UDC 615.31.074:543.42:547.789

L.M. Mosula [a, b], V.S. Mosula [a]V.V., Mazur [a], Y.V. Karpenko [c]

SPECTROPHOTOMETRIC DETERMINATION OF ANTIRADICAL POTENTIAL OF SOME 5-YLIDENE DERIVATIVES OF RHODANINE WITH A BENZOTHIAZOLE MOIETY IN THE MOLECULES

[a] I. Horbachevsky Ternopil National Medical University, Ternopil, Ukraine
[b] Ternopil Ivan Puluj National Technical University, Ternopil, Ukraine
[c] Zaporizhzhia State Medical and Pharmaceutical University, Zaporizhzhia, Ukraine

This study evaluates the antiradical potential of hybrid systems based on the benzothiazole-rhodanine scaffold, the synthesis of which was previously reported. Such heterocycles are promising objects in medicinal chemistry due to their broad spectrum of biological activities and the possibility of versatile functional substitution. Establishing structure–activity relationships is essential for the rational design of potent antioxidants. Preliminary DPPH screening of *N*-(4-oxo-2-thioxothiazolidin-3-yl)-2-(2-oxobenzo[*d*]thiazol-3(2*H*)-yl)acetamide and its 5-ylidene derivatives established that only the unsubstituted core heterocycle exhibits high activity comparable to ascorbic acid. Substitution at the 5-position of the rhodanine ring results in a decrease or loss of free radical scavenging activity. Among the derivatives, the compound bearing a 2,3-dichlorobenzylidene fragment exhibited the highest activity (62.85% at 2.0 mM), whereas other substituted benzylidene or isatinylidene derivatives displayed lower levels of inhibition. Modification of the 5-ylidene moiety appears to deactivate direct radical reactions. The observed effects are attributed to a complex interplay of electronic, thermodynamic, and delocalization factors, alongside limited steric accessibility of the reactive centers. Quantum chemical calculations using density functional theory for the most active heterocycle with a 5-unsubstituted rhodanine scaffold indicate that SPLET is the prevailing mechanism in methanol and substantiate its high radical scavenging activity in the DPPH test. The HAT pathway remains a plausible alternative under certain conditions or for different radicals, but it appears less favorable in MeOH. Similarly, SET-PT is thermodynamically possible but not initially favorable due to the higher ionization energy of the neutral molecule. Such synergy of approaches outlines the structure–activity relationship and provides a foundation for the rational design and targeted synthesis of the most promising agents for subsequent *in vivo* studies.

Keywords: rhodanine, benzothiazole, 5-ylidene derivatives, radical scavenging activity, 2,2-diphenyl-1-picrylhydrazyl, spectrophotometry, quantum chemical calculations, structure–activity relationship.

DOI: 10.32434/0321-4095-2026-166-3-89-99

Introduction

In recent years, a growing interest among scientists in the search for antioxidants to prevent the known harmful effects of free radicals on human metabolism has been observed. This is explained by the key role of antioxidants in the prevention and treatment of diseases associated with oxidative stress, which is an important factor in the development of many pathologies: cardiovascular diseases, neurodegenerative diseases (Alzheimer's, Parkinson's), diabetes, cancer. Antioxidants are able to neutralize free radicals, reducing damage to DNA, proteins, and lipids [1].

The efficacy of their action is determined by specific structural and functional factors, which can be categorized into four fundamental groups: electronic factors (governed by the inductive (–I) and mesomeric (+M) effects of substituents within the molecules, which redistribute electron density and determine the lability of active hydrogen atoms); thermodynamic factors (Bond Dissociation Enthalpy (BDE), Ionization Potential (IP), Proton Dissociation Enthalpy (PDE), Proton Affinity (PA, correlating with the pK_a acidity constant), and Electron Transfer Enthalpy (ETE)), which determine the energetic feasibility of the reaction following a specific free radical scavenging mechanism (Hydrogen Atom Transfer (HAT), Single Electron Transfer–Proton Transfer (SET-PT), or Sequential Proton Loss–Electron Transfer (SPLET)); steric factors (which determine the spatial accessibility of the reactive center to the free radical and depend on the bulkiness of substituents and the backbone rigidity of the molecular scaffold); and delocalization factors (associated with the ability of the conjugated bond system to effectively redistribute electron density, thereby ensuring the stability of the resulting radical intermediate) [2].

The development of new methods for evaluating antioxidant activity (AOA) enables the rapid and reliable comparison of various compounds and the identification of promising bioactive agents for disease prevention. Among the diverse approaches to assessing antioxidant efficacy, the determination of antiradical potential is of paramount importance, as it reflects the capacity of compounds for the direct neutralization of free radicals. For this purpose, model systems are widely employed, particularly the method utilizing the stable DPPH (2,2-diphenyl-1-picrylhydrazyl) radical. This method is characterized by high reproducibility and remains one of the most prevalent and widely used techniques. The primary criterion for the preliminary evaluation of the antiradical potential of compounds is their radical scavenging activity (RSA), expressed as the percentage of DPPH radical inhibition [1].

In the context of searching for potential drug-like molecules, derivatives of thiazolidine are of particular interest [3], among which derivatives of 2-thioxothiazolidin-4-one (rhodanine) occupy a special place. They are recognized as privileged scaffolds in medicinal chemistry. Due to the wide possibilities of functionalization (especially at positions 3 and 5), the rhodanine core is a promising scaffold for the design of drug-like molecules [4]. Various pharmacological activities of such derivatives are known: anticancer [5], antioxidant [6], anti-inflammatory [7], antidiabetic [4], antimicrobial and antiviral [8].

Among rhodanine subtypes, 5-ylidenerhodanines are of particular interest, among which hit and leads compounds have been found [4,5]. As demonstrated by our previous studies, the integration of such scaffolds with other pharmacologically attractive moieties, particularly benzothiazole, leads to enhanced pharmacological action and/or a broader spectrum of biological activity for their derivatives [9]. This is consistent with literature reports indicating that 3,5-disubstituted rhodanine derivatives exhibit superior biological activity compared to their 3- or 5-monosubstituted analogs [10].

Accordingly, our study focuses on rhodanine derivatives containing a 5-ylidene moiety (specificaly benzylidene or isatinylidene) and a benzothiazole moiety at the 3-position. We have previously reported the antitumor, antiviral, and antitubercular activities of such rhodanine derivatives [11]. At the same time, the antiradical activity (ARA) of these compounds remains insufficiently explored. The potential of molecules to scavenge free radicals, acting as an additional mechanism of biological activity, could significantly expand the pharmacological profile of these hybrids.

Based on this, it was appropriate to conduct an *in vitro* experiment using the DPPH test, which is a standard approach to assessing the antioxidant potential of a compound.

The aim of this work is the spectrophotometric evaluation of previously synthesized compounds for their RSA and the assessment of prospects for their structural modification.

Experimental

Chemistry

To investigate RSA, several synthesized 5-ylidene (benzylidene/isatinylidene) derivatives of *N*-(4-oxo-2-thioxothiazolidin-3-yl)-2-(2-oxobenzo[*d*]thiazol-3(2*H*)-yl)acetamide were selected. Our previous studies have experimentally demonstrated that such moieties are the most acceptable substituents in the rhodanine core, as they are well tolerated (i.e., do not cause a significant decrease in the established activity upon molecular modification); are structurally permissible within the pharmacophore or the active site; and can be used for variations in SAR analysis (structure–activity relationship) without compromising biological activity [9,11]. Furthermore, in the structure of the studied compounds, the benzothiazole moiety is coupled to the rhodamine ring via an acetamide linker, which may facilitate the binding of the molecules to multiple targets simultaneously. The –NH group of the acetamide linker can also serve as a hydrogen donor; however, we hypothesize that its activity is determined by the ability of the substituents at the 5-position to exert electron-withdrawing properties, thereby facilitating the cleavage of the N–H bond (Fig. 1).

The synthesis and structural characterization (elemental analysis and ^{1}H NMR) of these compounds were reported in our previous studies [11]. The structural formulas of the compounds with previously identified *in vitro* biological activity are presented in Table 1.

In vitro experiment – DPPH radical scavenging activity

The DPPH test is a method for neutralizing stable free radicals of 2,2-diphenyl-1-picrylhydrazyl. This method provides information about the activity of a molecule in scavenging DPPH radicals, whose alcohol solutions are colored in an intense violet color (λ_{max}=517 nm). Upon binding of compounds with free radicals, yellow-colored products are formed that do not absorb light at the above-mentioned wavelengths. The DPPH method allows for rapid identification of «hit compounds» with savings in time and quantities of substances.

Research methodology

Two millilitres of a 2.0 mM or 0.2 mM solution of the test compounds in dimethyl sulfoxide (DMSO) were mixed with 2.0 mL of a 0.1 mM methanolic solution of DPPH. The mixtures were shaken and incubated in the dark at 25^{0}C for 30 minutes. The absorbance of the test sample (A_{sample}) was measured at 517 nm [12]. The absorbance of the control sample ($A_{control}$), obtained by mixing 2.0 mL of DMSO with 2.0 mL of a 0.1 mM methanolic solution of DPPH, was determined simultaneously. Ascorbic acid was employed as a positive control (reference solution).

The RSA was calculated using the following formula:

$$\mathrm{RSA}(\%) = \frac{A_{control} - A_{sample}}{A_{control}} \cdot 100\%,$$

where $A_{control}$ is the absorbance of the DPPH solution without the test compound (control solution); A_{sample} is the absorbance of the DPPH solution in the presence of the test compound (test solution).

The calculated RSA values below 0% were treated as zero.

Reagents and synthesized compounds were weighed using a RADWAG AS 220/C analytical

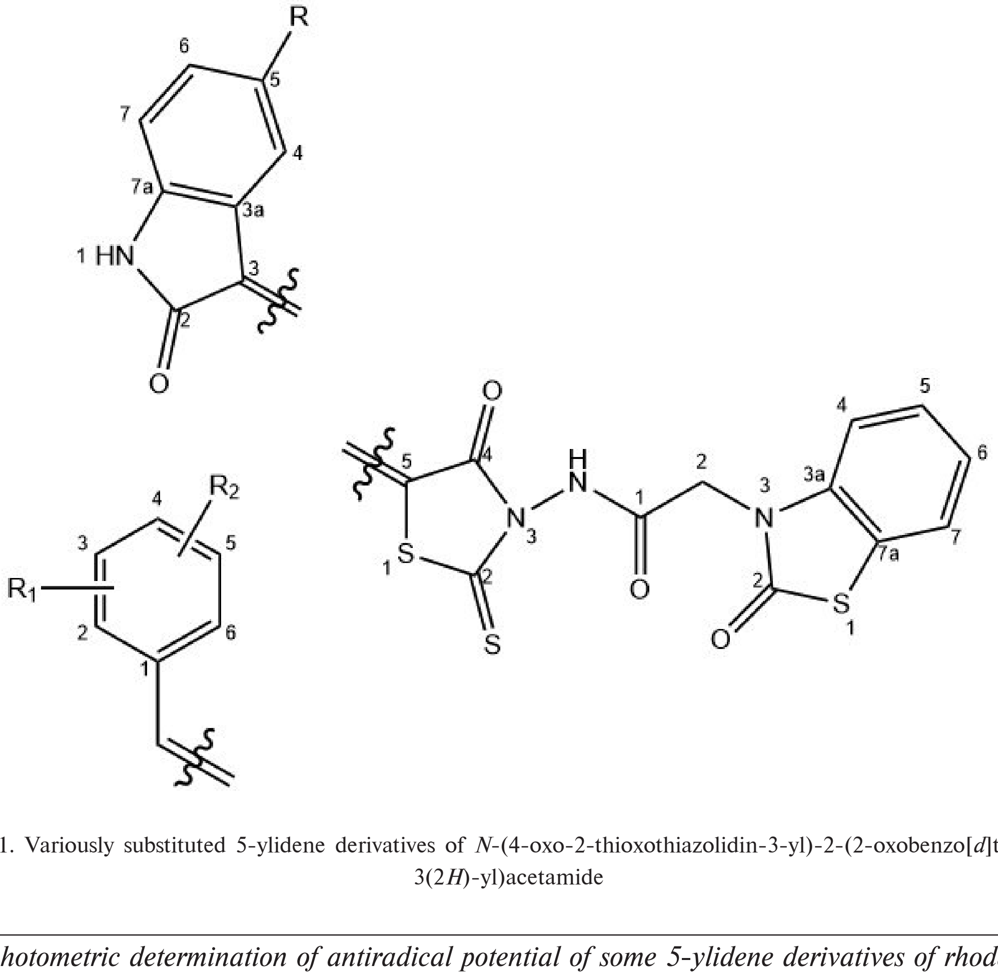
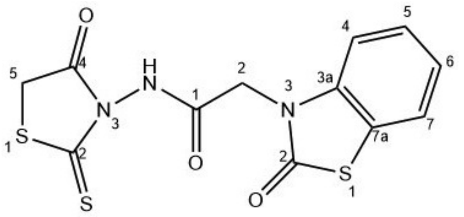
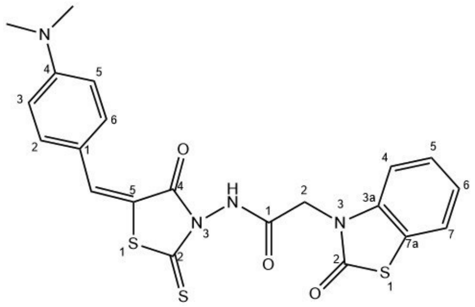
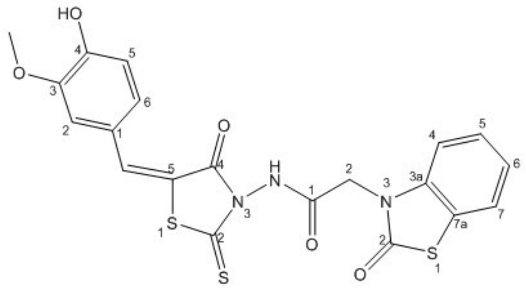
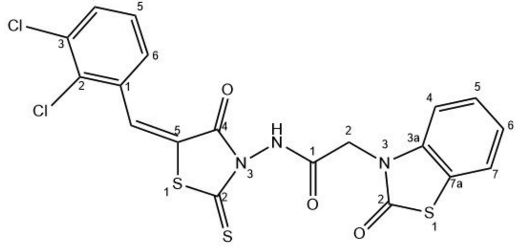


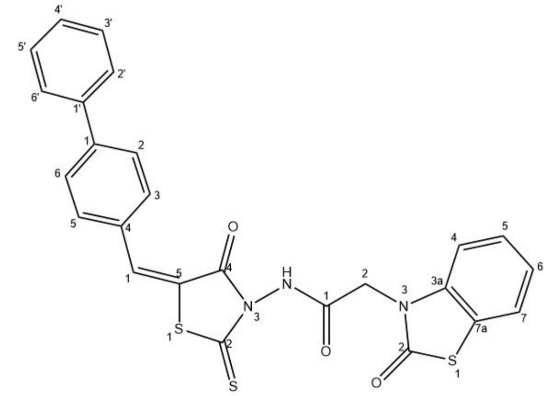
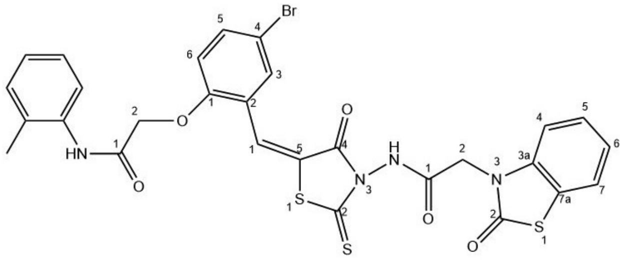
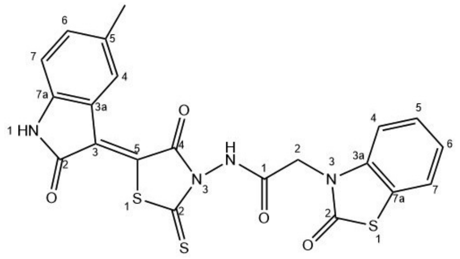
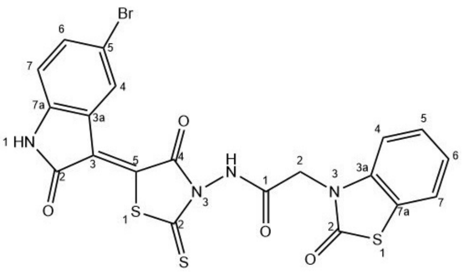
Fig. 1. Variously substituted 5-ylidene derivatives of *N*-(4-oxo-2-thioxothiazolidin-3-yl)-2-(2-oxobenzo[*d*]thiazol-3(2*H*)-yl)acetamide

Table 1

Structures and reported *in vitro* activity profiles of the synthesized derivatives

Compound No.	Structure and chemical name	Reported *in vitro* activity
1	2	3
Core heterocycle	 *N*-(4-oxo-2-thioxothiazolidin-3-yl)-2-(2-oxobenzo[*d*]thiazol-3(2*H*)-yl)acetamide	anticancer
Compound 1	 *(E)-N*-(5-(4-(dimethylamino)benzylidene)-4-oxo-2-thioxothiazolidin-3-yl)-2-(2-oxobenzo[*d*]thiazol-3(2*H*)-yl)acetamide	antitubercular
Compound 2	 *(E)-N*-(5-(4-hydroxy-3-methoxybenzylidene)-4-oxo-2-thioxothiazolidin-3-yl)-2-(2-oxobenzo[*d*]thiazol-3(2*H*)-yl)acetamide	anticancer, antitubercular
Compound 3	 *(E)-N*-(5-(2,3-dichlorobenzylidene)-4-oxo-2-thioxothiazolidin-3-yl)-2-(2-oxobenzo[*d*]thiazol-3(2*H*)-yl)acetamide	antitubercular

Continued Table 1

1	2	3
Compound 4	 *(E)*-*N*-(5-([1,1′-biphenyl]-4-ylmethylene)-4-oxo-2-thioxothiazolidin-3-yl)-2-(2-oxobenzo[*d*]thiazol-3(2*H*)-yl)acetamide	anticancer, antitubercular
Compound 5	 *(E)*-2-(4-bromo-2-((4-oxo-3-(2-(2-oxobenzo[*d*]thiazol-3(2*H*)-yl)acetamido)-2-thioxothiazolidin-5-ylidene)methyl)phenoxy)-*N*-(*o*-tolyl)acetamide	antiviral
Compound 6	 *(Z)*-*N*-(5-(5-methyl-2-oxoindolin-3-ylidene)-4-oxo-2-thioxothiazolidin-3-yl)-2-(2-oxobenzo[*d*]thiazol-3(2*H*)-yl)acetamide	anticancer, antitubercular
Compound 7	 *(Z)*-*N*-(5-(5-bromo-2-oxoindolin-3-ylidene)-4-oxo-2-thioxothiazolidin-3-yl)-2-(2-oxobenzo[*d*]thiazol-3(2*H*)-yl)acetamide	antiviral

balance (Poland). Absorbance was measured with a Shimadzu UV-1800 double-beam UV–Vis spectrophotometer (Japan).

DFT calculations

Quantum-chemical calculations were carried out in accordance with the ORCA (6.1.0) [13] using a composite DFT protocol for implicit methanol solvation. Geometry optimizations and harmonic frequency calculations (for stationary point confirmation and thermal/ZPE/entropic corrections at 298 K) were computed at the B97-3c level and single-point electronic energies were determined at the r2SCAN-3c level, while solvent phenomena were modeled with the CPCM continuum model and SMD parameters were estimated for methanol (MeOH). The composite thermodynamic quantities were constructed as $G_{comp}=E_{SP}+(G_{opt,freq}-E_{opt,freq})$ and $H_{comp}=E_{SP}+(H_{opt,freq}-E_{opt,freq})$, where E_{SP} is the single-point energy of r2SCAN-3c and thermal corrections taken from the B97-3c frequency calculations. In accordance with this principle, BDE/BDFE (HAT pathway) and IP, PDE, PA, ETE (SET-PT/SPLET pathways) antioxidant descriptors [14] were investigated for compatibility with the respective neutral, radical, cation-radical, and anionic species of core heterocycle. Literature reference terms for methanol were used for the proton solvation free energy and electron free-energy term (using the absolute NHE scale in MeOH) for solution-phase SET-PT/SPLET thermodynamic cycles. Further, reaction ΔG and ΔH parameters for DPPH-based model steps were calculated from the identical standardized thermodynamic model with consistently optimized and re-evaluated $DPPH^{\bullet}$, $DPPH^{-}$, and DPPH–H species in MeOH.

Results and discussion

The ability of synthesized compounds to neutralize free radicals was analyzed as one of the criteria for their prospects for further pharmacological screening, since it may be associated with various types of their biological effects.

The results of the primary screening of the synthesized compounds indicate that their RSA is significantly influenced by the nature of the ylidene substituent at the C5 position of the rhodanine ring. Table 2 presents the levels of DPPH radical inhibition at two concentrations.

According to the data presented in Table 2, the core heterocycle exhibited the highest RSA among the test compounds. It demonstrated a high level of inhibition of DPPH radicals at both tested concentrations (2.0 mM and 0.2 mM), exceeding 70%, which is comparable to the reference solution.

5-Ylidene derivatives are less active as radical scavengers compared to the basic heterocycle and ascorbic acid. Among them, 5-benzylidene derivatives (compounds 2–5), with the exception of compound 1, showed antiradical potential. In contrast, 5-isatinylidene derivatives (compounds 6 and 7) were found to be completely inactive under the tested conditions.

Among 5-benzylidene derivatives, the strongest radical scavenging potential was observed for compound 3, which contains two electron-withdrawing chlorine atoms in the benzylidene moiety. Its relatively high activity was observed at a concentration of 2.0 mM (62.85%), which is consistent with the inductive effect of the chlorine atoms capable of stabilizing radical intermediates. However, a tenfold reduction in concentration to 0.2 mM let to a sharp decrease in activity (20.99%).

A similar decrease in RSA with declining concentration was observed for other 5-benzylidene derivatives, confirming the concentration-dependent nature of their antioxidant properties. This indicates a complex mechanism of radical deactivation.

In addition, the activity level also depends on the substituents introduced into the benzylidene moiety. Specifically, compound 4 bearing a biphenyl substituent, demonstrated moderate DPPH radical inhibition (40.23%) at a concentration of 2.0 mM, which slightly exceeds the RSA value for compound 5 (30.92%) at the same concentration.

Compounds 1 and 2, bearing electron-donating substituents in the benzylidene moiety, failed to exhibit significant RSA at either concentration. Unexpectedly, compound 2 (bearing a 4-hydroxy-3-methoxybenzylidene substituent) displayed low RSA (14.42%), while compound 1 (bearing a 4-dimethylaminobenzylidene substituent) was found to be completely inactive. Despite this, the 5-benzylidene derivatives represent a promising scaffold for potential antioxidants and warrant further thorough investigation.

Table 2

Antiradical activity of the synthesized compounds *in vitro*

Compound	RSA, %	
	2.0 mM	0.2 mM
Ascorbic acid	82.23	76.74
Core heterocycle	73.66	73.05
Compound 1	0	0
Compound 2	14.42	2.27
Compound 3	62.85	20.99
Compound 4	40.23	12.16
Compound 5	30.92	11.31
Compound 6	0	0
Compound 7	0	0

We hypothesized that the incorporation of an ylidene substituent into the conjugated rhodamine-acetamide-benzothiazole scaffold generally leads to the deactivation of the molecule"s reactive centers, resulting in a diminished RSA for most derivatives compared to the parent structure. However, the significant activity observed for the 2,3-dichloro-substituted derivative suggests that specific electronic effects of the substituents can modulate this process, potentially by creating alternative sites for radical attack.

For a comprehensive assessment of antiradical potential, it is advisable to combine experimental methods with theoretical calculations. Quantum chemical calculations using the density functional theory (DFT) method allow determining key parameters specific to three possible mechanisms of action (HAT, SET-PT, and SPLET) and explaining the reasons for experimentally established activity. DFT calculations were performed for the core heterocycle, which demonstrated the highest RSA in the DPPH test. This approach enables the specification of the possible predominant mechanism and provides a robust justification for the observed antioxidant potential.

As shown in Table 3, the deprotonation step of the SPLET mechanism (PA=26.37 kcal/mol) is thermodynamically more favorable than the initial electron-transfer step of the SET-PT pathway (IP=42.31 kcal/mol), indicating that SPLET is the preferred initiation route for core heterocycle in methanol. Importantly, the thermodynamic cycle closes exactly within numerical precision, i.e.,

$$\mathrm{IP+PDE=PA+ETE=54.11\ kcal/mol},$$

which confirms internal consistency of the computed free-energy scheme and the adopted proton/electron reference terms.

The HAT pathway was assessed through the bond dissociation enthalpy and free energy of the relevant X–H bond in core heterocycle. The obtained values (BDE=102.95 kcal/mol, BDFE=94.64 kcal/mol) indicate that homolytic cleavage of this bond is thermodynamically possible but relatively energy-demanding in methanol. Thus, while HAT cannot be excluded as a mechanistic option, especially depending on the nature of the radical acceptor, it does not appear to be the most favorable initiation pathway under the present solvent conditions.

For the SET-PT mechanism, the first step is electron abstraction from neutral core heterocycle, quantified by the ionization potential (IP=42.31 kcal/mol). This value is significantly higher than the deprotonation-related term of the alternative SPLET route, indicating that direct ionization of the neutral molecule is thermodynamically costly in MeOH. The second SET-PT step, represented by PDE=11.79 kcal/mol, is substantially less demanding, which is a common pattern for SET-PT: the initial electron transfer is typically the limiting step, while subsequent proton release from the cation-radical is more facile.

In contrast, the SPLET mechanism is thermodynamically more favorable in methanol (Fig. 2).

The first SPLET step, deprotonation of neutral core heterocycle, is characterized by PA=26.37 kcal/mol, which is markedly lower than the ionization potential. The second SPLET step, electron transfer from the anion, has ETE=27.73 kcal/mol, which is thermodynamically accessible and, together with the lower PA value, supports SPLET as the preferred route in a polar protic solvent. The key mechanistic indicator is therefore the comparison of the first steps: PA<IP, which strongly favors initiation through deprotonation rather than direct electron transfer.

To further support the thermodynamic interpretation using a common radical probe, the reaction energetics with DPPH$^{\bullet}$ were also evaluated in methanol. A fully consistent set of composite

Table 3

Thermodynamic descriptors of antioxidant mechanisms for core heterocycle in MeOH (298 K)

Descriptor*	Definition	Value (kcal/mol)	Value (kJ/mol)
BDE	$AH \rightarrow A^{\bullet}+H^{\bullet}$ (enthalpy, HAT)	102.95	430.75
BDFE	$AH \rightarrow A^{\bullet}+H^{\bullet}$ (free energy, HAT)	94.64	395.96
IP	$AH \rightarrow AH^{\bullet+}+e^{-}$ (SET-PT, step 1)	42.31	177.04
PDE	$AH^{\bullet+} \rightarrow A^{\bullet}+H^{+}$ (SET-PT, step 2)	11.79	49.33
PA	$AH \rightarrow A^{-}+H^{+}$ (SPLET, step 1)	26.37	110.35
ETE	$A^{-} \rightarrow A^{\bullet}+e^{-}$ (SPLET, step 2)	27.73	116.03
Cycle closure check	IP+PDE vs. PA+ETE	54.11	226.38

Note: * – composite scheme: B97-3c (opt+freq) // r2SCAN-3c (SP), CPCM/SMD (MeOH).

thermodynamic functions was obtained for DPPH$^{\bullet}$, DPPH^{-}, and DPPH–H, enabling calculation of DG and DH for three representative elementary processes: 1) HAT from «*Core heterocycle*» to DPPH$^{\bullet}$, 2) SET from neutral «*Core heterocycle*» to DPPH^{-}, and 3) electron transfer from the deprotonated «*Core heterocycle*» anion to DPPH$^{\bullet}$ (the SPLET-associated ET step). The calculated reaction free energies are +33.45 kcal/mol for HAT, +35.77 kcal/mol for direct SET from the neutral form, and +21.18 kcal/mol for electron transfer from the anion. The corresponding reaction enthalpies follow the same trend.

All three modeled steps of interaction with DPPH are endergonic in the continuum-solvation thermodynamic framework; however, their relative ordering provides mechanistic information. The lowest ΔG was obtained for electron transfer from the anion of the core heterocycle to DPPH$^{\bullet}$, i.e., the step associated with the SPLET pathway. This is fully consistent with the descriptor-based analysis (lower PA than IP) and reinforces the conclusion that in methanol the antioxidant behavior of the core heterocycle is more likely to proceed via deprotonation followed by electron transfer rather than by direct HAT or direct electron transfer from the neutral species.

Overall, the combined descriptor analysis (BDE/BDFE, IP, PA, PDE, ETE) and DPPH-based reaction thermodynamics consistently indicate that SPLET is the thermodynamically preferred antioxidant mechanism for «*core heterocycle*» in methanol. The HAT pathway remains a plausible alternative under certain conditions or for different radicals, but it appears less favorable in MeOH. Similarly, SET-PT is thermodynamically possible, yet disfavored at the initiation stage due to the higher ionization energy of the neutral molecule.

The integration of the DPPH test and DFT calculations enabled not only the characterization of antiradical potential, but also the prediction of the mechanism underlying its activity.

SAR-analysis

Structure–antiradical activity relationship reveled that unsubstituted C5 position of the rhodanine ring is a key structural determinant for efficient radical scavenging. In our view, the RSA levels of the studied 5-ylidene derivatives result from a synergy of several parameters within their molecular systems.

Based on modern concepts of antiradical mechanisms [2], we suggest that the experimentally established percentages of radical inhibition are the result of a complex interplay of thermodynamic parameters (specifically BDE, IP, PA, and ETE), which determine the dominance of one of the free radical scavenging mechanism (HAT, SET-PT, or SPLET), and delocalization factors of molecules that ensure the stability of the resulting radical intermediates. Furthermore, the steric accessibility of the reactive centers may play a significant role, which is consistent with literature data regarding the critical impact of the conformational rigidity of the molecular scaffold on the reactivity of compounds. It is the synergy of these factors that explains why even a slight change in a substituent or the introduction of a bulky fragment can radically alter the DPPH inhibition percentage in our experiments.

SAR analysis allows tracking the influence of the nature of substituents at the 5-position of the rhodanine scaffold on the RSA of molecules. For

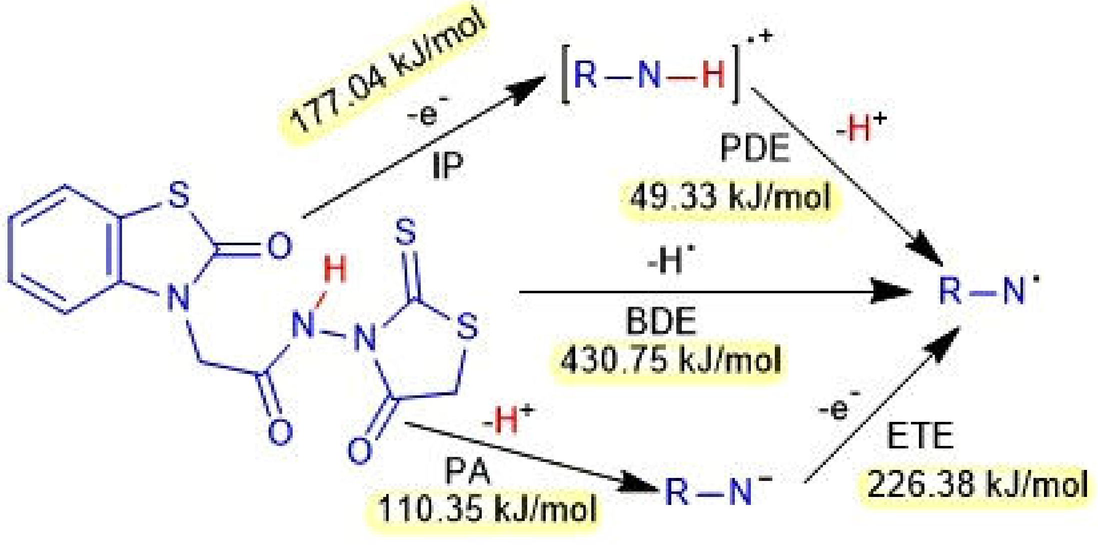


Fig. 2. Proposed antioxidant pathways for core heterocycle in methanol (MeOH)

instance, the established lack of activity in isatinylidenes (compound 6 and 7) can be explained by the dominance of the steric factor, such as conformational rigidity of the molecular backbone and the spatial shielding of the reactive center, which prevents effective interaction with the DPPH radical. At the same time, the lack of activity for compound 1 (with a dimethylamino group) indicates the dominance of electronic factors. The strong mesomeric effect (+M) of the amino group likely leads to significant electronic oversaturation of the system, which probably deactivates the reactive centers or hinders single-electron transfer (SET) due to excessive stabilization of the molecule's ground state.

The low activity of compound 2 (with a 4-hydroxy-3-methoxybenzylidene fragment), despite the presence of a phenolic hydroxyl, may be due to thermodynamic factors: the formation of an intramolecular hydrogen bond between the OH– and OCH_3– groups, which increases the BDE and hinders the realization of the HAT mechanism.

Instead, the moderate activity of compounds 3 and 4 may be due to favorable delocalization factors, which ensure the stability of radical intermediates through an extended conjugation system. Meanwhile, the highest activity of the core heterocycle confirms the impotance of delocalization factors. In these structures, the abcence of bulky or excessively donating substituents apparently facilitates the unimpeded distribution of electron density across both the benzothiazole and rhodanine rings, ensuring the maximum stability of the radical intermediates after interaction with DPPH.

Thus, the synergism between experimental and theoretical approaches provides a reliable rationale for SAR and offers new insights into the design and development of novel antioxidants.

Conclusions

Based on the primary screening results, the unsubstituted C5 position in the rhodanine core was identified as a key structural feature for effective free radical scavenging. The parent scaffold demonstrated a high level of RSA at both studied concentrations, comparable to that of the ascorbic acid. Modification at the 5 position with ylidene substituents significantly affects the antiradical profile. The introduction of a benzylidene moiety leads to a decrease in radical inhibition activity, which is most pronounced upon dilution to a concentration of 0.2 mM. The incorporation of a 5-substituted isatinylidene moiety negates RSA. SAR-analysis revealed that the activity of the synthesized derivatives is governed by the nature and position of substituents within the 5-ylidene moiety. It was established that the presence of electron-donating groups does not guarantee high activity. Compound 3, bearing electron-withdrawing chlorine atoms, exhibited the most potent effect. The sharp decline in activity upon dilution highlights a complex interplay of electronic, thermodynamic, and delocalization factors, alongside the steric accessibility of reactive centers. The investigated compounds may be of interest as potential multitarget agents, provided that their moderate direct antiradical effect is compensated by an impact on key enzymatic defense pathways and genoprotection. This outlines the directions for further investigation. DFT calculations substantiated the *in vitro* findings for the core heterocycle. The obtained data identify *(E)*-*N*-(5-(2,3-dichlorobenzylidene)-4-oxo-2-thioxothiazolidin-3-yl)-2-(2-oxobenzo[*d*]thiazol-3(2*H*)-yl)acetamide as a promising antioxidant candidate whose reactivity in polar protic media is primarily governed by anion formation and subsequent electron transfer. Mechanistic modeling provides deeper insights into the structure–antiradical activity relationship and established a foundation for the rational design of promising antioxidants. Future efforts will be focused on an in-depth quantum chemical analysis of the 5-ylidene derivatives. This will enable the theoretical substantiation of the dominant antiradical mechanisms and provide a detailed understanding of the electronic effects within the 5-ylidene moiety. An important step will involve verifying potential multitarget profile of the compounds through *in silico* prediction. Such a chemoinformatic approach facilitates the rational design and targeted synthesis of the most promising agents for future *in vivo* evaluation.

Acknowledgments

The authors are grateful to the Armed Forces of Ukraine for providing the safety and opportunity to work and conduct this research.

Conflict of interest

The authors declare no conflict of interest.

REFERENCES

1. *Gulcin I., Alwasel S.H.* DPPH radical scavenging assay // Processes. – 2023. – Vol.11. – No. 8. – Art. No. 2248.

2. *Galano A., Alvarez-Idaboy J.R.* Computational strategies for predicting free radical scavengers' protection against oxidative stress: where are we and what might follow? // Int. J. Quantum Chem. – 2018. – Vol.119. – No. 2. – Art. No. e25665.

3. *Synthesis* and evaluation of biological activity of 1-[2-amino-4-methylthiazol-5-yl]-3-arylpropenones / Lozynskyi A.V., Yushyn I.M., Konechnyi Y.T., Roman O.M., Matiykiv O.V., Smaliukh O.V., et al. // Biopolym. Cell. – 2021. – Vol.37. – No. 5. – P.389-399.

4. *Rhodanine* scaffold: A review of antidiabetic potential and structure—activity relationships (SAR) / Bin Ahmad Kamar A.K.D., Yin L.J., Liang C.T., Fung G.T., Avupati V.R. // Med. Drug Discov. — 2022. — Vol.15. — Art. No. 100131.

5. *Synthesis* and in vitro anticancer potential of new thiazole-containing derivatives of rhodanine / Los O.V., Sinenko V.O., Kobzar O.L., Zhirnov V.V., Vovk A.I., Brovarets V.S. // Chem. Heterocycl. Compd. — 2023. — Vol.59. — P.484-493.

6. *Saturated* five-membered thiazolidines and their derivatives: from synthesis to biological applications / Sahiba N., Sethiya A., Soni J., Agarwal D.K., Agarwal S. // Top. Curr. Chem. — 2020. — Vol.378. — No. 2. — Art. No. 34.

7. *Thiazolidines* derivatives and their anti-inflammatory activity in LPS-induced RAW 264.7 macrophages: a systematic review and meta-analysis / Bramorski Mohr E.T., Lubschinski T.L., Oliveira J.M.D.D., Oliveira P.G.F.D., Borba B.G.M., Demarchi I.G., et al. // Nat. Prod. Res. — 2025. — Vol.39. — No. 10. — P.2895-2911.

8. *Rhodanine* derivatives: an insight into the synthetic and medicinal perspectives as antimicrobial and antiviral agents / Chaurasyia A., Chawla P., Monga V., Singh G. // Chem. Biol. Drug Des. — 2023. — Vol.101. — No. 3. — P.500-549.

9. *Synthesis* and anticancer activity evaluation of 4-thiazolidinones containing benzothiazole moiety / Havrylyuk D., Mosula L., Zimenkovsky B., Vasylenko O., Gzella A., Lesyk R. // Eur. J. Med. Chem. — 2010. — Vol.45. — P.5012-5021.

10. *Anticancer* profile of rhodanines: structure—activity relationship (SAR) and molecular targets — a review / Szczepanski J., Tuszewska H., Trotsko N. // Molecules. — 2022. — Vol.27. — No. 12. — Art. No. 3750.

11. *Syntez* ta poperednia otsinka farmakolohichnoho potentsialu pokhidnykh rodaninu z benztiazolnym frahmentom u molekulakh / Mosula L.M., Havryliuk D.Ya., Kazmirchuk G.V., Lesyk R.B. // Farmatsevtychnyi Zhurnal. — 2009. — Vol.1. — P.54-60.

12. *Synthesis*, tautomerism, and antiradical activity of novel pteridinetrione derivatives / Kazunin M.S., Voskoboynik O.Yu., Nosulenko I.S., Berest G.G., Sergeieva T., Okovytyy S., et al. // J. Heterocycl. Chem. — 2018. — Vol.55. — No. 4. — P.1033-1041.

13. *Neese F.* Software update: the ORCA program system — version 6.0 // *WIREs Comput. Mol. Sci.* — 2025. — Vol.15. — Art. No. e70019.

14. *Design*, synthesis, molecular docking, and antioxidant properties of a series of new S-derivatives of ((1,2,4-triazol-3(2H)-yl)methyl)thiopyrimidines / Karpenko Y., Medvedeva K., Solomennyi A., Rudenko O., Panasenko O., Parchenko V., et al. // ScienceRise Pharm. Sci. — 2025. — Vol.1. — No. 53. — P.62-70.

Received 24.02.2026
Accepted 12.05.2026
Published 26.06.2026

СПЕКТРОФОТОМЕТРИЧНЕ ВИЗНАЧЕННЯ АНТИРАДИКАЛЬНОГО ПОТЕНЦІАЛУ ДЕЯКИХ 5-ІЛІДЕНПОХІДНИХ РОДАНІНУ З БЕНЗОТІАЗОЛЬНИМ ФРАГМЕНТОМ

Л.М. Мосула, В.В. Мазур, В.С. Мосула, Ю.В. Карпенко

Робота присвячена оцінюванню антирадикального потенціалу гібридних систем на основі бензотіазол-роданінового скафолду, синтез яких описано раніше. Такі гетероцикли є перспективними об'єктами медичної хімії завдяки широкому спектру біологічної активності та можливості варіативного функціонального заміщення. Актуальність дослідження зумовлена необхідністю встановлення закономірностей «структура—активність» для дизайну ефективних антиоксидантів. Первинний скринінг антирадикальної активності *N*-(4-оксо-2-тіоксотіазолидин-3-іл)-2-(2-оксобензо[*d*]тіазол-3(2*H*)-іл)ацетаміду та його 5-іліденпохідних показав, що лише незаміщений базовий гетероцикл виявляє високу активність, сумірну з аскорбіновою кислотою. Заміщення у 5-ому положенні роданінового циклу призводить до зниження або втрати здатності до поглинання вільних радикалів. Найвищу активність серед похідних демонструє сполука з 2,3-дихлоробензилідeновим фрагментом (62,85% при 2,0 мМ), тоді як інші бензиліден- або ізатиніліденпохідні виявляють нижчі рівні інгібуванння. Модифікація 5-іліденового фрагмента, схоже, є деактивуючим фактором для прямого перебігу радикальних реакцій. Виявлені ефекти пояснюються комплексною взаємодією електронних, термодинамічних та делокалізаційних чинників разом із обмеженою стеричною доступністю реакційних центрів. Квантово-хімічні розрахунки з використанням методу теорії функціоналу густини для найактивнішого гетероциклу із 5-незаміщеним роданіновим ядром вказують на механізм SPLET як переважаючий у метанолі та обґрунтовують його високу радикал-поглинаючу активність у DPPH-тесті. Шлях HAT залишається ймовірною альтернативою за певних умов або для різних радикалів, але він видається менш сприятливим у MeOH. Аналогічно, SET-PT є термодинамічно можливим, але не сприятливим на початковому етапі через вищу енергію іонізації нейтральної молекули. Така синергія підходів окреслює взаємозв'язок «структура—активність» і створює основу для раціонального дизайну та цілеспрямованого синтезу найбільш перспективних агентів для подальших *in vivo* досліджень.

Ключові слова: роданін, бензотіазол, 5-іліденпохідні, радикал-поглинаюча активність, 2,2-дифеніл-1-пікрилгідразил, спектрофотометрія, квантово-хімічні розрахунки, взаємозв'язок «структура—активність».

SPECTROPHOTOMETRIC DETERMINATION OF ANTIRADICAL POTENTIAL OF SOME 5-YLIDENE DERIVATIVES OF RHODANINE WITH A BENZOTHIAZOLE MOIETY IN THE MOLECULES

L.M. Mosula [a, b, *], V.S. Mosula [a], V.V. Mazur [a], Y.V. Karpenko [c]

[a] **I. Horbachevsky Ternopil National Medical University, Ternopil, Ukraine**

[b] **Ternopil Ivan Puluj National Technical University, Ternopil, Ukraine**

[c] **Zaporizhzhia State Medical and Pharmaceutical University, Zaporizhzhia, Ukraine**

[*] **e-mail: mosula@tdmu.edu.ua**

This study evaluates the antiradical potential of hybrid systems based on the benzothiazole-rhodanine scaffold, the synthesis of which was previously reported. Such heterocycles are promising objects in medicinal chemistry due to their broad spectrum of biological activities and the possibility of versatile functional substitution. Establishing structure–activity relationships is essential for the rational design of potent antioxidants. Preliminary DPPH screening of N-(4-oxo-2-thioxothiazolidin-3-yl)-2-(2-oxobenzo[d]thiazol-3(2H)-yl)acetamide and its 5-ylidene derivatives established that only the unsubstituted core heterocycle exhibits high activity comparable to ascorbic acid. Substitution at the 5-position of the rhodanine ring results in a decrease or loss of free radical scavenging activity. Among the derivatives, the compound bearing a 2,3-dichlorobenzylidene fragment exhibited the highest activity (62.85% at 2.0 mM), whereas other substituted benzylidene or isatinylidene derivatives displayed lower levels of inhibition. Modification of the 5-ylidene moiety appears to deactivate direct radical reactions. The observed effects are attributed to a complex interplay of electronic, thermodynamic, and delocalization factors, alongside limited steric accessibility of the reactive centers. Quantum chemical calculations using density functional theory for the most active heterocycle with a 5-unsubstituted rhodanine scaffold indicate that SPLET is the prevailing mechanism in methanol and substantiate its high radical scavenging activity in the DPPH test. The HAT pathway remains a plausible alternative under certain conditions or for different radicals, but it appears less favorable in MeOH. Similarly, SET-PT is thermodynamically possible but not initially favorable due to the higher ionization energy of the neutral molecule. Such synergy of approaches outlines the structure–activity relationship and provides a foundation for the rational design and targeted synthesis of the most promising agents for subsequent in vivo studies.

Keywords: rhodanine; benzothiazole; 5-ylidene derivatives; radical scavenging activity; 2,2-diphenyl-1-picrylhydrazyl; spectrophotometry; quantum chemical calculations; structure–activity relationship.

REFERENCES

1. Gulcin I, Alwasel SH. DPPH radical scavenging assay. *Processes.* 2023; 11: 2248. doi: 10.3390/pr11082248.

2. Galano A, Alvarez-Idaboy JR. Computational strategies for predicting free radical scavengers' protection against oxidative stress: where are we and what might follow? *Int J Quantum Chem.* 2018; 119: e25665. doi: 10.1002/qua.25665.

3. Lozynskyi AV, Yushyn IM, Konechnyi YT, Roman OM, Matiykiv OV, Smaliukh OV, et al. Synthesis and evaluation of biological activity of 1-[2-amino-4-methylthiazol-5-yl]-3-arylpropenones. *Biopolym Cell.* 2021; 37(5): 389-399. doi: 10.7124/bc.000A64.

4. bin Ahmad Kamar AKD, Yin LJ, Liang CT, Fung GT, Avupati VR. Rhodanine scaffold: a review of antidiabetic potential and structure–activity relationships (SAR). *Med Drug Discov.* 2022; 15: 100131. doi: 10.1016/j.medidd.2022.100131.

5. Los OV, Sinenko VO, Kobzar OL, Zhirnov VV, Vovk AI, Brovarets VS. Synthesis and in vitro anticancer potential of new thiazole-containing derivatives of rhodanine. *Chem Heterocycl Compd.* 2023; 59: 484-493. doi: 10.1007/s10593-023-03220-z.

6. Sahiba N, Sethiya A, Soni J, Agarwal DK, Agarwal S. Saturated five-membered thiazolidines and their derivatives: from synthesis to biological applications. *Top Curr Chem.* 2020; 378: 34. doi: 10.1007/s41061-020-0298-4.

7. Bramorski Mohr ET, Lubschinski TL, Dias de Oliveira JM, Fragoso de Oliveira PG, Borba BGM, Demarchi IG, et al. Thiazolidines derivatives and their anti-inflammatory activity in LPS-induced RAW 264.7 macrophages: a systematic review and meta-analysis. *Nat Prod Res.* 2025; 39: 2895-2911. doi: 10.1080/14786419.2024.2394103.

8. Chaurasyia A, Chawla P, Monga V, Singh G. Rhodanine derivatives: an insight into the synthetic and medicinal perspectives as antimicrobial and antiviral agents. *Chem Biol Drug Des.* 2023; 101(3): 500-549. doi: 10.1111/cbdd.14163.

9. Havrylyuk D, Mosula L, Zimenkovsky B, Vasylenko O, Gzella A, Lesyk R. Synthesis and anticancer activity evaluation of 4-thiazolidinones containing benzothiazole moiety. *Eur J Med Chem.* 2010; 45: 5012-5021. doi: 10.1016/j.ejmech.2010.08.008.

10. Szczepanski J, Tuszewska H, Trotsko N. Anticancer profile of rhodanines: structure–activity relationship (SAR) and molecular targets – a review. *Molecules.* 2022; 27(12): 3750. doi: 10.3390/molecules27123750.

11. Mosula LM, Havryliuk DYa, Kazmirchuk GV, Lesyk RB. Syntez ta poperednia otsinka farmakolohichnoho potentsialu pokhidnykh rodaninu z benztiazolnym frahmentom u molekulakh [Synthesis and preliminary evaluation of pharmacological potential rhodamine derivatives with benzothiazole moiety in molecules]. *Farmatsevtychnyi Zhurnal.* 2009; 1: 54-60. (*in Ukrainian*).

12. Kazunin MS, Voskoboynik OYu, Nosulenko IS, Berest GG, Sergeieva T, Okovytyy S, et al. Synthesis, tautomerism, and antiradical activity of novel pteridinetrione derivatives. *J Heterocycl Chem.* 2018; 55: 1033-1041. doi: 10.1002/jhet.3135.

13. Neese F. Software update: the ORCA program system – version 6.0. *WIREs Comput Mol Sci.* 2025; 15: e70019. doi: 10.1002/wcms.70019.

14. Karpenko Y, Medvedeva K, Solomennyi A, Rudenko O, Panasenko O, Parchenko V, et al. Design, synthesis, molecular docking, and antioxidant properties of a series of new S-derivatives of ((1,2,4-triazol-3(2H)-yl)methyl)thiopyrimidines. *ScienceRise Pharm Sci.* 2025; 1(53): 62-70. doi: 10.15587/2519-4852.2025.312075.