinding method and catalytic amounts of ethanol. This procedure successfully yielded the desired products S1-S8. The synthesized derivatives' chemical structures were deduced based on their spectral data.

Section A-Research Paper
ISSN 2063-5346

The ^{1}HNMR spectrum of S1 showed a singlet at δ 6 ppm and a multiplet δ 7- 8 ppm for aromatic hydrogens. The IR spectrum of the S1 compound showed bands at 1675 cm^{-1} for (C=N stretching) and at 3285 cm^{-1} due to N-H stretching. The ^{1}HNMR spectrum of S2 (fig 1) showed significant singlet signals at δ 3 - 4 ppm, another singlet signal at δ 6 ppm due to proton attached to carbon of the imine group (H-C=N) and multiplet signals from δ 7 – 8.5 ppm due to aromatic hydrogens. The IR spectrum of compound S2 (fig 2) showed strong absorption bands at 1570 cm^{-1} and 830 cm^{-1}due to C=N stretching and C-Cl stretching, respectively, with a mass of 349.04. The NMR spectrum of S3 showed significant multiple singlets at 2.5 ppm, 6 ppm (H-C=N proton) and multiple peaks at 8 ppm for aromatic hydrogens. Compound S3's IR spectrum exhibited strong absorption bands at 1580 cm^{-1} and 503cm^{-1}due to (C=N) (C–Cl meta substituted) stretching, respectively, with a mass of 349.04. The NMR spectrum S4 showed singlet peaks at δ 2 - 4 ppm, 6 ppm and multiplet from δ 7 - 8 ppm for aromatic hydrogens Similarly, the IR spectrum showed bands at 525cm^{-1} (C-Br stretching) with a mass of 392.99.

The NMR spectrum S5 showed two singlet signals at δ 3-2 ppm and multiple peaks with doublet peaks at 7-8 ppm for aromatic hydrogen, with the IR spectrum showing bands at 503 cm-1 (C-Br stretching meta-substituted). The NMR spectra of the S6 compound showed singlets signals at 3-2 ppm, singlet at 6ppm, multiplet for 7-8 ppm, and IR showed absorption at 525 cm-1(C –I stretching) with a mass of 440.98. The NMR spectrum of compound S7 showed singlets at δ 2-3ppm and 6.5 ppm and multiplet signals at 7.5 - 8.2 ppm, which are due to aromatic hydrogens. The NMR spectrum of compound S8 showed from multiplet signals at 8 ppm for aromatic hydrogens, with the IR spectrum showing 3467cm^{-1} (OH stretching).

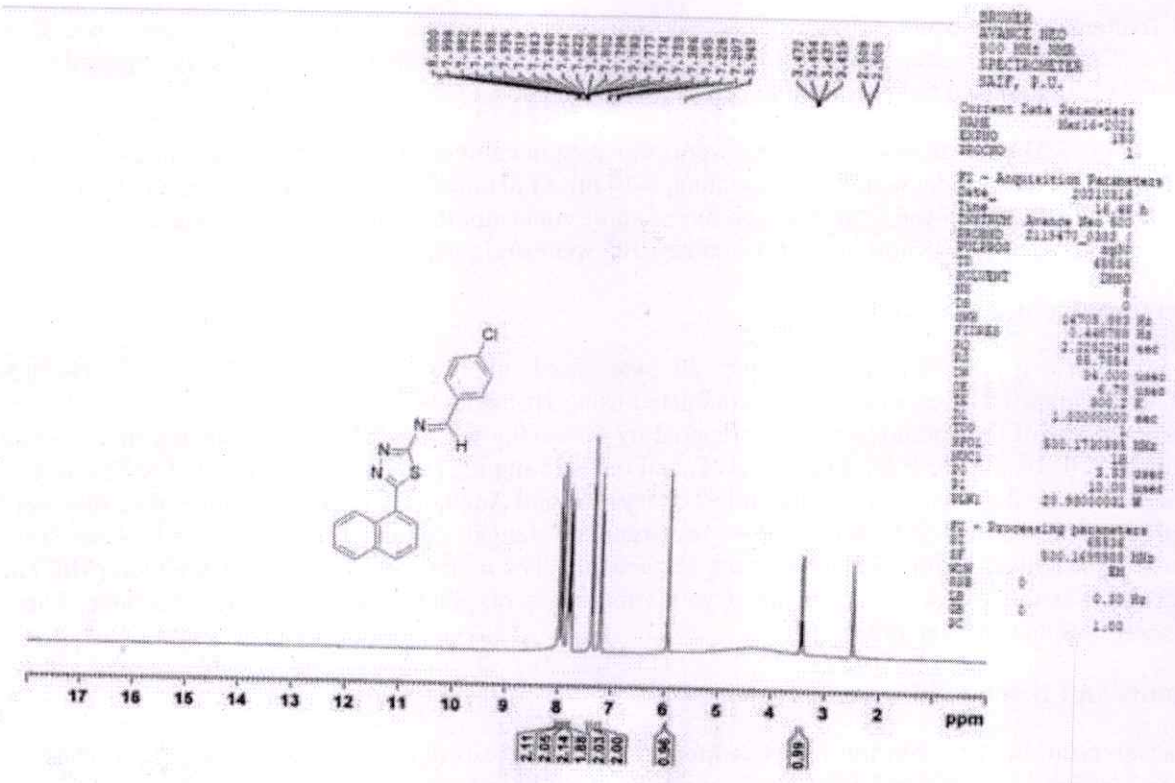


Fig. 1. NMR Spectrum of S2 compound

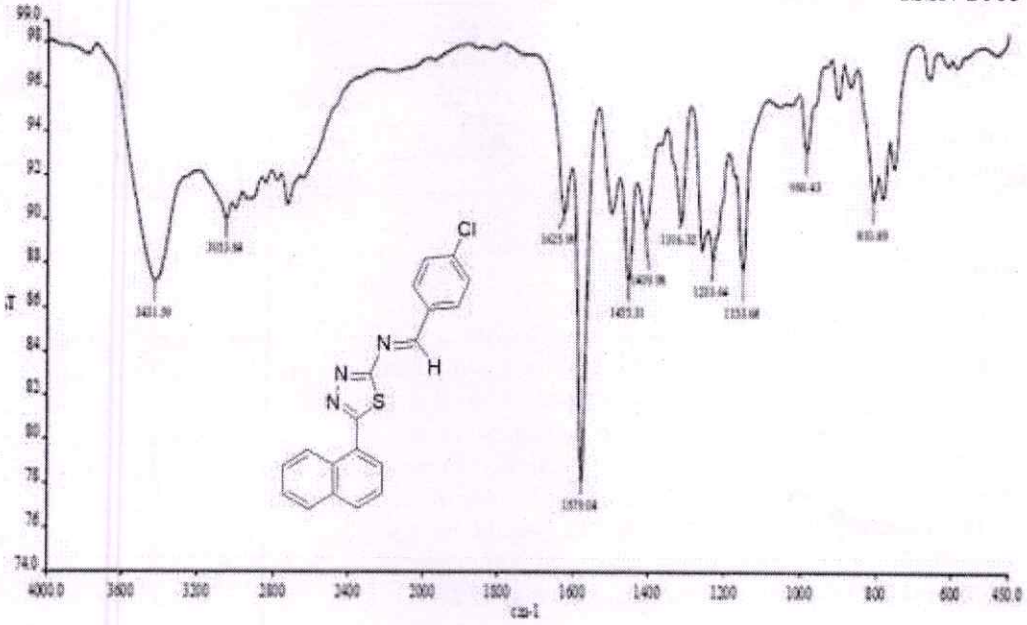


Fig.2 IR spectrum of the S2 compound

Antimicrobial Screening

The exponential growth of antimicrobial resistance is widely recognized as a significant health issue, as pathogenic microorganisms are progressively acquiring resistance to antimicrobial or anti-infective substances (O'Meara, 2020). Eight synthesized 1,3,4-thiadiazole compounds were tested for their antibacterial efficacy against various pathogens in the present investigation. Against all of the pathogens we studied (*P. aeruginosa, E. coli, A. fumigatus,* and *A. flavus*), substances S2 and S4 showed antibacterial activity. Almost all S2, S3 and S4 showed a positive effect on *E.coli.* Furthermore, compounds S2 and S3 also showed considerable action against *A. Fumigatus*, while they did not have any activity toward bacterial pathogens *(B. Subtilis).* In addition, substances S8, S6, and S5 showed no antimicrobial activity against the whole range of bacteria and fungi examined. Studies have shown that 1,3,4-thiadiazole compounds have antimicrobial properties, namely significant antibacterial and antifungal activities (Kamoutsis et al., 2021). The MIC was also configured, as displayed in **Table 2.**

Table 2: Antimicrobial activity and MIC of synthesized thiadiazole derivatives.

Compound codes	*P. aeruginosa*	*E. coli*	*B. subtilis*	*A. niger*	*A. fumigatus*	*A. flavus*
S-1	1.25	1.25	1.25	1.25	1.25	1.25
S-2	2.5	2.5	1.25	1.00	2.5	1.25
S-3	1.25	2.5	2.5	2.5	2.5	2.5
S-4	2.5	2.5	1.25	1.00	--	--
S-5	1.25	1.25	1.25	1.25	1.25	1.25
S-6	1.25	1.25	1.25	--	--	--

S-7	1.0	1.25	1.25	2.5	1.25	1.25
S-8	1.25	1.25	1.00	1.25	1.25	1.25
Tetracycline	0.00125	0.01	0.00125	-	-	-
Amphotericin B	-	-	-	0.00125	0.000156	0.000156

Conclusion

After synthesizing Schiff bases tagged with a 1,3,4-thiadiazole moiety and testing them for antibacterial activity, we have concluded that these compounds are physiologically significant. Molecules with specific antibacterial and antifungal activity, as well as those with activity directed toward pathogens such as *P. aeruginosa, E. coli, B. subtilis, A. fumigatus, A. niger,* and *A. flavus* may be differentiated in the pool of examined compounds. The most significant benefit of the newly produced bioactive compounds is their antibacterial specificity. Molecules S2 and S3, with action against all infections, have the best therapeutic potential. Many studies should be conducted to create novel antimicrobials and treatment techniques to combat opportunistic infections, such as new chemicals and targets in microbial cells or new antimicrobial tactics in clinical development.

References

1. Basher, N., Flifel, I. A., & Mashaf, A. K. (2020). *Synthesis, Characterization and antibacterial Study of some Complexes Derivatives from1,3,4– Thiadiazole Schiff base. 928*(5), 052009–052009. https://doi.org/10.1088/1757-899x/928/5/052009
2. Bhongade, B. A., Talath, S., Gadad, R. A., & Gadad, A. K. (2016). Biological activities of imidazo[2,1-b][1,3,4]thiadiazole derivatives: A review. *Journal of Saudi Chemical Society*, *20*, S463–S475. https://doi.org/10.1016/j.jscs.2013.01.010
3. Hameed, S. A., Varkey, J., & Jayasekhar, P. (2019). Schiff bases and Bicyclic derivatives comprising 1, 3, 4-thiadiazole moiety-A Review on their Pharmacological activities. *Asian Journal of Pharmaceutical Research*, *9*(4), 299. https://doi.org/10.5958/2231-5691.2019.00047.9
4. Kamoutsis, C., Fesatidou, M., Petrou, A., Geronikaki, A., Poroikov, V., Ivanov, M., Soković, M., Ćirić, A., Carazo, A., & Mladěnka, P. (2021). Triazolo Based-Thiadiazole Derivatives. Synthesis, Biological Evaluation and Molecular Docking Studies. *Antibiotics*, *10*(7), 804. https://doi.org/10.3390/antibiotics10070804.
5. Malik, S., & Nema, B. (2016). Antimicrobial activities of Schiff Bases: A review. https://www.researchtrend.net/ijtas/pdf/7%20BHARTI%20NEMA.pdf
6. O'Meara, S. (2020). Antimicrobial resistance. *Nature*, *586*(7830), S49–S49. https://doi.org/10.1038/d41586-020-02883-4
7. Senthil Kumar Raju, Archana Settu, Archana Thiyagarajan, Divya Rama, Praveen Sekar, & Shridharshini Kumar. (2022). Biological applications of Schiff bases: An overview. *GSC Biological and Pharmaceutical Sciences*, *21*(3), 203–215. https://doi.org/10.30574/gscbps.2022.21.3.0484
8. Sharma, P. C., Bansal, K. K., Sharma, A., Sharma, D., & Deep, A. (2020). Thiazole-containing compounds as therapeutic targets for cancer therapy. *European Journal of Medicinal Chemistry*, *188*, 112016. https://doi.org/10.1016/j.ejmech.2019.112016.