

A. V. Lozynskyi <https://orcid.org/0000-0003-1351-7183>
 Yu. T. Konechnyi <https://orcid.org/0000-0003-4789-1675>
 N. M. Lebyak <https://orcid.org/0009-0007-6768-301X>
 I. M. Yushyn <https://orcid.org/0000-0001-7625-4531>
 I. R. Tymechko <https://orcid.org/0000-0002-7022-9412>
 R. B. Lesyk <https://orcid.org/0000-0002-3322-0080>

SYNTHESIS OF PYRAZOLO-PYRROLO-THIOPYRANO[2,3-*D*]THIAZOLE AS A POTENTIAL BIOLOGICAL ACTIVE COMPOUND

Danylo Halytsky Lviv National Medical University, Lviv, Ukraine;

UDC 547.789.1:615.012.1|073/.076

A. V. Lozynskyi, Yu. T. Konechnyi, N. M. Lebyak, I. M. Yushyn, I. R. Tymechko, R. B. Lesyk
 SYNTHESIS OF PYRAZOLO-PYRROLO-THIOPYRANO[2,3-*d*]THIAZOLE AS A POTENTIAL BIOLOGICAL ACTIVE COMPOUND

Danylo Halytsky Lviv National Medical University, Lviv, Ukraine

The purpose of the present study is the synthesis of 6-(1,5-dimethyl-3-oxo-2-phenyl-2,3-dihydro-1*H*-pyrazol-4-yl)-8-(4-methoxyphenyl)-3-methyl-2-thioxo-3,4a,7a,8-tetrahydropyrrolo[3',4':5,6]thiopyrano[2,3-*d*]thiazole-5,7(2*H*,6*H*)-dione and its biological evaluation as a potential bioactive compound.

Materials and methods: organic synthesis, ¹H, and ¹³C NMR spectroscopy, liquid chromatography-mass spectrometry, *in vitro* antimicrobial and anticancer screening, drug-like prediction.

Results. The title compound was obtained via a one-pot/two-step protocol of 4-aminoantipyrine and maleic anhydride with 5-(4-methoxybenzylidene)-3-methylthiazolidine-2,4-dithione. The structure of the synthesized compound (yield 69%) was confirmed by ¹H, ¹³C NMR, and LC-MS spectra. According to US NCI protocols, the compound displayed a moderate level of antimitotic activity against melanoma MDA-MB-435 and breast cancer cell lines HS 578T. The synthesized derivative 4 was evaluated for antimicrobial activity against 17 strains of Gram-positive and Gram-negative bacteria, as well as yeasts, demonstrating moderate efficacy against drug-resistant *Klebsiella* strains. The drug-like properties of the synthesized compound were assessed using SwissADME, showing favorable drug-like parameters.

Conclusions. The one-pot/two-step protocol for synthesizing thiopyrano[2,3-*d*]thiazole derivative is proposed. The title compound was fully characterized by spectral analysis methods. The tested compound displayed moderate activity against melanoma and breast cancer cell lines and moderate antimicrobial activity against drug-resistant *Klebsiella* strains. The synthesized thiopyrano[2,3-*d*]thiazole exhibits favorable predicted physicochemical and drug-like properties based on *in silico* analysis and demonstrates promising antimicrobial activity *in vitro*. The obtained results indicate the potential of this compound for further investigation against antibiotic-resistant microbial strains and tumors that are unresponsive to conventional anticancer agents.

Keywords: thiopyrano[2,3-*d*]thiazole, hetero-Diels-Alder reaction, antimicrobial activity, anticancer activity, drug-like prediction.

УДК 547.789.1:615.012.1|073/.076

А. В. Лозинський, Ю. Т. Конечний, Н. М. Леб'як, І. М. Юшин, І. Р. Тимечко, Р. Б. Лесик
 СИНТЕЗ ПІРАЗОЛО-ПІРОЛО-ТІОПІРАНО[2,3-*D*]ТІАЗОЛУ ЯК ПОТЕНЦІЙНО БІОЛОГІЧНО АКТИВНОЇ СПОЛУКИ

Львівський національний медичний університет імені Данила Галицького, Львів, Україна

Метою цієї роботи був синтез тіопірано[2,3-*d*]тіазолу шляхом трьохкомпонентної одностадійної реакції 4-аміноантипірину, малеїнового ангідриду та 5-(4-метоксибензиліден)-3-метилтіазолідин-2,4-дитіону, а також дослідження його біологічної активності. Структуру синтезованої сполуки підтверджено за допомогою спектрів ¹H, ¹³C ЯМР-спектроскопією та хромато-мас-спектрометрією. На основі результатів скринінгу Національного інституту раку США синтезована сполука продемонструвала помірний рівень антимітотичної активності проти меланоми MDA-MB-435 та клітинних ліній раку молочної залози HS 578T. Синтезована молекула досліджувалася на протимікробну активність щодо 17 штамів грампозитивних і грамнегативних бактерій, а також дріжджів, демонструючи помірну ефективність відносно стійкого до лікарських засобів штаму *Klebsiella*. Лікоподібність синтезованої сполуки оцінювалася за допомогою онлайн-сервісу SwissADME, що дало можливість охарактеризувати її потенційні лікоподібні параметри. Отримані результати свідчать про перспективу дослідження цієї сполуки проти антибіотикорезистентних штамів мікроорганізмів та пухлин, які нечутливі до лікування класичними протипухлинними агентами.

Ключові слова: тіопірано[2,3-*d*]тіазолі; реакції *гетеро*-Дільса-Альдера, протимікробна активність, протиракова активність; прогнозування лікоподібності.



Introduction. Thiazolidinone derivatives are a well-known class of compounds that lay the groundwork for numerous drugs and biologically active molecules [1-3]. An important aspect of analysing these molecules is the study of the biological activity of condensed thiazolidinone derivatives, particularly thiopyrano[2,3-*d*]thiazoles [4-7], which have several advantages over their non-condensed analogues. Highly active anticancer, antimicrobial, antitrypanosomal, antiviral, and antioxidant agents have been identified among thiopyranothiazole derivatives [8] (Fig. 1.). The anticancer potential of this class of condensed compounds deserves special attention [9]. Among some of them, a mechanism of biological activity was identified through the activation of peroxisome proliferator-activated receptor gamma (PPAR γ), inhibition of TGF β , and DNA-binding ability [8, 10]. Accordingly, this study aims at the synthesis of new functionally substituted thiopyrano[2,3-*d*]thiazole derivatives and their biological evaluation as potential drug-like molecules in modern medicinal chemistry.

Materials and Methods

General Information and Compound 4 Synthesis

Melting points were measured in open capillary tubes on an IA 9200 Electrothermal melting point apparatus (Bibby Scientific Limited, Stone, UK) and are uncorrected. The elemental analyses (C, H, N) were performed using the FlashSmart CHNS/O analyzer (Thermo Scientific, Waltham, MA, USA) and were within $\pm 0.4\%$ of the theoretical values. The 400 MHz- ^1H and 126 MHz- ^{13}C spectra were recorded on Varian Unity Plus 400 (400 MHz) spectrometer (Varian Inc., Palo Alto, CA, USA). Chemical shifts (δ) are quoted in ppm and coupling constants (*J*) are reported in Hz. LC-MS spectra were obtained on Agilent 1260 Infinity II with single-quadrupole mass-selective detector Agilent 6125 (Agilent Technologies, Santa Clara, CA, USA). The reaction mixture was monitored by thin layer chromatography (TLC) using commercial glass-backed TLC plates (Merck Kieselgel 60 F₂₅₄). Solvents and reagents (4-aminoantipyrine, CAS number: 83-07-8; maleic anhydride, CAS number: 108-31-6) that are commercially available were used without further purification. The 5-(4-methoxybenzylidene)-3-methylthiazolidine-2,4-dithione **3** was prepared according to the method described in [10].

6-(1,5-Dimethyl-3-oxo-2-phenyl-2,3-dihydro-1H-pyrazol-4-yl)-8-(4-methoxyphenyl)-3-methyl-2-thioxo-3,4a,7a,8-tetrahydropyrrolo[3',4':5,6]thiopyrano[2,3-*d*]thiazole-5,7(2H,6H)-dione (**4**)

A mixture of 4-aminoantipyrine (5 mmol), maleic anhydride (5 mmol) was heated at reflux for 1 h in AcOH

(10 mL), and afterward addition of 5-(4-methoxybenzylidene)-3-methylthiazolidine-2,4-dithione (5 mmol) and a catalytic amount of hydroquinone and reflux else for 2 h (monitored by TLC). The obtained solid product was collected after cooling by filtration and recrystallized from the mixture DMF: ethanol (1:2).

Yellow crystals, yield: 69%, *R*_f = 0.81 (ethyl acetate/benzene: 1/2), mp 229–231 °C (DMF : EtOH). ^1H NMR (400 MHz, DMSO-*d*₆): δ (ppm) 2.17 (s, 3H, CH₃), 3.17, 3.19 (2*s, 3H, CH₃), 3.52, 3.54 (2*s, 3H, CH₃), 3.74, 3.75 (2*s, 3H, OCH₃), 4.38 (dd, 1H, *J* = 4.3, 8.2 Hz, 7a-H, *exo*), 4.62 (dd, 1H, *J* = 4.6, 8.9 Hz, 7a-H, *endo*), 4.64 (m, 1H, 4a-H, *endo*), 4.88 (d, *J* = 8.9 Hz, 4a-H, *exo*), 4.89 (m, 1H, 4a-H, *endo*), 4.90 (t, 1H, *J* = 3.3 Hz, 8-H, *endo*) 5.02 (d, *J* = 8.9 Hz, 8-H, *exo*), 6.91, 6.93 (2*m, 2H, arom.), 7.31, 7.34 (2*m, 5H, arom.), 7.38, 7.53 (2*m, 2H, arom.). ^{13}C NMR (126 MHz, DMSO-*d*₆, δ): 9.30, 34.97, 43.75, 44.21, 48.87, 49.26, 55.10, 99.22, 113.85, 114.01, 124.37, 124.81, 127.25, 128.73, 129.17, 130.12, 152.15, 158.39, 158.50, 159.62, 173.36, 174.37. LCMS (ESI): *m/z* 565.0 (100.00%, [M+H]⁺). Anal. Calc. for C₂₇H₂₄N₄O₄S₃: C 57.43%; H 4.28%; N 9.92%. Found: C 57.60%; H 4.37%; N 9.81%.

In vitro anticancer assay

The primary anticancer screening was carried out on roughly sixty human tumor cell lines representing nine types of neoplastic diseases, following the methodology outlined by the Drug Evaluation Branch of the National Cancer Institute in Bethesda [11–14]. The compounds under investigation were applied to the cultures at a single concentration (10–5 M) and incubated for 48 hours. Growth was assessed using the Sulphorhodamine B (SRB) protein-binding dye, with results expressed as the percentage of growth in treated cells relative to untreated controls. Measurements were taken spectrophotometrically, comparing treated cells to those not exposed to the test substances.

Molecular and pharmacokinetic properties

The physical properties and adsorption, distribution, metabolism, elimination, and toxicity (ADMET) parameters of (*E*)-2-((5-(3-(2-fluorophenyl)acryloyl)-4-methylthiazol-2-yl)amino)isoindoline-1,3-dione was calculated using the SwissAdme online server of the Swiss Institute of Bioinformatics [<http://www.swissadme.ch/index.php>].

Antimicrobial Activity

The synthesized compound **4** was evaluated in vitro for its antibacterial and antifungal activities using agar diffusion and resazurin-based microdilution assays [15–17]. Dimethyl sulfoxide (DMSO) and vancomycin were used as controls. Both reference and clinical microbial strains were tested, with identification confirmed by the MALDI-TOF

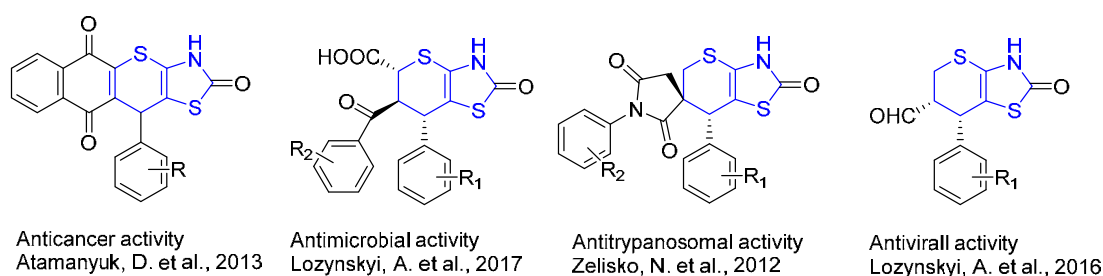


Fig. 1. Structures of biologically active thiopyranothiazole derivatives

system (Bruker, Bremen, Germany) and 16S rRNA gene sequencing. All clinical strains exhibited multidrug-resistant (MDR) or extensively drug-resistant (XDR) profiles with diverse antibiotic resistance patterns. *Raoultella ornithinolytica* (VIM) harbored the VIM metallo-beta-lactamase gene, conferring resistance to carbapenems. Clinical *Staphylococcus* strains b2 and b5 were identified as carriers of the *icaA* biofilm formation gene. These clinical strains were isolated from patients with healthcare-associated infections in regional hospitals. All experiments were conducted in triplicate to ensure reproducibility.

Results and Discussion

Synthesis of the Title Compound 4

The synthesis of compound **4** was accomplished via one-pot/two-steps protocol which included the interaction of the 4-aminoantipyrine **1** with maleic anhydride **2** (reflux during 1 h, AcOH) at the first stage and with the next addition of 5-(4-methoxybenzylidene)-3-methylthiazolidine-2,4-dithione **3** and refluxing during 2 h at the second stage (Fig. 2).

The reaction of **1** and **2** with 5-(4-methoxybenzylidene)-3-methylthiazolidine-2,4-dithione proceeded via *hetero*-Diels-Alder reaction with moderate diastereoselectivity providing mixtures of *endo/exo* adducts **4**. The ratio between *endo/exo* diastereoisomeric pairs of compound **4** (Scheme 1) was calculated from the integration of signals in the ¹H NMR spectra of the crude mixtures. The stereochemistry was determined from the coupling constants of the C-8 protons (*rel*-4aR,7aR,8R-diastereoisomer *J* = 3.3 Hz and *rel*-4aR,7aR,8S-diastereoisomer *J* = 8.9 Hz). The stereochemical outcome can be attributed to the transition state geometry, which determines the *trans* or *cis* configuration of the protons at the C-8 position. Given this limitation and assuming a concerted mechanism, the reaction can proceed via either an *endo* or *exo* transition state. Each path results in a specific stereochemical relationship between the pro-

tons at the C-8 positions of the adducts. Consequently, the *endo* pathway yields an all *anti*-configuration, whereas the *exo* pathway produces a *syn* configuration of the C-8 proton relative to the other two.

Furthermore, in the ¹³C NMR spectrum of compound **4**, the signals of carbon atoms within the carbonyl (C = O) and thiocarbonyl (C = S) groups were observed at δ 158.3-174.3 ppm.

The molecular ion peak detected at an *m/z* value of 565.0 [M+H]⁺ in positive ionization mode within the mass spectrum confirmed the synthesis of the title compound **4**.

In vitro evaluation of the anticancer activity of compound **4**

Compound **4** was evaluated by the National Cancer Institute (NCI) under the Developmental Therapeutic Program (DTP) using a single-dose assay (10⁻⁵ M). This evaluation was conducted across a panel of roughly sixty cancer cell lines, following the standard NCI protocol previously outlined [11–14]. The synthesized 6-(1,5-dimethyl-3-oxo-2-phenyl-2,3-dihydro-1H-pyrazol-4-yl)-8-(4-methoxyphenyl)-3-methyl-2-thioxo-3,4a,7a,8-tetrahydropyrrolo[3',4':5,6]thiopyrano[2,3-*d*]thiazole-5,7(2*H*,6*H*)-dione (**4**) demonstrated moderate anticancer activity, with an average cell growth inhibition rate (GP_{mean}) of 103.32%. Compound **4** demonstrated its most potent cytotoxic effects against the melanoma MDA-MB-435 (GP = 27.98%), and breast cancer cell lines HS 578T (GP = 57.81%) (Table 1, Figure S7).

Molecular and pharmacokinetic properties

ADME prediction for the evaluated compound was performed using the SwissAdme online server [18]. Compound **4** has low gastrointestinal absorption and permeability through the blood-brain barrier. According to the results of the ADME analysis, the following parameters are critical in evaluating the drug-likeness of compound **4**: molecular weight (564.70), number of heavy atoms (38), number of

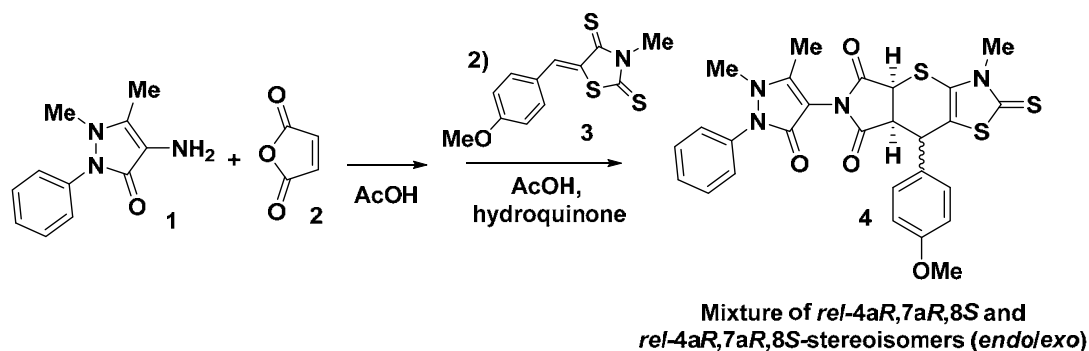


Fig. 2. Synthesis of target compound 4

Table 1

Anticancer screening data in concentration 10⁻⁵ M

Compound	60 Cell Lines Assay in One Dose, 10 μM		
	Mean Growth, %	Range of Growth, %	Most Sensitive Cell Line(s) Growth Inhibition Percent ¹ /Line/Panel ²
4	103.32	27.98 to 131.01	27.98/ MDA-MB-435 /NSCLC 57.81/ HS 578T/BC

¹ Percent growth (GP%) ≤ 60%.

² Abbreviations: NSCLC — Non-Small Cell Lung Cancer; BC — Breast Cancer.

aromatic heavy atoms (22), number of rotatable bonds (4), number of hydrogen bond acceptors (4), number of hydrogen bond donors (0), and consensus log Po/w (3.92). These parameters are all essential in determining the compound's potential as a drug-like molecule. Based on these results, compound **4** complies with Lipinski's Rule of Five, indicating that it meets the key criteria for drug-likeness, suggesting that the compound has favourable properties for oral bioavailability and could be a viable candidate for further drug development (Table 2). The use of *in silico* methodologies, is crucial in drug development, especially among heterocyclic compounds, as they help predict interactions, optimize structure, and enhance the overall drug design process [19, 20].

In vitro evaluation of the antimicrobial activity of compound **4**

The antimicrobial activity of compound **4** was tested on 17 strains, including both Gram-positive and Gram-negative bacteria as well as yeasts, to cover a broad spectrum of clinically relevant microorganisms. The selected strains include *Pseudomonas aeruginosa* ATCC 10145, *Raoultella ornithinolytica* ATCC 31898, *Klebsiella pneumoniae* (N216, N215), *Pseudomonas aeruginosa* (N197, N50), *Raoultella ornithinolytica* (VIM), *Staphylococcus epidermidis* ATCC 12228, *Staphylococcus aureus* subsp. *aureus* ATCC 25923, *Streptococcus agalactiae* ATCC 13813, *Limosilactobacillus fermentum*, *Lactobacillus acidophilus*, *Staphylococcus aureus* b2 and b5, and yeasts such as *Candida albicans* ATCC 885-653, *Candida guilliermondii* N94, and *Candida krusei* N71 (Table 3). The selection of these microorganisms was based on their clinical relevance, with a focus on drug-resistant strains, as well as their involvement in a variety of infections, ranging from hospital-acquired infections to opportunistic infections caused by

Table 2
Physicochemical and pharmacokinetics properties of studied compound **4**

Physicochemical properties		
1	Molecular weight	564.70
2	Num. heavy atoms	38
3	Num. arom. heavy atoms	22
4	Num. rotatable bonds	4
5	Num. H-bond acceptors	4
6	Num. H-bond donors	0
7	Molar Refractivity	154.33
8	TPSA Å ²	164.10
9	Consensus log Po/w	3.92
10	Lipinski' Rule	Yes
Pharmacokinetics		
11	GI absorption	Low
12	BBB permeant	No
13	P-gp substrate	No
14	CYP1A2 inhibitor	No
15	CYP2C19 inhibitor	Yes
16	CYP2C9 inhibitor	Yes
17	CYP2D6 inhibitor	No
18	CYP3A4 inhibitor	Yes
19	Log Kp (SP) (cm/s) (skin permeation)	-6.80
20	Bioavailability Score	0.55

yeasts. This diverse group of microorganisms allows for a comprehensive evaluation of the compound's antimicrobial potential. The antimicrobial activity was evaluated in terms of the diameter of the inhibition zone of microbial growth and minimum inhibitory concentrations (MICs).

Compound **4** demonstrated moderate antimicrobial activity against both reference and clinical strains, exhib-

Table 3
In vitro antimicrobial activity of compound **4** (zone of growth inhibition at conc. 1 mg/mL after 24–48 h)

Type of Species		No.	Species of Bacteria and Fungi	Zone of Growth Inhibition (mm ± SE)		
				4	DMSO	Vancomycin
Gram-negative bacteria	Reference strains	1	<i>Pseudomonas aeruginosa</i> ATCC 10145	–	–	–
		2	<i>Raoultella ornithinolytica</i> ATCC 31898	11.8 ± 0.2	–	–
	Clinical strains	3	<i>Klebsiella pneumoniae</i> N216	13.5 ± 0.2	–	–
		4	<i>Klebsiella pneumoniae</i> N215	9.5 ± 0.2	–	–
		5	<i>Pseudomonas aeruginosa</i> N 197	–	–	–
		6	<i>Pseudomonas aeruginosa</i> N 50	–	–	–
		7	<i>Raoultella ornithinolytica</i> (VIM)	11.5 ± 0.2	–	–
Gram-positive bacteria	Reference strains	8	<i>Staphylococcus epidermidis</i> ATCC 12228	–	–	32 ± 0.5
		9	<i>Staphylococcus aureus</i> subsp. <i>aureus</i> ATCC 25923	6.5 ± 0.2	–	32 ± 0.5
		10	<i>Streptococcus agalactiae</i> ATCC 13813	–	–	–
		11	<i>Limosilactobacillus fermentum</i>	10.4 ± 0.2	–	–
		12	<i>Lactobacillus acidophilus</i>	10.4 ± 0.2	–	–
	Clinical strains	13	<i>Staphylococcus aureus</i> b2	7.8 ± 0.2	6.3 ± 0.4	16.2 ± 0.2
		14	<i>Staphylococcus aureus</i> b5	–	–	11.4 ± 0.3
Fungi	Reference strain	15	<i>Candida. albicans</i> ATCC 885-653	10.4 ± 0.2	9.2 ± 0.2	–
	Clinical strains	16	<i>Candida guilliermondii</i> N94	11.5 ± 0.3	–	–
		17	<i>Candida krusei</i> N71	–	–	–

“–” – no inhibition was observed in the experiment; “–” – not tested; Vancomycin 30 µg (inhibition zone 17–21 mm for *S. aureus*).

iting the highest efficacy against clinical strains of *Klebsiella pneumoniae* (N 216 and VIM, with inhibition zones of 13.5 mm and 11.5 mm, respectively). For Gram-positive bacteria and yeast, the activity was negligible. Notably, the compound also showed antimicrobial effects against probiotic, non-pathogenic strains of *Lactobacillus* and *Limosilactobacillus* (Table 3).

MIC to *Klebsiella pneumoniae* N216 та *Raoultella ornithinolytica* (VIM) was in the range of 250–500 µg/mL (< 885.4 µM).

Conclusions. In the present paper, the one-pot/two-step protocol for the synthesis of thiopyrano[2,3-d]thiazole derivative is proposed. The title compound was fully characterized by spectral analysis methods. The tested compound displayed moderate activity against melanoma and breast cancer cell lines and moderate antimicrobial activity against drug-resistant *Klebsiella* strains. The synthesized compound **4** exhibits favourable predicted physicochemical and drug-like properties

based on *in silico* analysis and demonstrates promising antimicrobial activity *in vitro*. The obtained results indicate the potential of this compound for further investigation against antibiotic-resistant microbial strains and tumors that are unresponsive to conventional anticancer agents.

Funding. The study was funded by the Ministry of Health of Ukraine [grant number: 0123U100153], National Research Foundation of Ukraine 2023.03/0104; 2023.05/0021.

Acknowledgments. This research was supported by the Danylo Halytsky Lviv National Medical University, which is gratefully acknowledged. We express our gratitude to the National Contact Point of Horizon Europe (Health) for supporting our aspirations to conduct high-quality scientific research and for fostering educational and scientific transformations in Ukraine.

Conflicts of Interest. The authors declare no conflict of interest.

BIBLIOGRAPHY

1. Tripathi AC, Gupta SJ, Fatima GN, Sonar PK, Verma A, Saraf SK. 4-Thiazolidinones: The advances continue. *Eur J Med Chem.* 2014; 72: 52–77. Available from: <https://doi.org/10.1016/j.ejmech.2013.11.017>.
2. Nirwan S, Chahal V, Kakkar R. Thiazolidinones: Synthesis, reactivity, and their biological applications. *J Heterocycl Chem.* 2019; 56: 1239–1253. Available from: <https://doi.org/10.1002/jhet.3514>.
3. Konechnyi YT, Lozynskyi AV, Horishny VY, et al. Synthesis of indoline-thiazolidinone hybrids with antibacterial and antifungal activities. *Biopolym Cell.* 2020; 36: 381–391. Available from: <https://doi.org/10.7124/bc.000A3A>.
4. Narigara R, Joshi D, Bhola Y, Jani G. Synthesis and biological activity of some various aldehyde and 1,2,3-triazole containing heterocyclic compounds. *World Sci News.* 2019; 123: 246–257.
5. Metwally NH, Badawy MA, Okpy DS. Synthesis, biological evaluation of novel thiopyrano[2,3-d]thiazoles incorporating arylsulfonate moiety as potential inhibitors of tubulin polymerization, and molecular modeling studies. *J Mol Struct.* 2022; 1258: 132648. Available from: <https://doi.org/10.1016/j.molstruc.2022.132648>.
6. Metwally NH, El-Desoky EA. Novel thiopyrano[2,3-d]thiazole-pyrazole hybrids as potential nonsulfonamide human carbonic anhydrase IX and XII inhibitors: Design, synthesis, and biochemical studies. *ACS Omega.* 2023; 8: 5571–5592. Available from: <https://doi.org/10.1021/acsomega.2c06954>.
7. Lozynskyi A, Zimenkovsky B, Gzella AK, Lesyk R. Arylidene pyruvic acids motif in the synthesis of new 2H,5H-chromeno[4',3':4,5]thiopyrano[2,3-d]thiazoles via tandem hetero-Diels–Alder-hemiacetal reaction. *Synth Commun.* 2015; 45: 2266–2270. Available from: <https://doi.org/10.1080/00397911.2015.1076004>.
8. Kryshchysyn A, Roman O, Lozynskyi A, Lesyk R. Thiopyrano[2,3-d]thiazoles as new efficient scaffolds in medicinal chemistry. *Sci Pharm.* 2018; 86: 26. Available from: <https://doi.org/10.3390/scipharm86020026>.
9. Zhang Y, Wang S, Wu S, et al. Facile construction of structurally diverse thiazolidinedione-derived compounds via divergent stereoselective cascade organocatalysis and their biological exploratory studies. *ACS Comb Sci.* 2013; 15: 298–308. Available from: <https://doi.org/10.1021/co400022r>.
10. Lozynskyi A, Senkiv J, Ivasechko I, et al. 1,4-Naphthoquinone motif in the synthesis of new thiopyrano[2,3-d]thiazoles as potential biologically active compounds. *Molecules.* 2022; 27: 7575. Available from: <https://doi.org/10.3390/molecules27217575>.
11. Monks A, Scudiero D, Skehan P, et al. Feasibility of a high-flux anticancer drug screen using a diverse panel of cultured human tumor cell lines. *J Natl Cancer Inst.* 1991; 83: 757–766. Available from: <https://doi.org/10.1093/jnci/83.11.757>.
12. Boyd MR, Paull KD. Some practical considerations and applications of the National Cancer Institute *in vitro* anticancer drug discovery screen. *Drug Dev Res.* 1995; 34: 91–109. Available from: <https://doi.org/10.1002/ddr.430340203>.
13. Boyd MR. The NCI *in vitro* anticancer drug discovery screen. In: Teicher BA, editor. *Anticancer drug development guide*. Totowa, NJ: Humana Press; 1997, p. 23–42. Available from: https://doi.org/10.1007/978-1-4615-8152-9_2.
14. Shoemaker RH. The NCI60 human tumour cell line anticancer drug screen. *Nat Rev Cancer.* 2006; 6: 813–823. Available from: <https://doi.org/10.1038/nrc1951>.
15. Balouiri M, Sadiki M, Ibsouda SK. Methods for *in vitro* evaluating antimicrobial activity: A review. *Pharm Anal.* 2016; 6: 71–79. Available from: <https://doi.org/10.1016/j.jpha.2015.11.005>.
16. EUCAST. Disk Diffusion—Manual v 9.0 (1 January, 2021). Available online: https://www.eucast.org/ast_of_bacteria/disk_diffusion_methodology/ (accessed on 01 September 2024).
17. Rebriy J, Sukhodub L, Kasianchuk V. Characteristics of nanometric particles of metal oxides. *Eastern Ukrainian Medical Journal.* 2023; 11(2): 103–112. [https://doi.org/10.21272/eumj.2023;11\(2\):103-112](https://doi.org/10.21272/eumj.2023;11(2):103-112).
18. SwissADME. Available online: <http://www.swissadme.ch/> (accessed on 10 September 2024).

19. Rauf A, Khan H, Khan M, Abusharha A, Serdaroğlu G, Daglia M. In silico, SwissADME, and DFT studies of newly synthesized oxindole derivatives followed by antioxidant studies. *Journal of Chemistry*. 2023; 2023(1): 5553913. <https://doi.org/10.1155/2023/5553913>.
20. Kalchenko VV, Shcherbina RO, Panasenko OI, Salionov VO, Morozova LP. Evaluation of the antioxidant potential of some 5-(2-bromo-4-fluorophenyl)-4-ethyl-1,2,4-triazole-3-thiol derivatives. *Odesa Medical Journal*. 2024; 5: 89–94. <https://doi.org/10.32782/2226-2008-2024-5-15>.

Надійшла до редакції 07.02.2025.

Прийнята до друку 15.05.2025.

Електронна адреса для листування dr_r_lesyk@org.lviv.net