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Synthesis and Biological Assessment of New Thiazolo[3,2-a] Pyrimidine Derivatives as Potential Antimicrobial Agents

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Abstract

New compound 6-Cyano-3-(4-cyanophenyl)-7-(methylthio)-5-oxo-5H-thiazolo[3,2-a] pyrimidine (**3**) was synthesized in this study by refluxing the ethyl 2-cyano-3,3-bis(methylthio)acrylate and 4-(2-aminothiazol-4-yl) benzonitrile in anhydrous K₂CO₃ and DMF solvent. IR, ¹H NMR, and mass spectrometry were used to confirm the structure of the new compound. Antibacterial and antifungal activity of derivatives studied by using different bacteria and fungi. Out of all derivatives, compounds **3a** and **3c** have antimicrobial potential. The synthesized compounds were evaluated for lipophilicity and ADME properties by log P and in silico ADME estimation.

Keywords: ADME, antimicrobial properties, 4-(2-aminothiazol-4-yl)benzonitrile, spectroscopy, anhy. K₂CO₃.

Introduction

Heterocyclic compounds containing nitrogen and sulfur atoms are important in the synthesis of different medicines and agrochemicals. The researchers were attracted to study the thiazole and its derivatives because of their biological activities, like antioxidant, anti-inflammatory, antimalarial, antibacterial, anti-allergic, antimicrobial, and anticancer activities¹⁻⁴. In the presence of a weak base, ketene dithioacetals and 2-amino heterocyclic compounds undergo condensation reactions to yield pyrimidine derivatives^{5,6}. According to a literature survey, the pyrimidine molecule is an important structural element of a number of compounds that are physiologically active. In the present work, we describe how aminothiazole reacts with ethyl 2-cyano-3,3-bis(methylthio) acrylate to synthesize and derivatize thiazolopyrimidine⁷⁻¹¹. In order to confirm the structures of these new compounds, spectroscopic methods, including mass spectrometry, ¹H NMR, and FTIR, are required. The systematic synthesis and characterization of derivatives **3a**, **3b**, **3c**, and **3d** and the study of their antibacterial and antifungal properties are the aim of this investigation. The new compounds' drug-likeness and bioavailability were predicted based on their ADME characteristics¹². The objective is to clarify the structural and spectroscopic properties of these compounds in order to provide an understanding of their potential uses in medicinal chemistry.

Experimental

To track the reaction progress, TLC was used. Using TMS as an internal standard, the ¹H spectra were captured using a Bruker (400 MHz) spectrometer. A Bruker mass spectrometer was used to perform HRMS of substances.

Synthesis of 3-(4-cyanophenyl)-7-(methylthio)-5-oxo-5H-thiazolo[3,2-a] pyrimidine-6-carbonitrile (**3**)

The 3-(4-cyanophenyl)-7-(methylthio)-5-oxo-5H-thiazolo[3,2-a] pyrimidine-6-carbonitrile (**3**) was prepared by refluxing 5–6 hours ethyl 2-cyano-3,3-bis(methylthio)acrylate and 4-(2-aminothiazol-4-yl) benzonitrile in 10 mg of K₂CO₃ and 15 mL of DMF. After the completion of the reaction, the mixture stands for cooling, and then it transfers into crushed ice. After the filtration the resulting precipitate recrystallized in the ethanol solvent. The color of the pure product was yellow color with a melting point of 212°C.

IR: 3086.80 cm⁻¹, 2751.72 cm⁻¹, 2211.10 cm⁻¹, 1632.27 cm⁻¹, and 1668.17 cm⁻¹ for (C–H), (SCH₃), (CN), (C=O), and (C=N) respectively.

¹H NMR (DMSO-d₆, δ): 2.55 (s, 3H, SCH₃), 7.09 (d, 1H, J = 8 Hz), 7.20 (d, 1H, J = 8 Hz), 7.46 (d, 2H, J = 8 Hz), 8.03 (s, 1H).

HRMS (m/z): 325.22 [M + H]⁺. C₁₅H₈N₄OS₂.

Synthesis of derivatives **3a-d**.

Different substituted hydrazines and newly prepared compound (**3**) was reflux for 3–4 hours in 15 ml DMF and anhydrous K₂CO₃. After completion of reaction, mixture stands for cooling at room temperature and then it transfers to crushed ice.

The derivatives (**3a-d**) were obtained by recrystallization from the ethanol after the precipitate was filtered.

4-(3-imino-4-oxo-1,2,3,4-tetrahydropyrazolo[3,4-d] thiazolo[3,2-a] pyrimidin-6-yl) benzonitrile (3a). Yellow solid with melting point (206–208 °C) IR: 3340 cm⁻¹, 3237 cm⁻¹, 2196 cm⁻¹, 1672 cm⁻¹ for (C=NH), (–NH), (CN), (C=O) respectively.

¹H NMR (DMSO-d₆) δ 7.243–9.061 (m, 5H), 12.712–12.997 (s, 2H, Ar–NH), 14.051 (s, 1H, C=NH). HRMS (m/z): 309.13 [M+H]⁺. C₁₄H₈N₆OS.

4-(3-imino-4-oxo-2-phenyl-1,2,3,4-tetrahydropyrazolo[3,4-d] thiazolo[3,2-a] pyrimidin-6-yl) benzonitrile (3b). Yellow solid with melting point (189–191 °C)

IR: 3178 cm⁻¹, 3110 cm⁻¹, 2227 cm⁻¹, 1655 cm⁻¹, 1582 cm⁻¹ for (C=NH), (–NH), (CN), (C=O), (C=N) respectively.

¹H NMR (DMSO-d₆) δ 7.283–9.173 (m, 10H), 13.109 (s, 1H, Ar–NH), 14.548 (s, 1H, C=NH).

HRMS (m/z): 385.12 [M+H]⁺. C₂₀H₁₂N₆OS:

4-(2-(2,4-dichlorophenyl)-3-imino-4-oxo-1,2,3,4-tetrahydropyrazolo[3,4-d] thiazolo[3,2-a] pyrimidin-6-yl) benzonitrile (3c). Yellow solid with melting point (216–218 °C)

IR: 3293 cm⁻¹, 3139 cm⁻¹, 2232 cm⁻¹, 1661 cm⁻¹, 1590 cm⁻¹ for (C=NH), (Ar–NH), (CN), (C=O), (C=N) respectively.

¹H NMR (DMSO-d₆) δ 6.942–8.634 (m, 8H), 10.427 (s, 1H, Ar–NH), 12.106 (s, 1H, C=NH).

HRMS (m/z): 453.95 [M+H]⁺. C₂₀H₁₀Cl₂N₆OS:

4-(2-(3,4-dichlorophenyl)-3-imino-4-oxo-1,2,3,4-tetrahydropyrazolo[3,4-d] thiazolo[3,2-a] pyrimidin-6-yl) benzonitrile (3d). Yellow solid with melting point (201–203 °C).

IR: 3380 cm⁻¹, 3056 cm⁻¹, 2117 cm⁻¹, 1573 cm⁻¹, 1521 cm⁻¹ for (C=NH), (–NH), (CN), (C=O), (C=N).

¹H NMR (DMSO-d₆) δ 7.301–8.897 (m, 8H), 13.053 (s, 1H, Ar–NH), 14.452 (s, 1H, C=NH).

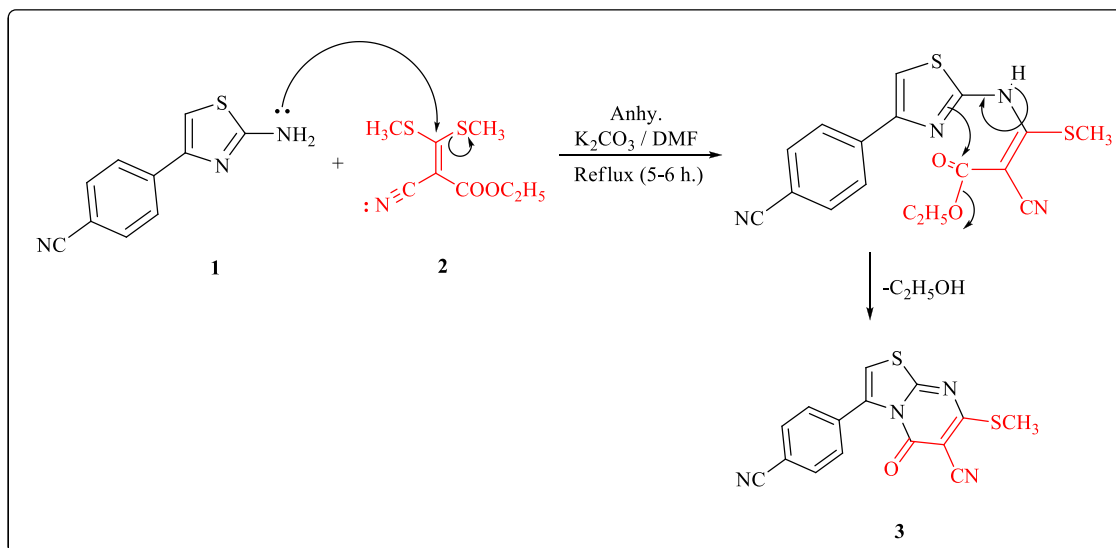
HRMS (m/z): 453.16 [M+H]⁺.

Antimicrobial Activities

Agar well diffusion method was used to study the antimicrobial activities of new compounds. The subjects for the tests were bacterial strains and fungal strains. Bacterial strains were cultivated on Mueller-Hinton agar and fungal strains on Sabouraud dextrose agar. Sterile wells (6 mm) were punched into agar plates and 100 µL of each test solution (1 mg/mL in DMSO) was added. Streptomycin (10 µg/mL) was used as antibacterial reference and fluconazole (10 µg/mL) as antifungal reference. DMSO alone was used as negative control. The plates were incubated at 37 °C for 24 hours for bacteria and at 28 °C for 48 hours for fungi. The antimicrobial activity was evaluated by measuring the diameter of the inhibition zones (mm) and each experiment was done in triplicate.

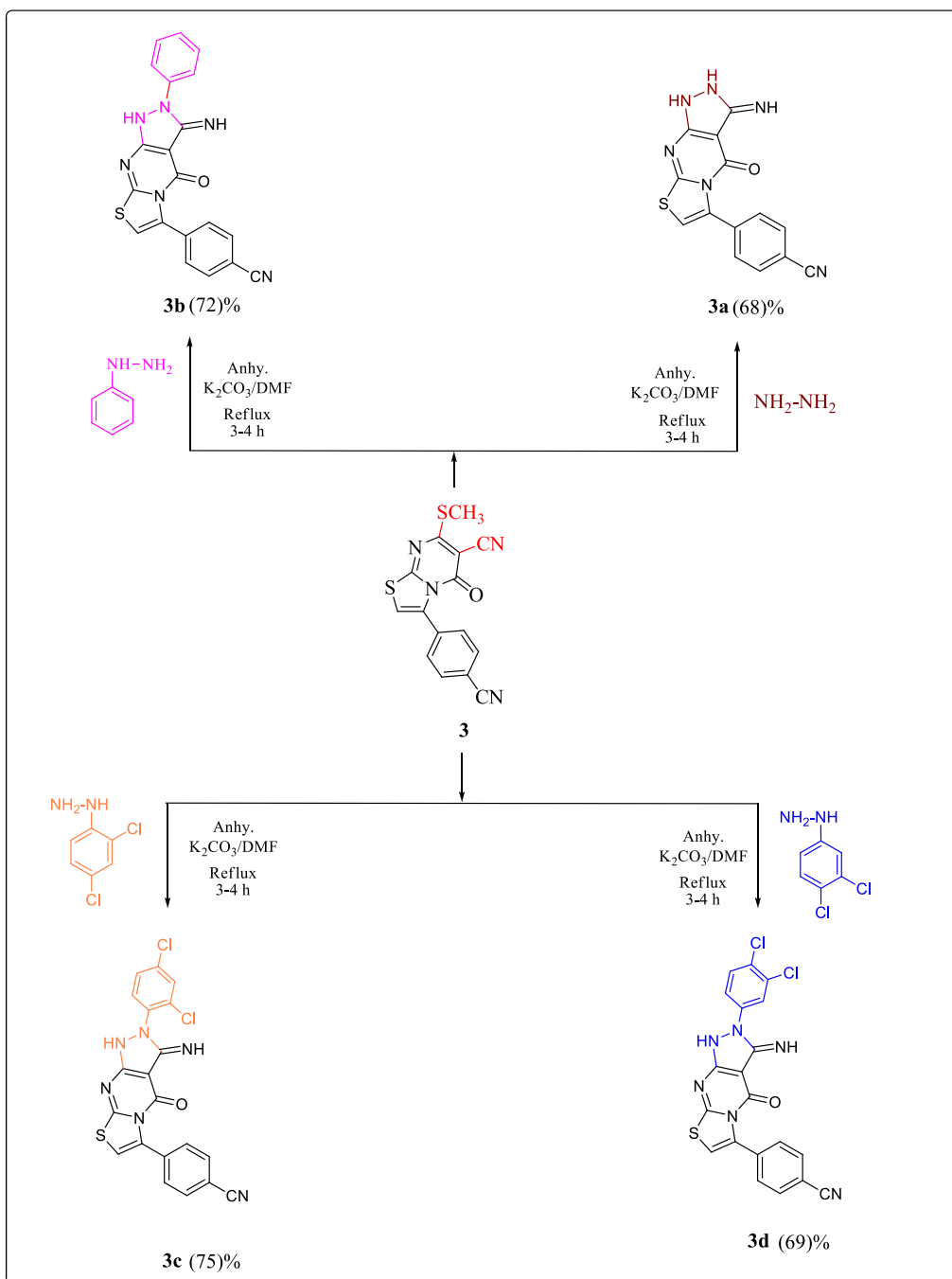
Results and Discussion

The compound (**3**) was prepared by Refluxing 4-(2-aminothiazol-4-yl) benzonitrile and ethyl 2-cyano-3,3-bis(methylthio)acrylate in DMF solvent and anhydrous K₂CO₃ for 5–6 hours. Different spectroscopic methods were used to confirm the structure of the new compound. The yield of the product was 71%, and the melting point was 211–213 °C. **Scheme 1** illustrates the reaction mechanism for (**3**).



Scheme 1. Synthesis of a new compound (**3**)

Different substituted hydrazine reflux with 6-Cyano-3-(4-cyanophenyl)-7-(methylthio)-5-oxo-5H-thiazolo[3,2-a] pyrimidine (**3**) for 3–4 hours in DMF and anhydrous K₂CO₃ to form substituted derivatives (**3a-d**) (**Scheme 2**).



Scheme 2. Synthesis of a new derivative (3a-d)

Antimicrobial Screening

Against *B. subtilis*, compounds 3b and 3d shows zone of inhibition 15 mm and 16 mm, respectively which outperformed the zone of inhibition demonstrated by the conventional medication, streptomycin 14 mm. In the case of *S. aureus*, 3b and 3d shows 15 mm and 14 mm inhibited growth respectively, which is significantly more than streptomycin 12 mm, indicating greater efficacy.

With a zone of inhibition of 17 mm as compared to 15 mm for streptomycin, compound 3b showed superior antibacterial efficacy against *P. aeruginosa*. Against *E. coli* compounds 3b and 3d shows 17 mm zone of inhibition as compared to streptomycin 16 mm. Compound 3b demonstrated superior efficacy with a 15 mm zone of inhibition over fluconazole (12 mm) against fungal strain *S. cerevisiae*. Compounds 3b and 3d showed wide-spectrum antibacterial activity against fungi and bacteria. The compounds 3a and 3c, on the other hand, displayed minimal to no activity.

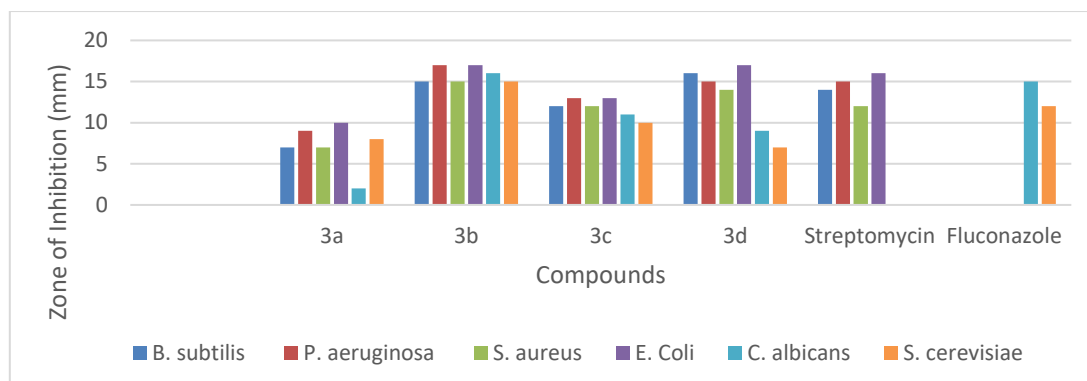


Figure 1. Zone of Inhibition in mm

Compounds 3b and 3d show great antibacterial efficacy against *B. subtilis*, having an MIC value of 1.56 µg/mL, in contrast to the standard streptomycin at 3.12 µg/mL. Compound 3b demonstrated exceptional antibacterial efficacy against *S. aureus*, with an MIC value of 1.56 µg/mL, as compared to the reference drug, streptomycin, at 3.12 µg/mL. Against *P. aeruginosa*, compounds 3b and 3d exhibited outstanding antibacterial potency with an MIC value of 3.12 µg/mL, demonstrating higher activity than the standard drug streptomycin (6.25 µg/mL). Against *E. coli*, compound 3b showed excellent antibacterial activity with an MIC value of 3.12 µg/mL, confirming it is more potent than the streptomycin (6.25 µg/mL). Compound 3d exhibited higher antifungal efficacy against *C. albicans*, showing an MIC of 3.12 µg/mL, as compared to fluconazole at 6.25 µg/mL. Compound 3b exhibited exceptional antifungal efficacy against *S. cerevisiae*, exhibiting an MIC of 1.56 µg/mL, compare to the reference drug fluconazole (3.12 µg/mL).

The results indicate that compounds 3b and 3d are the most promising candidates for broad-spectrum antibacterial effectiveness. Their low MIC values suggest that they could be good candidates for further structural optimization and biological testing.

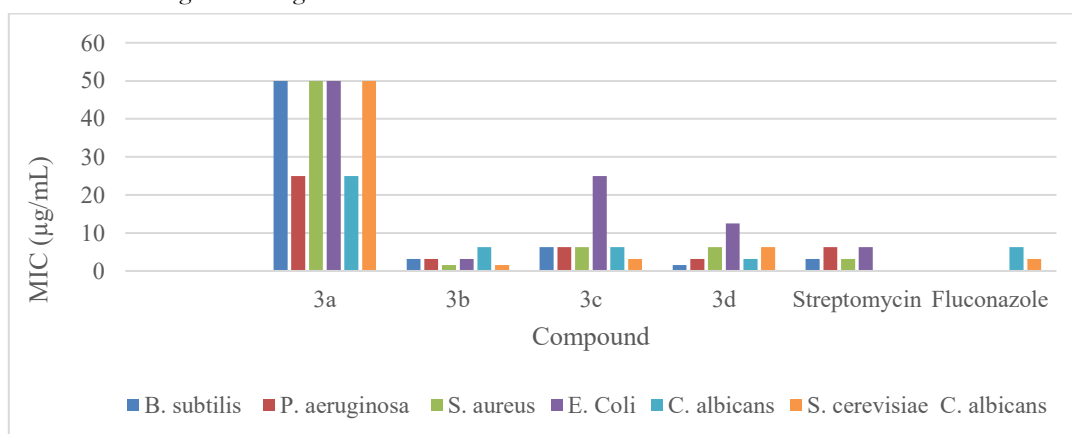


Figure 2. MIC values in µg/mL

ADME Analysis

The physicochemical and pharmacokinetic parameters of the derivatives 3a to 3d were investigated using the online tool SwissADME to determine their potential bioavailability and drug resemblance. All novel compounds had molecular weights less than 500 Da, which met the drug-like requirements for molecular size. The number of hydrogen bond acceptors (≤ 5) and donors (≤ 3) fulfilled Lipinski's rule of five, indicating a good balance of polarity and permeability. The TPSA values for the derivatives varied from 130.97 Å² to 141.83 Å², indicating effective passive membrane diffusion and acceptable oral bioavailability. The computed Log P values (2.80-4.40) fell within the ideal lipophilicity range, indicating balanced hydrophilic-lipophilic behavior. None of the three analogs violated Lipinski's rule, indicating that they met the drug-likeness criteria. The uniform bioavailability score of 0.55 for all compounds indicated a moderate absorption potential. Furthermore, the synthetic accessibility ratings (2.87-3.23) indicated that the compounds are synthetically feasible and lack significant structural complexity, making them suitable candidates for additional pharmacological testing.

Table 5: ADME Analysis

Molecule	MW	HBA	HBD	TPSA	Log P	Lipinski's rules of violations	Bioavailability Score	Synthetic Accessibility
Swiss ADME Ranges	<500 Dalton or gram/mol	≤10	≤5	130 Å	-0.7-5	0 - 2	0 – 1	1-10
3a	308.32	4	3	141.83	2.80	0	0.55	2.87
3b	384.41	4	2	130.97	2.80	0	0.55	3.20
3c	453.30	4	2	130.97	4.40	0	0.55	3.23
3d	453.30	4	2	130.97	4.40	0	0.55	3.23

Conclusion

The nucleophilic substitution of a new molecule (3) with hydrazine produces structurally distinct thiazolo[3,2-a] pyrimidine derivatives (3a-d). Spectral analysis confirmed the expected structural frameworks, indicating the successful inclusion of many substituents into the core heterocyclic scaffold. A biological investigation confirmed that freshly produced compounds have high antibacterial properties, with varying efficiency depending on substituents utilized. Compounds 3b and 3d had significant antibacterial activity against bacterial and fungal strains, with MIC values ranging from 1.56 µg/ml to 6.25 µg/ml. The study found that structural changes to the thiazolo[3,2-a] pyrimidine nucleus had a considerable effect on biological activity.

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Nil.

Conflicts of interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

References

1. Pathania S., Narang R.K. (2019). European Journal of Medicinal Chemistry, 180: 486-508. <https://doi.org/10.1016/j.ejmech.2019.07.043>
2. Obaid R.J., Mughal E.U., Naeem N., Al-Rooqi M.M., Sadiq A., Jassas R.S., Moussa. Z., Ahmed S. A. (2022). Process Biochemistry, 120: 250-259. <https://doi.org/10.1016/j.procbio.2022.06.009>
3. Shah M.S., Rahman M.M., Islam M.D., Al-Macktuf A., Ahmed J.U., Nishino H., Haque M.A. (2022). Journal of Molecular Structure, 1248: 131465. <https://doi.org/10.1016/j.molstruc.2021.131465>
4. Sharma P.C., Bansal K.K., Sharma A., Sharma D., Deep A. (2020). European Journal of Medicinal Chemistry, 188: 112016. <https://doi.org/10.1016/j.ejmech.2019.112016>
5. Huang L., Wu J., Hu J., Bi Y., Huang D. (2020). Tetrahedron Letters, 61: 151363. <https://doi.org/10.1016/j.tetlet.2019.151363>
6. Kohra S., Tominaga Y., Hosomi. (1988). Journal of Heterocyclic Chemistry, 25: 959-968. <https://doi.org/10.1002/jhet.5570250349>
7. Abdelghani E., Said S. A., Assy M.G., Abdel Hamid A. M. (2017). Arabian Journal of Chemistry, 10: 10S2926-S2933. <https://doi.org/10.1016/j.arabjc.2013.11.025>
8. Gawande M., Auti M., Halikar N., Vartale S. Russ J Gen Chem. 96, 195 (2026). <https://doi.org/10.1134/S107036322560715X>
9. Auti M., Gawande M., Halikar N., Vartale S. Russ J Gen Chem. 96, 130 (2026). <https://doi.org/10.1134/S1070363225605824>
10. Abolibda T. Z., El-Sayed A. A.A., Farag B., Zaki M.E.A., Alrehaily A., Elbadawy H.M., Al-Shahri A.A., Alsenani S.R., Gomha S.M. (2025). Results in Chemistry, 13: 102008. <https://doi.org/10.1016/j.rechem.2024.102008>
11. Ewieda S.Y., Salem M., Elshewy A., Abdalla M., El-Manawaty M., Abdelhady H.K., Haredy H.H., Fadaly W.A.A., Mohamed M.F.A., Nemr M.T.M. (2025). Bioorganic Chemistry, 163: 108672. <https://doi.org/10.1016/j.bioorg.2025.108672>
12. Kumar H., Kumar D., Kumar P. (2022). BMC Chemistry, 16: 68. <https://doi.org/10.1186/s13065-022-00861-7>