MAAREF UNIVERSITY RESEARCH JOURNAL RJMU

Research Journal of Maaref University of Applied Sciences (RJMU)

Research Paper

Vol 1, Issue 1, pages.07-11, August (2025) **ISSN:** 0000-0000

Synthesis of Thiazolidine Derived from Ninhydrin and 3-Methoxyphenyl Isothiocyanate and Study of Their Biological Effect

Deaa Gamal Alden Alasaad [1] Ayham Aljnde [2] Anber M. ANBER [3]

1. Department of Chemistry, Faculty of Science, University of Idlib, Syria
2. Faculty of Pharmacy, Alshmal Private University, Idlib, Syria
3. Department of Chemistry, Faculty of Science, Erciyes University, Kayseri 38039, Turkey- Department of Chemistry, Faculty of Pharmacy, Idlib University, Idlib, Syria.

*Corresponding Author: Deaa Gamal Alden Alasaad: e-mail: deaaalas3d@gmail.com

Available online at: https://journal.mas.edu.sy/MASV01E01D08202502

Received: 20 January 2025 Revised: 05 April 2025 Accepted: 02 July 2025

***Abstract*—** A biologically effective chemical structure was obtained from ninhydrin and isothiocyanate. At the first stage of the synthesis, isothiocyanate was combined with hydrazine to obtain a hydrazine derivative, and then its reactivity in the second stage with ninhydrin was used to obtain a new derivative of the Schiff base. At the third stage, dimethylacetylene dicarboxylate (DMAD)was added to the prepared Schiff base, and oxathiozolidine derivatives were obtained. The prepared compounds were characterized using proton nuclear magnetic resonance (^{1}H NMR), carbon nuclear magnetic resonance (13C NMR), Mass spectrometry, and infrared spectroscopy (FT-IR). Also, the biological activity of these compounds was studied at a series of concentrations against Escherichia coli (Gram-negative) and Staphylococcus aureus (Gram-positive) bacterial strains. Using the diffusion method, some compounds showed biological effects that ranged from medium to good on these germs.

***Keywords*—** Schiff base, Thiazolidine-4-one, Isothiocyanates, Escherichia coli, Staphylococcus aureus.

I. INTRODUCTION

Thiazolidinone derivatives are widely studied for their biological activities, including anticancer, anti-inflammatory, antimicrobial, antioxidant, and antileishmanial effects. Known for their role as active chromophores, these compounds are essential in drug development and have shown efficacy comparable to marketed medications (Tawfeek et al., 2022; Mech et al., 2021).

Thiazolidines (TZs) are an essential class of heterocyclic compounds known for exhibiting a wide range of pharmaceutical and biological activities (Zare Fekri et al, 2024)

Many reagents and catalysts have been used to facilitate the efficient and straightforward synthesis of thiazolidin-4-ones. These include anhydrous $ZnCl_2$, CH_3COONa, CH_3COOH/Ac_2O, DCC, support systems, nanocatalysts, microwave irradiation, ultrasonication, ionic liquids, and more. All of these methods offer certain advantages compared to traditional approaches (Goswami & Chaudhary, 2023).

Recently, 4-thiazolidinones have gained attention for their diverse biological activities. Their presence in penicillin highlighted their medicinal potential. These compounds act as COX-1 inhibitors, MurB enzyme inhibitors, HIV-RT non-nucleoside inhibitors, and anti-histaminics. Some derivatives, like chromenyl-based 2-imino thiazolidin-4-ones, also inhibit tubulin polymerization and show cytotoxicity against various cancer cell lines, including liver, lung, breast, and colon cancers.(Khan et al., 2022)

Schiff bases are important in organic chemistry for their simple synthesis and diverse applications, including anticancer, antibacterial, antiviral, and enzyme immobilization, as well as in metal complexes and agriculture (Alkorbi et al., 2024; Elkanzi et al., 2019).

Schiff bases are key in synthesizing industrial and biological compounds, with known antimicrobial, antifungal, and anticancer activities. They are also used as ligands for metal complexes and in dye production. We aim to synthesize new Schiff bases with potential biological benefits; their properties and complexation abilities are currently being studied.(Siddiqui et al., 2006)

Ninhydrin and its related indanone scaffolds are extensively used as versatile building blocks in organic and bioorganic chemistry. The reactivity of ninhydrin has been harnessed to synthesize a wide range of cyclic and acyclic compounds using the mortar and pestle grinding method (Das et al., 2023).

II. MATERIALS AND METHODS

2-1 Equipment and Materials Used:

2-1-1- The chemical substance: Hydrazine (80%),3-methoxyphenyl isothiocyanate (98%), Ninhydrin (98%), dimethylacetylene dicarboxylate (DMAD), Methanol (99.9%), Acetonitrile (99.9%), Dimethyl sulfoxide (99.9%)

2-1-2-Bacterial strains: Staphylococcus aureus (ATCC6538) Escherichia coli (ATCC 8739)

2-1-3-Germ transplant community:

Tryptic Soy Broth) HIMEDIA®)
soybean-casein digest agar) HIMEDIA®)
Mueller Hinton Agar) CONDAL A®) (USP47-NF42, 2024)
2-1-4-Devices used:

Table (1): Devices used in the Experiment

Heater	Mass Spectrometer	Electrothermal Melting Point Apparatus
sensitive scale	biological safety cabinet	Nuclear Magnetic Resonance (NMR) Meter
Autoclave	Incubators	Infra-Red (IR) Spectrometer
Oven	Water bath	Magnetic thermal mixer

2-2-Chemistry Reactions:
2-2-1- The first step: Synthesis of N-(3-methoxyphenyl) hydrazinecarbothioamide:
A quantity of 3 mmol (0.4956 g) of 3-methoxyphenyl isothiocyanate was added dropwise to 3 mmol (0.1201 g) of hydrazine in a flask placed within an ice bath, in the presence of 15 mL of acetonitrile as a solvent. The addition was performed slowly over 15 minutes, followed by allowing the reaction to proceed at room temperature with continuous stirring for 3 hours. The progress of the reaction was monitored using TLC and mobile phase (methanol: chloroform) (99:1). Upon completion, 15 mL of distilled water was added, followed by filtration and washing the precipitate with distilled water. The precipitate was dried to obtain the A derivative, a white solid with a 74% yield and a melting point of 180-182°C. The reaction equation is shown in Figure 1.

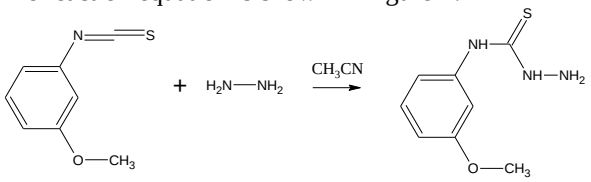


Figure (1): equation of the reaction of hydrazine with 3-methoxyphenyl isothiocyanate

2-2-2- The second step: Synthesis of 1H-indene-1,2,3-trione 2-[N-(3-methoxyphenyl) thiosemicarbazone]:

A sieved flask equipped with a reflux condenser and an electric heater with a magnetic stirrer was prepared. Then, two mmol (0.3945 g) of A and two mmol (0.3562 g) of ninhydrin were added, along with 10 mL of absolute methanol as a solvent. The mixture was then boiled for 5 hours with continuous stirring, and the reaction progress was monitored using TLC and mobile phase (methanol: chloroform) (99:1).

The end of the reaction was confirmed by the appearance of a single spot of the reaction product and the disappearance of spots resulting from the compounds involved in the response.

Upon completion of the reaction, the product was cooled, filtered to collect the formed precipitate, and then dried. The precipitate B was a yellow color, with a yield of 65%, and a melting point of 211-213°C. The equation of the reaction obtained is shown in Figure 2.

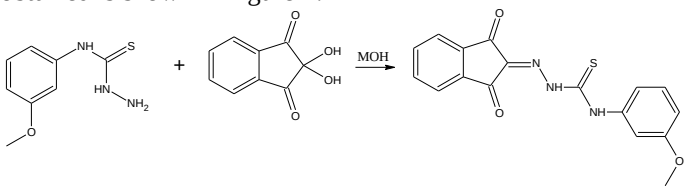


Figure (2): Equation of the reaction of A with ninhydrin

2-2-3- The third step: Synthesis of methyl (2Z)-[(2Z)-2-[(1,3-dioxo-1,3-dihydro-2H-inden-2-ylidene) hydrazono]-3-(3-methoxyphenyl)-4-oxo-1,3-thiazolidin-5-ylidene] acetate:
A sieved flask equipped with a reflux condenser and an electric heater with a magnetic stirrer was prepared. Then, one mmol (0.3393 g) of B and two mmol (0.2842 g) of DMAD were added, along with 10 mL of absolute methanol as a solvent. The mixture was then boiled continuously for 5 hours with constant stirring, and the reaction progress was monitored using TLC and mobile phase (methanol: chloroform) (99:1). The end of the reaction was confirmed by the appearance of a single spot of the reaction product and the disappearance of spots resulting from the compounds involved in the response. Upon completion of the reaction, the product was cooled, and the resulting precipitate C, which was red, was filtered out, with a yield of 78% and a melting point of 273-275°C. The equation of the reaction obtained is shown in Figure 3.

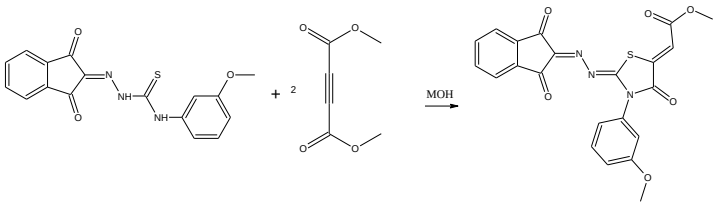


Figure (3): Equation of the reaction of B with DMAD

2-3- Biological Study
The diffusion method measured the biological activity against Escherichia coli (Gram-negative) and Staphylococcus aureus (Gram-positive) bacteria. This method was used to measure the effectiveness of compounds B and C.
2-3-1-Preparation of solutions of the synthesized compounds and the reference antibiotic: Samples of compounds B and C were prepared in four concentrations of each compound (5-10-15-20) µg/50 µL using (DMSO) as a solvent and A sample of the Ampicillin titer was prepared at a concentration of 10 µg/50 µL, which is the minimum inhibitory concentration (MIC) of Ampicillin, with the same solvent DMSO.
2-3-2- Preparation of Agar Muller-Hinton: Muller Hinton medium was prepared by taking the weight of gr 38 of Muller Hinton powder, dissolved with 1liter of distilled water in a glass flask and heated with stirring to a boil, sterilized with a Autoclave device at a temperature of oC 120 and a pressure of 15 bar for 15 minutes, then left to cool, then poured into petri dishes by 25 ml per dish, and left to harden with exposure to ultraviolet radiation for an hour.
2-3-3- Preparation of microbial suspensions: Standard bacterial strains were used according to the recommendations of the American Laboratory Standards Institute (ELSI), and these bacterial strains are: Staphylococcus Aureus (ATCC 6538)& Escherichia Coli (ATCC 8739). Using sterile instruments, a quantity of colonies grown on Agar was taken by a sterile metal lug, placed in a sterile glass tube containing 5 mL sterile distilled water, mixed well, and its turbidity was measured by a McFarland scale, where it should indicate a turbidity equal to 0.5 McFarland.

© 2025, RJMU All Rights Reserved

2-3-4-Microbial transplantation: A sterile scanner was dipped into the bacterial suspension and brushed on the surface of the prepared culture culture, petri dishes were left for 30 minutes to absorb the suspension, then drilling was performed in the culture using an agar drilling device, 50 µL of solutions of the studied compounds in the prepared concentrations were placed in the pits, then they were placed in the incubator at a temperature of 37°C for (20) hours and the antibiotic Ampicillin was used for comparison in this test, where efficacy results were obtained by measuring the diameters of growth inhibition the germ around the updated pits utilizing a ruler is inserted and these diameters are recorded.

III. RESULTS AND DISCUSSION:

3-1- characterization of N-(3-methoxyphenyl) hydrazinecarbothioamide:

The FT-IR infrared spectrum of compound A is shown in Figure 4.

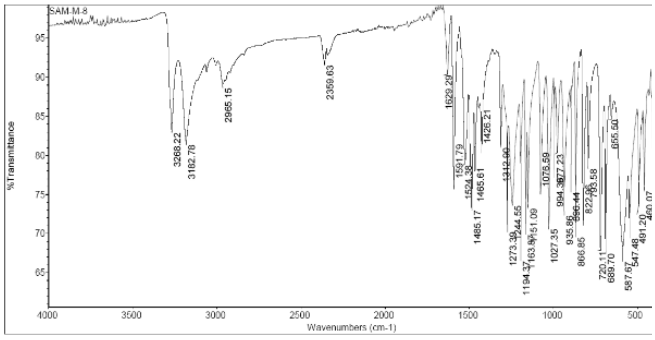


Figure (4): The FT-IR infrared spectrum of compound A

The FT-IR infrared spectrum of compound A gave an intermediate absorption beam at (3268) cm-1 belonging to the (NH2) Group, an intermediate absorption beam at (3182) cm-1 belonging to the (N-H) Group, an absorption beam at (2965) cm-1 belonging to the (C-H)sp3 aliphatic group and an absorption beam at (3050) cm-1 belonging to the (Ar-H) aromatic group.

3-2- characterization of 1H-indene-1,2,3-trione 2-[N-(3-methoxyphenyl) thiosemicarbazone]:

The FT-IR infrared spectrum of compound B, as shown in Figure 5:

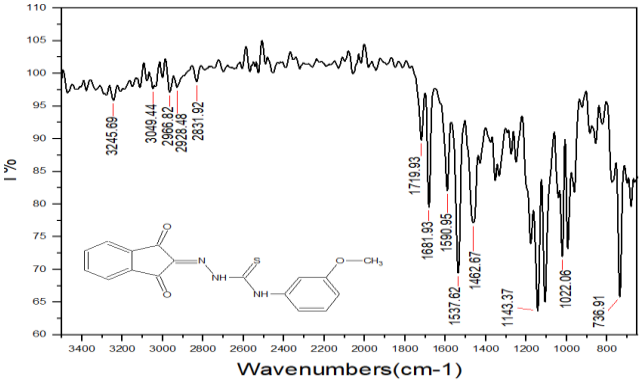


Figure (5): The FT-IR infrared spectrum of compound B

The FT-IR infrared spectrum of compound B gave two weak absorption beams at (2831-2928) cm-1 belonging to the aliphatic (C-H) sp3 Group, a weak absorption beam at (3245) cm-1 belonging to the (N-H) Group, a weak absorption beam at (3049) cm-1 belonging to the aromatic (Ar-H) Group, and a medium and narrow absorption beam at (1731) cm-1 belonging to the (C=O).

3-2- characterization of methyl (2Z)-[(2Z)-2-[(1,3-dioxo-1,3-dihydro-2H-inden-2-ylidene) hydrazono]-3-(3-methoxyphenyl)-4-oxo-1,3-thiazolidin-5-ylidene] acetate:

The FT-IR infrared spectrum of compound C is shown in Figure 6:

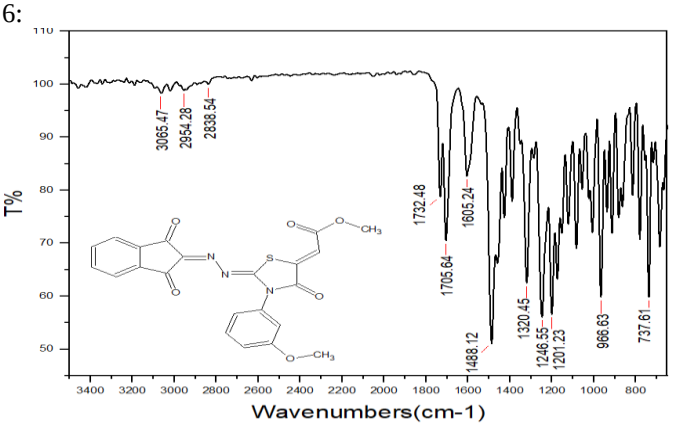


Figure (6): The FT-IR infrared spectrum of compound C

The FT-IR infrared spectrum of compound C gave two weak absorption beams at (2838-2954) cm-1 belonging to the aliphatic (C-H) group and a weak absorption band at (3065) cm-1 belonging to the aromatic (C-H) group, and two absorption beams at (1705-1732) cm-1 belonging to the (C=O) groups.

The 1H-NMR spectrum of compound C is shown in Figure 7:

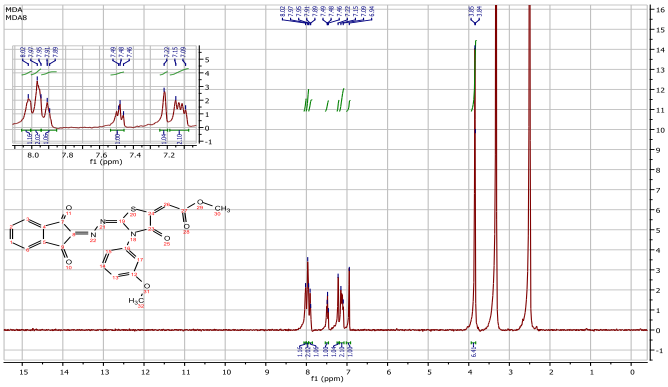


Figure (7): The 1H-NMR spectrum of compound C

1H NMR (400 MHz, DMSO) δ 8.02 (s, 1H), 7.96 (d, J = 8.8 Hz, 2H), 7.90 (d, J = 5.7 Hz, 1H), 7.53 – 7.46 (m, 1H), 7.22 (s, 1H), 7.12 (d, J = 23.1 Hz, 2H), 6.94 (s, 1H), 3.84 (d, J = 4.0 Hz, 6H).

The prepared compound C was diagnosed by a (1H-NMR) spectrum where DMSO was used as a solvent and figure 7 showed a binary peak at the (δ:3.84 ppm) belonging to six protons(C-H)sp3 of the aliphatic (CH3) group and a single peak at the (δ:6.9423 ppm) belonging to the olefinic proton (C-H)sp2 Group and a single peak at the (δ:7.22 ppm) belonging to the aromatic Proton of the (Ar-H) Group and the appearance of a binary peak at the (δ:7.15-7.09 ppm) due to an aromatic Proton of the (Ar -H) Group and an aromatic Proton of the (Ar -H) Group and the appearance of a triple peak at the (δ:7.46-7.48-7.49 ppm) is due to a Proton of the Ar -H Group and a triple peak at the (δ:7.97-7.95-7.91 ppm) belongs to the four aromatic protons of the ninhydrin ring.

© 2025, RJMU All Rights Reserved

The 13C-NMR spectrum of compound C is shown in Figure 8:

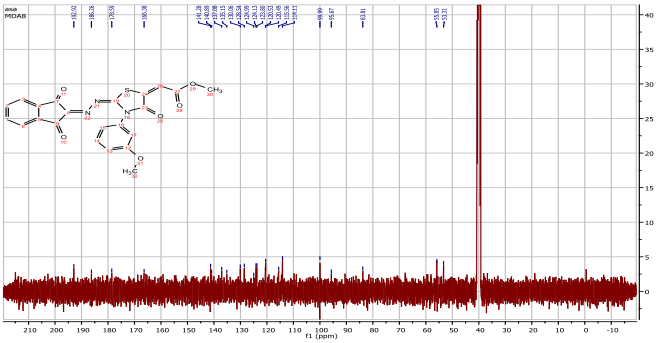


Figure (8): The 13C-NMR spectrum of compound C

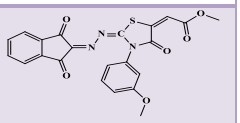
13C NMR (101 MHz, DMSO) δ 192.92, 186.26, 178.59, 166.38, 141.28, 140.89, 137.08, 135.15, 130.06, 128.54, 124.99, 124.13, 123.80, 120.53, 120.49, 115.56, 114.11, 99.99, 95.67, 83.81, 55.85, 53. 31.

Table (2): The Structural Formula of the Compound C

C	δ ppm	C	δ ppm
27	192.92	8	99.99
23	186.26	19	95.67
7+9	178.59	26	114.11
24	166.38	12-16-17-13-15-14	115.56-120.49-120.52-123.80-124.13-124.99
32	53.31	1-2-6-3-4-5	128.54-130.05-135.14-137.08-140.89-141.28
30	55.85		

Mass spectrometry results for compound C:
Table 1 shows the structural formula of the compound C and Table 2 shows the molecular weight of compound C.

Table (3): The Molecular Weight of Compound C.

Code	Molecular formula	Mw	Structural formula
8	C22H15N3O6S	449.43600	

Formula Calculator Results

Table (4): the Diameters of the Inhibition Zone

Formula	Best	Mass	Tat Mass	Diff (ppm)	Ion Species	Score
C22 H15 N3 O6 S	TRUE	449.06818	449.06816	0	C22 H16 N3 O6 S	85.57

3-4- Biological activity results: Table 3 shows the diameters of the inhibition zone for each of B, C, and Ampicillin (Goswami & Chaudhary, 2023).

Table (5): Bacterial Strains

Bacterial strains	The sample	Concentration	Inhibition zone diameter (mm)
Escherichia coli	Ampicillin	10µg/50µl	17
	B	5µg/50µl	-
		10µg/50µl	-
		15µg/50µl	-
		20µg/50µl	7mm
	C	5µg/50µl	-
		10µg/50µl	-
		15µg/50µl	-
		20µg/50µl	-
Staphylococcus aureus	Ampicillin	10µg/50µl	26
	B	5µg/50µl	12
		10µg/50µl	13
		15µg/50µl	14
		20µg/50µl	15
	C	5µg/50µl	-
		10µg/50µl	-
		15µg/50µl	-
		20µg/50µl	-

The researchers prepared a thiazolidine ring, and it turned out that at a concentration of 2.5 µgr/50 µL, the diameter of the inhibition zone for Escherichia coli spores of compound 5a was 15mm, and compound 5B was 16mm. At a concentration of 3.125 µgr/50 µL, the diameter of the inhibition zone for Staphylococcus aureus spores of compound 5a was 14mm, and compound 5B was 16mm. We find that the effectiveness is approximately the same for both types of bacteria.

IV. CONCLUSION

New derivatives of ninhydrin can be prepared from derivatives of isothiocyanate and hydrazine using simple steps. The thiazolidine ring can be prepared from ninhydrin derivatives and DMAD bacterial strains. Compound 1H-indene-1,2,3-trione 2-[N-(3-methoxyphenyl) thiosemicarbazone] recorded biological activity against Staphylococcus aureus spores in all prepared concentrations (5-10-15-20) µg/50 µL and did not record any biological activity against Escherichia coli spores. Compound methyl (2Z)-[(2Z)-2-[(1,3-dioxo-1,3-dihydro-2H-inden-2-ylidene) hydrazine]-3-(3-methoxyphenyl)-4-oxo-1,3-thiazolidin-5-ylidene] acetate is not effective against Staphylococcus aureus and Escherichia coli. The biological activity of the synthesized compounds can be studied on a different set of bacteria and fungi.

Declaration of Conflicting Interests

The author declared no potential conflicts of interest.

Funding

The author received no financial support for this book review.

Human Participants

This paper does not involve human participants.

Originality Note

The author confirms that the manuscript is his original work, and if others' works are used, they are properly cited/quoted.

V. REFERENCES

1. Alkorbi, F., Abdelaziz, M. A., Mazi, W., Omer, N., Jame, R., Gad, M. A., Omran, O. A., & Ali, A. M. (2024). Design, Synthesis of Novel Schiff Bases As Potential Insecticidal

© 2025, RJMU All Rights Reserved

Agents Against Spodoptera Frugiperda (Lepidoptera: Noctuidae). Bulletin of the Chemical Society of Ethiopia, 38(3), 765–774. https://doi.org/10.4314/bcse.v38i3.17
2. Das, S., Das, P., Maity, S., Ghosh, P., & Dutta, A. (2023). Mechanochemical reaction of ninhydrin with aromatics, enols, and amines: Synthesis, crystal structure, and supramolecular self-assembly of cyclic and acyclic adducts. Results in Chemistry, 5(October 2022), 100713. https://doi.org/10.1016/j.rechem.2022.100713
3. Elkanzi, N. A. A., Ghoneim, A. A., & Hrichi, H. (2019). Synthesis and antimicrobial evaluation of novel pyrazole, imidazole, and pyrimidine derivatives possessing imidazo[4,5-b]indol moiety. Chemistry Journal of Moldova, 14(2), 105–116. https://doi.org/10.19261/cjm.2019.638
4. Goswami, S., & Chaudhary, R. P. (2023). Expeditious mechanochemical conversion of dihydronaphthalenyl-N-phenylhydrazine-1-carbothioamides into hydrazono-thiazolidin-4-one and hydrazono-dihydrothiazole derivatives—Spectroscopic, DFT, X-ray diffraction, and antibacterial studies. Phosphorus, Sulfur and Silicon and the Related Elements, 198(7), 615–626. https://doi.org/10.1080/10426507.2023.2189252
5. Khan, S. A., Ali, M., Latif, A., Ahmad, M., Khan, A., & Al-Harrasi, A. (2022). Mercaptobenzimidazole-Based 1,3-Thaizolidin-4-ones as Antidiabetic Agents: Synthesis, in Vitro α-Glucosidase Inhibition Activity, and Molecular Docking Studies. ACS Omega, 7(32), 28041–28051. https://doi.org/10.1021/acsomega.2c01969
6. Mech, D., Kurowska, A., & Trotsko, N. (2021). The bioactivity of thiazolidin-4-ones: A short review of the most recent studies. International Journal of Molecular Sciences, 22(21). https://doi.org/10.3390/ijms222111533
7. Siddiqui, H. L., Iqbal, A., Ahmad, S., & Weaver, G. W. (2006). Synthesis and spectroscopic studies of new Schiff bases. Molecules, 11(2–3), 206–211. https://doi.org/10.3390/11020206
8. Tawfeek, H. N., Hassan, A. A., Bräse, S., Nieger, M., Mostafa, Y. A., Gomaa, H. A. M., Youssif, B. G. M., & El-Shreef, E. M. (2022). Design, synthesis, crystal structures, and biological evaluation of some 1,3-thiazolidin-4-ones as dual CDK2/EGFR potent inhibitors with potential apoptotic antiproliferative effects. Arabian Journal of Chemistry, 15(11). https://doi.org/10.1016/j.arabjc.2022.104280
9. USP47-NF42 (p. Chapter 621). (2024).
10. Zare Fekri, L., Hassan Zadeh, L., Nikpassand, M., Zeinali, S., & Varma, R. S. (2024). Recent advances include synthesizing thiazolidines using ionic liquids as a green catalyst and media. Journal of Sulfur Chemistry, 45(4), 569–611. https://doi.org/https://doi.org/10.1080/17415993.2024.2350381

About the authors

Dr. Ayham Al-Jundi is a seasoned academic and industry professional in the field of applied chemistry and pharmaceutical sciences. He earned his Bachelor's degree in Applied Chemistry from the University of Aleppo, followed by a Master's in Organic Chemical Industries (2012), and a PhD in Applied Chemistry (2016). Dr. Al-Jundi has over 15 years of combined experience in academia and the pharmaceutical industry. He has taught at several universities, including Aleppo and Ibla Private University, and currently serves as Head of the Pharmacy Department at Al-Shamal Private University. His academic portfolio includes subjects such as pharmaceutics, pharmaceutical synthesis, and industrial pharmacy. In parallel, he has held roles in manufacturing and pharmaceutical sales with notable companies like Rama Pharma. Dr. Al-Jundi is also known for organizing academic symposia and advancing scientific discourse. He brings bilingual proficiency (Arabic and English), strong technical expertise, and a deep commitment to pharmaceutical education and applied research.

Dr. Anber M. Anber is a distinguished scholar in chemistry and education, holding dual PhDs—one in Organic Chemistry from Erciyes University (Turkey) and another in Curriculum and Instruction from Al-Madinah International University (Malaysia). His academic and professional journey spans teaching roles in Idlib and Al-Shamal Universities, supervising development at JOCHEM Chemicals, and participating in international workshops through the Cara Syria Programme. With a Master's in Education and Chemistry, and a strong background in laboratory research and pedagogy, Dr. Anber combines scientific rigor with educational innovation. His publication record includes impactful research in drug synthesis, cancer treatment agents, and science education challenges in conflict-affected regions. He is fluent in Arabic and English and has advanced Turkish proficiency; he actively bridges chemistry and curriculum development. Dr. Anber also plays a consultative role in chemical R&D and educational reform. He remains a respected academic voice advancing scientific knowledge and education quality in the Middle East.

Prof. Diaa Jamal Al-Deen Al-Asaad is a dedicated chemistry educator with over a decade of teaching experience across public and private institutions in Syria. He holds a Bachelor's degree in Pure Chemistry and a Diploma in Educational Qualification from Al-Baath University. Currently, he is pursuing his Master's degree in General Chemistry at Idlib University. His teaching career includes roles at Al-Baath University, Idlib University, and Al-Shamal University, where he has consistently demonstrated his ability to simplify complex scientific content for learners of all levels. Diaa is ICDL-certified and brings strong communication and instructional skills to his work, making him a valued contributor to academic settings. He is proficient in Arabic and has intermediate English and beginner-level Turkish proficiency. With a passion for scientific education and curriculum delivery, he plays a crucial role in developing students' understanding of chemistry in northern Syria's higher education sector.

This is an open-access article distributed under the terms of the Creative Commons Attribution-NonCommercial 4.0 International (CC BY-NC 4.0), which allows reusers to distribute, remix, adapt, and build upon the material in any medium or format for noncommercial purposes only, and only so long as attribution is given to the creator.