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EVALUATION OF BIOPHARMACEUTICAL PROPERTIES AND *IN SILICO* MODELING OF THE ANTIOXIDANT POTENTIAL OF RHODANINE-BENZOTHIAZOLE HYBRIDS

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Oxidative stress is involved in the pathogenesis of many systemic disorders; therefore, the search for new multifunctional antioxidants remains a relevant task. In this study, seven rhodanine–benzothiazole hybrids, 5-ylidene derivatives of N-(4-oxo-2-thioxothiazolidin-3-yl)-2-(2-oxobenzo[d]thiazol-3(2H)-yl)acetamide, were evaluated using ADMET prediction, target profiling, and DFT-based quantum-chemical calculations. The compounds showed moderate lipophilicity, with predicted Log P values of 1.576–3.446, and a high probability of antioxidant response element activation; SR-ARE values for 5-ylidene derivatives exceeded 0.800 and reached 0.978 for compound 1, 0.927 for compound 2, and high values for other active derivatives. Compound 2, bearing a 4-hydroxy-3-methoxybenzylidene moiety, demonstrated the most favorable direct radical-scavenging profile: $\Delta E_{H\text{-}loss}$ for the phenolic OH was 405.90 kcal·mol^{-1} in water and 407.07 kcal·mol^{-1} in methanol, while $\Delta E_{from\ anion}$ values were 107.62 and 106.84 kcal·mol^{-1}, respectively. Compound 3, containing a 2,3-dichlorobenzylidene moiety, was distinguished by increased lipophilicity and a promising indirect antioxidant/multitarget profile, which may support membrane-associated redox protection. Overall, compounds 2 and 3 can be considered the most promising representatives for further optimization as multifunctional antioxidant agents.

Keywords: rhodanine, benzothiazole, 5-ylidene derivatives, ADMET properties, quantum-chemical calculations, SAR analysis, antioxidant activity.

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Introduction

Oxidative stress is a key factor in the pathogenesis of a number of systemic diseases (cardiovascular, diabetes, cancer, multiple sclerosis, etc.) [1]. The current antioxidant strategy aims to create covalent modulators acting as inhibitors of specific regulator proteins and activators of endogenous call defense systems. The mechanism of action of such compounds involves initial reversible recognition of the therapeutic target, followed by the formation of a strong, irreversible bond between the electrophilic moiety and the cysteine residue in the active site of the protein (in particular, the sensor protein Keap1). Thus, the concept of covalent inhibition of the Keap1 protein forms the basis for the development of potent activators of cellular defence via the Nrf2/ARE pathway. The advantage of

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covalent modulators is their high efficacy and prolonged therapeutic effect, even at low doses. Successful examples of FDA-approved covalent inhibitors include a number of ancancer (for example, Ibrutinib), Osimertinib, Afatinib, Acalabrutinib, Sotorasib) and antivaral (Nimatrelvir, Boceprevir) agents [2–4].

The search for new generation antioxidants among rhodanine-benzothiazole hybrids with variously substituted aromatic moieties that have shown radical-scavenging activity *in vitro* is relevant [5]. The phenolic moiety is known to carry antioxidant potential and is present in the structures of many natural and synthetic antioxidants. The phenolic moiety acts on a single electron transfer (SET) or hydrogen atom transfer (HAT) mechanism to absorb radicals by donating an electron or H atom, and the resulting phenolic radical is resonantly stabilized by an extended conjugated system [1].

In this work, the physicochemical, pharmacokinetic, toxicological parameters of rhodanino-benzothiazole hybrids containing in the 5 position of the rhodanine cycle a benzylidene or isatinylidene moiety with electron-donating or electron-withdrawing substituents are investigated using *in silico* tools [6,7]. In addition, possible interactions of the studied compounds with biological targets are predicted, which determine the body's antioxidant response to oxidative stress. The influence of the structural features of these hybrids on their potential antioxidant activity (AOA) is evaluated. The parameters calculated *in silico* could provide a starting point for rational modelling of new potential antioxidants.

Experimental

In silico prediction of ADMET properties of molecules

To predict the ADMET properties (A – absorbtion, D – distribution, M – metabolism, E – excretion, T – toxicity) and potential interactions of *N*-(4-oxo-2-thioxothiazolidin-3-yl)-2-(2-oxobenzo[*d*]thiazol-3(2*H*)-yl)acetamide (core heterocycle) and some of its 5-benzylidene and 5-isatinylidene derivatives with therapeutic targets responsible for AOA, we have used a number of freely available *in silico* tools: ADMETlab 3.0 [8], SwissADME [9], SwissTargetPrediction [10], SuperPred 3.0 [11] i ProTox 3.0 [12]. This enabled us to compare the prediction results obtained on various online platforms and to focus on modelling the desired potential of molecules. The general structural formula of the studied molecules is shown in Fig. 1.

Quantum-chemical calculations

DFT calculations were performed using ORCA 6.1.0 [13]. The geometries of neutral molecules, cation radicals, anions formed after deprotonation, and radicals formed after hydrogen atom abstraction were prepared for two polar protic media – water (H_2O) and methanol (MeOH). Geometry optimization and frequency analysis were carried out at the B97-3c level using the CPCM/SMD solvent model [14]. To refine the energies, single-point calculations were performed at the r2SCAN-3c/CPCM/SMD level.

The antioxidant potential was evaluated through three classical free-radical neutralization mechanisms: HAT (hydrogen atom transfer), SET-PT (single electron transfer–proton transfer), and SPLET (sequential proton loss electron transfer). For this purpose, the following energetic parameters were analyzed:

$$\Delta E_{ion} = E(N^{\cdot +}) - E(N),$$

$$\Delta E_{deprot} = E(A^-) - E(N),$$

$$\Delta E_{H\text{-}loss} = E(R^{\cdot}) - E(N),$$

$$\Delta E_{from\ anion} = E(R^{\cdot}) - E(A^-),$$

where N is the neutral form; $N^{\cdot +}$ is the cation radical; A^- is the anion formed after deprotonation of the corresponding center; and $R^{\cdot}$ is the radical formed after H-atom abstraction.

All energy differences were converted from Hartree to kcal/mol using the factor 627.5095. Lower values of the corresponding descriptor were interpreted as indicating a more thermodynamically favorable process.

Results and discussion

The synthesis, structural characterization (elemental analysis and ^{1}H NMR) and certain types of biological activity of *N*-(4-oxo-2-thioxothiazolidin-3-yl)-2-(2-oxobenzo[*d*]thiazol-3(2*H*)-yl)acetamide and its 5-ylidene derivatives have been described by us previously [15]. Preliminary *in vitro* studies have demonstrated the radical-scavenging activity of the core heterocycle and some of its 5-ylidene derivatives [5].

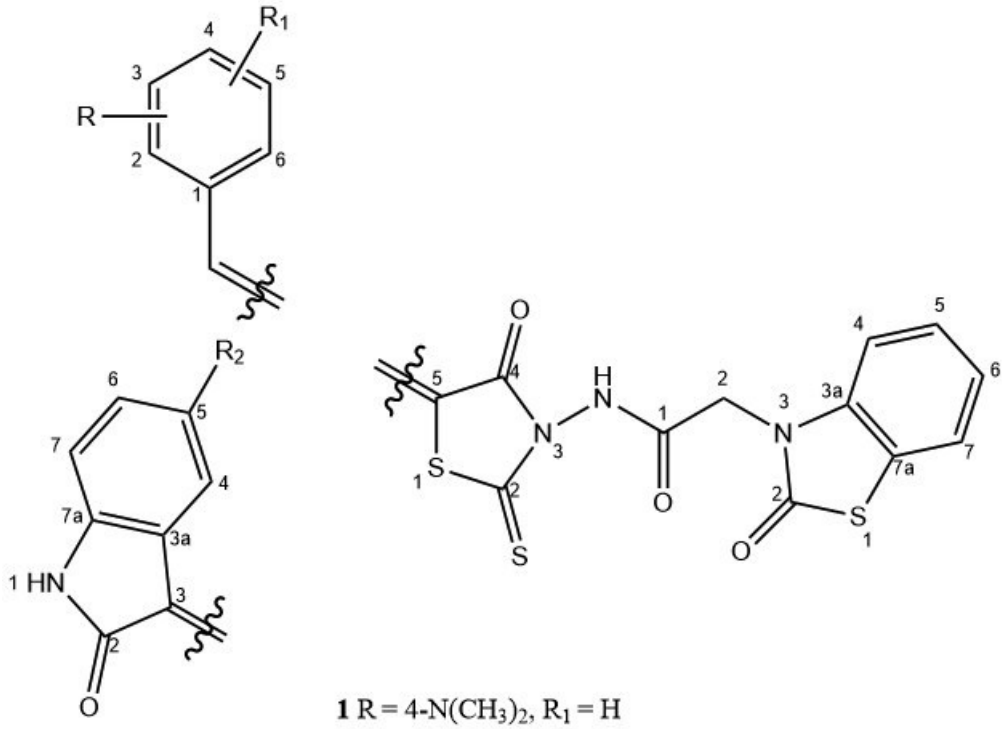
During the investigation of the ADMET properties of these hybrids using standard chemoinformatics filtering protocols, several structural alerts (rhodanine core, thiocarbonyl group and enone fragment (O=C–C=CH–Ar)) in their molecules, which meet the criteria of the PAINS (Pan-Assay Interference Compounds) and Brenk filters [9]. According to SwissADME, these fragments are classified as potential sources of false-positive results or Michael acceptors (MAs). However, the current paradigm in drug discovery shows a clear trend away from the dogmatic use of *in silico* as absolute hard

cut-off criteria. Modern medicinal chemistry successfully incorporates such reactive fragments into the design of targeted covalent inhibitors and multitarget agents, refuting the outdated view of them as exclusively non-selective interferents.

Assessing the antioxidant potential of hybrid systems through the prism of ADMET parameters is critical to understanding their biological fate. The realization of the antioxidant effect is not limited only to the direct neutralization of free radicals, but can also occur by activating endogenous protective systems of the cell. To function effectively by this mechanism, the molecule must successfully overcome barriers to the therapeutic target, that is, be characterized by high reactivity and an optimal ADMET profile.

To predict the behavior of the studied molecules in the human body, their safety profiles and probable toxicity classes, we calculated the main physicochemical, pharmacokinetic and toxicological parameters using three separate platforms (ADMETlab 3.0, SwissADME and ProTox 3).

According to predictions by various *in silico* tools, the studies molecules are characterized by optimal lipophilicity, Log *P* indicators range from 1.576 to 3.446. Such a moderately lipophilic character indicates the amphiphilic potential of the molecules. Compounds 3, 4, 5 with higher Log *P* values (3.3−3.4) have the potential to effectively integrate into the hydrophobic lipid bilayer of cell membranes, providing protection against lipid peroxidation. At the same time, core heterocycle and compounds 2 and 6 (Log $P \leq 2.5$) exhibit greater hydrophilicity, as confirmed by higher solubility values (Log *S* is −3.343, −3.494 and −3.985, respectively), which can contribute to their functioning as redox agents in the aqueous environment of the cytoplasm. Compounds 1 and 2 exhibit the most balanced lipophilicity and solubility values (Log *P*=2.573, Log *P*=2.066, Log *S*=−4.224 and Log *S*=−3.494, respectively). This provides them with better predicted bioavailability ($F_{50\%}$ 0.334 and 0.689, respectively). The oral



1 R = 4-N(CH$_3$)$_2$, R$_1$ = H
2 R = 4-OH, R$_1$ = 3-OCH$_3$
3 R = 2-Cl, R$_1$ = 3-Cl
4 R = 1-Ph, R$_1$ = H
5 R = 4-Br, R$_1$ = 1-OCH$_2$CONH-PhCH$_3$
6 R$_2$ = CH$_3$
7 R$_2$ = Br

Fig. 1. Substituted 5-isatinylidene and 5-benzylidene derivatives of *N*-(4-oxo-2-thioxothiazolidin-3-yl)-2-(2-oxobenzo[*d*]thiazol-3(2*H*)-yl)acetamide

bioavailability score for the entire series, according to SwissADME, is high enough (BS=0.55).

Studies of molecular permeability using the Caco-2 line model demonstrated moderate permeability coefficients (ranging from −4.785 to −5.289). The best permeability in Caco-2 (a human colon adenocarcinoma cell line) is predicted for compounds 2 and 6 (Log P_{app}>−5.15 cm/s). A low human intestinal absorption (HIA) value is predicted for molecules. None of the compounds is a P-glycoprotein substrate (P-gp substrate=0.0), which rules out the mechanism of active efflux of molecules from the cell. The prediction for penetration across the blood-brain barrier (BBB) is negative (0.000−0.319). This is a positive factor for somatic antioxidants, as it indicates the absence of any undesirable neurotropic effects on the central nervous system [8].

The molecules are predicted to exhibit inhibitory activity against most of the cytochrome isoforms (CYP450). This indicates their high affinity for the catalytic sites of hemoproteins. The active interaction of compounds with various CYP450 isoforms is an indirect confirmation of high biochemical activity in redox reactions.

The estimated plasma half-life ($T_{1/2}$) is short, ranging from 0.403 to 0.649 hours, and clearance (CL_{plasma}) is within 3.177−5.790, indicating rapid elimination of substances from the body and no cumulative effect.

A sign of the druglikeness of the molecules under investigation is their correspondence to the Lipinski Rule of Five (MW≤500, N-HBD≤5, N-HBA≤10, Log P≤5, N-RB≤10). The reasons why compounds do not pass through other drug-like filters are deviations from the optimal ranges of physicochemical parameters such as MW and MR. Regarding the TPSA scores calculated using different *in silico* methods, the values vary. Thus, according to calculations on the ADMETlab 3.0 platform, this indicator is optimal for all studied molecules and is in the range of 70−110 Å^2, and according to SwissADME, molecules are characterized by high polarity (TPSA>130 Å^2). Compounds whose TPSA approaches the upper limit may be more active than less polar molecules, but this makes it difficult to absorb them in the gastrointestinal tract (GIT). Importantly, both resources predict easy synthesis of compounds.

In our studies, we paid special attention to the analysis of parameters that indicate the antioxidant potential of molecules. A possible detection of AOA is indicated by such a direct molecular parameter as SR-ARE (Stress Response−Antioxidant Response Element), which is the main marker of the antioxidant response. The closer the numerical value of this indicator is to 1, the greater the antioxidant potential of the compound. According to ADMETlab 3.0 predictions, all studied molecules are highly likely to activate the ARE. The calculated SR-ARE for the core heterocycle is 0.711, and for 5-ylidene derivatives this value exceeds 0.800 and reaches a maximum for compound 1 (0.978), compound 5 (0.959), compound 7 (0.953) and compound 2 (0.927). Consequently, the introduction of a 5-ylidene moiety enhances the ability of the molecules to activate cell defense systems.

At the same time, this requires mandatory verification of the molecules for cytotoxicity, as the line between an active antioxidant and a reactive toxicant is very fine. According to ProTox 3.0 predictions, the studied compounds are not cytotoxic [12]. For most molecules, toxicity class 4 is assumed, except for the basic heterocycle and compound 3 (toxicity class 3). The best safety profile is predicted for compound 2 with a 4-hydroxy-3-methoxybenzylidene moiety.

A number of indirect indicators testify to the potential ability of molecules to counteract oxidative stress. Some cytotoxicity tests can be virtually performed on the ADMETlab 3.0 platform. This makes it possible to predict the specific toxic effects of compounds on various types of human cells and organs: lung adenocarcinoma (A549), embryonic kidney (Hek293), myeloma (RPMI-8226). The low values of these parameters indicate a low probability of direct toxic action of molecules on these cell lines, which emphasizes their safety. There is some risk of cytotoxicity on the embryonic kidney cell line due to increased Hek293 in individual molecules (compounds 1, 3 and 4).

To understand the possible mechanisms of AOA of compounds, we analyzed their SR-p53, SR-MMP and HLM values.

SR-p53 (Stress Response p53) is a marker of the stress response associated with the p53 protein, which is activated in response to DNA damage. A low probability of p53 activation by the compounds (SR-p53 level close to zero) indicates the absence of a genotoxic stress response and no interference with the processes of programmed cell death. This is a positive characteristic of the safety of molecules and indicates the absence of their direct pro-oxidant effect on DNA. Unlike some antioxidants, which under in vivo conditions can exhibit pro-oxidant properties, the mechanism of action of the studied molecules aims to protect cells through activation of the antioxidant element (SR-ARE) without creating additional genotoxic stress.

The SR-MMP (Stress Response−Mitochondrial Membrane Potential) quantifies the probability that a

compound will disrupt the mitochondrial membrane potential, that is, cause membrane depolarization. The lowest effect on mitochondrial function is predicted for the core heterocycle.

The high SR-MMP values (0.641–0.957) for the 5-ylidene derivatives indicate their significant potential for interacting with mitochondrial membranes. Given that mitochondria are the main site of endogenous radical formation, such affinity can contribute to localization of the antioxidant effect directly at sites of intense oxidative stress. In the context of oxidative stress, this suggests that 5-ylidene derivatives actively interact with mitochondrial signaling systems, stabilizing the respiratory chain and preventing electron leakage, thereby minimizing the intracellular generation of reactive oxygen species (ROS). Importantly, this interaction is not accompanied by the activation of pro-apoptotic pathways (low SR-p53 levels), confirming the regulatory, rather than destructive, nature of the effect on cellular energy metabolism. It is known that many effective antioxidants are mitochondria-targeted.

Another key molecular parameter for predicting the antioxidant profile is HLM Stability (Human Liver Microsomes stability). This indicator reflects the metabolic stability of substances in human liver microsomes and the rate of their biotransformation during first-pass metabolism. The high probability values calculated indicate intensive conversion of molecules by the enzymatic systems of hepatocytes. Although rapid metabolic transformation may limit the duration of systemic action, for antioxidants, high reactivity in the microsomal environment often correlates with their ability to readily engage in redox interactions. Such molecules are able to neutralize free radicals and reactive metabolites even before they damage hepatocytes. Thus, a certain metabolic lability can be considered as a positive characteristic, which indicates the high chemical activity of the studied compounds and their ability to quickly interact with oxidants under the influence of liver enzymes.

Alongside a comprehensive assessment of the probable pharmacokinetic profile and safety of compounds (ADMETlab 3.0, SwissADME, ProTox 3.0), we tried to predict the mechanism of antioxidant protection by analyzing the potential interactions of the molecules under investigation with therapeutic targets responsible for the development of oxidative stress or modulating the antioxidant response, using the *in silico* tools SwissTargetPrediction and SuperPred 3.0.

Experimentally detected *in vitro* and previously described by us [5], the anti-radical activity of some studied rhodanine-benzothiazole hybrids finds a logical theoretical complement in the SwissTargetPrediction prediction results. The presence of several classes of biological targets in pie charts may indicate a multitarget mechanism of action of compounds. Diagrams of 5-ylidene derivatives are dominated by kinases (>50% in pie charts for each derivative).

The predicted probability of interaction of the synthesized compounds with Family A receptors conjugated to G-proteins (Family A G protein-coupled receptor (GPCR)) indicates the presence of potent indirect antioxidant potential. In living chemical trapping systems (scavenging), free radicals are often insufficient to completely level oxidative stress, as pathogens induce their continuous generation. Family A GPCR includes critical redox regulatory targets such as adenosine receptors. Modulation of these membrane sensors triggers intracellular cascades that block the work of the NADPH oxidase complex and suppress the so-called oxidative burst in immune cells, completely stopping the excess release of ROS into the focus of inflammation.

According to SwissTargetPrediction, the common biological targets for the series of compounds have been identified as MAP kinase cascades (MAP kinase p38 alpha (MAPK14), c-Jun N-terminal kinases 1, 2 and 3 (MAPK8), c-Jun N-terminal kinase 2 (MAPK9), c-Jun N-terminal kinase 3 (MAPK10), MAP kinase ERK2 (MAPK1)) and G-protein-coupled receptors of the A family (in particular adenosine receptors, such as ADORA2A (Adenosine A2a receptor), Adenosine A2b receptor (ADORA2B), Adenosine A1 receptor (by homology) (ADORA1), and Adenosine A3 receptor (ADORA3).

Thus, the rhodanine-benzothiazoles show signs of promising multitarget agents. They are able to act synergistically at two levels: as direct chemical traps for already existing radicals (direct action) and as biological redox modulators (indirect action). This complex combination of properties fully justifies their further development as means for the therapy of pathologies burdened with oxidative stress and inflammation.

It is worth noting that unlike other 5-ylidene derivatives, for compound 2, the SwissTargetPrediction tool does not predict direct interaction with adenosine receptors. However, this does not reduce its antioxidant potential, but may indicate an alternative, kinase-induced mechanism of cytoprotection. The predicted profile of compound 2 is characterized by the dominance of targets such as PIM kinases (Serine/threonine-protein kinases PIM1, PIM2, PIM3), Polo-like kinase 1 (Serine/threonine-protein kinase PLK1 (PLK1), Aurora kinases (Serine/threonine-protein kinase Aurora-A (AURKA), Serine/

threonine-protein kinase Aurora-B (AURKB)) and regulatory survival protein (Induced myeloid leukemia cell differentiation protein Mcl-1 (MCL1)). In living systems, these protein complexes act as critical mediators of the cell's response to oxidative stress and genotoxic damage caused by ROS. Modulation of PIM kinases and mitochondrial factor MCL1 under the action of compound 2 is able to provide effective protection of the mitochondrial matrix and DNA from peroxide destruction, preventing the launch of proapoptotic cascades. Compound 2 is likely to realize its antioxidant potential not through receptor inhibition of primary radical release (like adenosine ligands), but through potent intracellular protection of vital organelles from the effects of oxidative stress.

The results of the modeling carried out on the SuperPred 3.0 platform provided further bioinformatic evidence supporting the antioxidant potential of the target molecules. Unlike the ligand-based prediction (SwissTargetPrediction), the prediction based on structural similarity to known drugs (SuperPred 3.0) identified a series of common biological targets with a high level of predictive reliability, directly involved in the enzymatic redox processes of the cell [11].

The key common targets for most 5-ylidene derivatives and the core heterocycle have been identified as AP-endonuclease (DNA-apurinic or apyrimidinic site lyase (APE1)), regulatory subunit p105 of nuclear factor NF-kappa-B (Nuclear factor NF-kappa-B p105 subunit (NF-kB1)) and Aldose reductase (AKR1B1).

The highest probability of interaction with APE1 is characteristic for compound 2 (98.21% with a prediction model accuracy of 91.11%), with the NF-κB1 for compound 6 (96.16% with a prediction model accuracy of 96.09%), and with AKR1B1 for compound 4 (97.87% with a prediction model accuracy of 92.38%). Interaction with APE1 indicates the ability of compounds to maintain the redox status of transcription factors and stimulate the repair of DNA damaged by ROS. In turn, the predicted interaction with AKR1B1 and NF-κB1 supports the ability of molecules to prevent the depletion of endogenous glutathione and block the pro-inflammatory generation of radicals.

A separate important aspect of the antioxidant potential of studied compounds, including the core heterocycle, is the predicted interaction (probability above 50%) with the Nuclear factor erythroid 2-related factor 2 (Nrf2). This means that the ability to interact with Nrf2 is embedded in the chemical nature of the rhodanine-benzothiazole skeleton and the introduction of various ylidenic substituents only modulates this property without destroying it. The highest probability of interaction with this target is predicted for compound 5 and is 88.31% with a prediction model accuracy of 96.00%. Thus, interaction with Nrf2 implies a prolonged effect of the compounds, aimed at enhancing the cells' own (endogenous) defense mechanisms under conditions of peroxide stress.

To explain the antioxidant potential of the rhodanine–benzothiazole hybrids, a quantum-chemical analysis was performed, which made it possible to assess the favorability of the main radical-neutralization mechanisms: HAT, SET-PT, and SPLET. The $\Delta E_{H\text{-}loss}$ parameter characterized the direct abstraction of a hydrogen atom, ΔE_{deprot} reflected the ease of deprotonation, and $\Delta E_{from\ anion}$ described electron transfer from the anionic form at the second stage of the SPLET mechanism.

The analysis of ΔE_{ion} showed that compound 1 had the most favorable SET component: 114.38 kcal·mol^{-1} in water and 115.42 kcal·mol^{-1} in methanol, which is associated with the electron-donating effect of the 4-$N(CH_3)_2$ group. In contrast, compound 3, bearing a 2,3-dichlorobenzylidene moiety, showed the highest ΔE_{ion} values, 143.09 and 143.73 kcal·mol^{-1}, respectively, indicating less favorable primary electron transfer. However, for this compound, an indirect antioxidant mechanism may be more likely, associated with its lipophilicity, the electron-withdrawing character of the chlorinated fragment, and potential interaction with redox-regulatory biological targets.

The best direct radical-scavenging profile was established for compound 2, which contains a 4-hydroxy-3-methoxybenzylidene moiety. The phenolic OH group is the main H-donor center: $\Delta E_{H\text{-}loss}$ for OH was 405.90 kcal·mol^{-1} in water and 407.07 kcal·mol^{-1} in methanol, whereas for the NH center the corresponding values were 423.27 and 424.59 kcal·mol^{-1}. Thus, hydrogen atom abstraction from OH is more favorable by 17.37–17.52 kcal·mol^{-1}, confirming the leading role of the phenolic moiety in the HAT mechanism and the stabilization of the phenoxyl radical with the participation of the methoxy group.

Compound 2 also showed the most favorable profile for the SPLET mechanism. Although primary deprotonation was slightly more favorable for the NH center, the second stage, electron transfer from the anionic form, was considerably more favorable for the phenolic OH group. The $\Delta E_{from\ anion}$ values for OH were 107.62 kcal·mol^{-1} in water and 106.84 kcal·mol^{-1} in methanol, which were the lowest values among the evaluated centers. Therefore, the NH fragment may participate in the acid–base stage, whereas the OH group provides the main redox-active stage.

Among the other derivatives, compound 3 should be highlighted separately. Despite less favorable parameters for direct electron transfer and the absence of a phenolic OH group, its 2,3-dichlorobenzylidene moiety may increase lipophilicity, membrane affinity, and the ability for indirect redox modulation. Therefore, compound 3 should be considered not as a direct radical scavenger, but as a promising candidate with a potential multitarget or membrane-associated antioxidant mechanism.

Compounds 4–7 generally showed lower direct HAT/SPLET potential compared with compound 2, which is due to the absence of an effective phenolic H-donor center. Compound 5 is characterized by distributed acid–redox behavior involving two N–H centers, whereas for the isatinylidene derivatives 6 and 7, the moderate HAT/SPLET profile is mainly associated with the NH_2 center. In particular, for compound 6, $\Delta E_{H\text{-}loss}$ was 412.15 and 414.08 kcal·mol^{-1} in water and methanol, respectively, which is better than for compounds 3–5 but still inferior to phenolic compound 2.

To summarize the results, a «compound–mechanism» heatmap was constructed (Fig. 2), combining the key energetic descriptors of AOA: the ability for primary electron transfer, the favorability of the HAT mechanism, and the efficiency of the second stage of SPLET.

Overall, the quantum-chemical results confirm that the antioxidant potential of the studied hybrids strongly depends on the nature of the substituent in the 5-ylidene moiety. Thus, two different types of potential antioxidant behavior can be distinguished within the studied series (Table). The first type is represented by compound 2, for which the direct radical-scavenging mechanism involving the phenolic OH center predominates. The second type is represented by compound 1 and, partially, by compounds 3–7, for which the SET component, electron delocalization, or indirect interaction with biological targets involved in the antioxidant response are more likely. This distribution is in good agreement with the structural features of the molecules and highlights the key role of the phenolic moiety in the formation of direct antiradical activity of rhodanine–benzothiazole hybrids.

SAR analysis

Chemoinformatic analysis allowed tracing a clear evolution of the pharmacophore profile in the transition from the core heterocycle to its 5-ylidene derivatives in the context of their antioxidant potential. If the core structure is characterized mainly by monoamine oxidase (MAOA) and narrow kinase vector, then the chemical modification at the position 5 of the rhodanine cycle significantly expanded the spectrum of redox regulatory targets.

For most modified hybrids (compounds 1, 3, 4, 5, 6, and 7), the modification opened the possibility

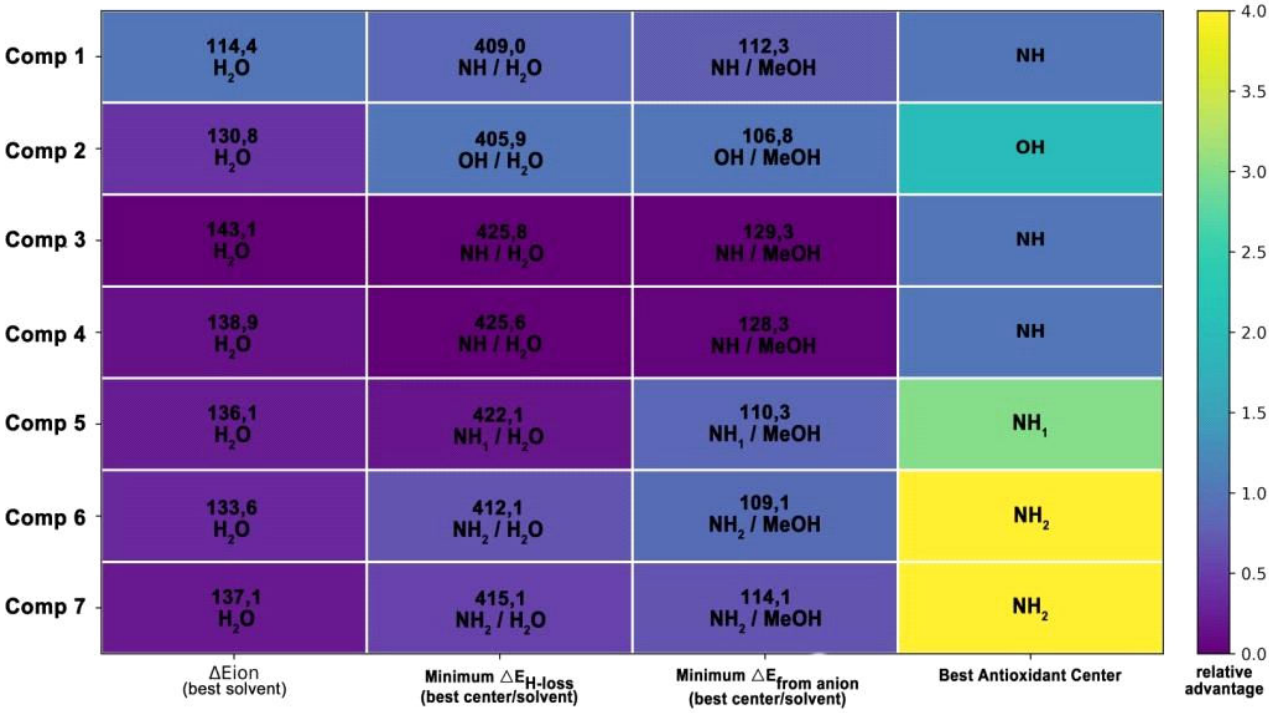


Fig. 2. Compound–mechanism heatmap of quantum-chemical descriptors associated with the antioxidant potential of rhodanine–benzothiazole hybrids 1–7

of a highly probable interaction with membrane sensors of oxidative stress – Family A receptors conjugated to G-proteins (Family A GPCR: ADORA2A, ADORA3), which are responsible for suppressing the NADPH oxidase complex and extinguishing inflammation.

In the case of compound 2 with a 4-hydroxy-3-methoxybenzylidene moiety, the modification directed the profile towards the intracellular survival systems of PIM kinases and the mitochondrial protector MCL1. Furthermore, according to SuperPred 3.0, the introduction of ylidene substituents allowed the entire series of compounds to maintain or increase (probability >90%) affinity for the fundamental enzymes of antioxidant protection of the nucleus and cytoplasm: DNA-reparating AP-endonuclease (APE1), transcription regulator NF-kappa-B (NF-κB1) and aldoreductase (AKR1B1).

The obtained *in silico* data are in harmony with previous *in vitro* test results, confirming that the rational design of 5-ylidene derivatives of rhodanine-benzothiazole will allow combining direct direct radical-scavenging action with multitarget receptor-enzymatic redox protection of the cell.

In addition, the introduction of 4-(dimethylamino)benzylidene (compound 1, 74.79%), 4-hydroxy-3-methoxybenzylidene (compound 2, 81.71%), 2,3-dichlorobenzylidene (compound 3, 79.93%), 4-phenylbenzylidene (compound 4, 72.97%), and 4-bromo-1-methylphenoxy)-*N*-(*o*-tolyl)acetamide-benzylidene (compound 5, 88.31%) substituent at the position 5 of the rhodanine ring led to an increased probability of interaction with Nrf2, compared with the unsubstituted core heterocycle (60.47%). The introduction of 5-methyl-2-oxoindolin-3-ylidene (compound 6) and 5-bromo-2-oxoindolin-3-ylidene (compound 7) substituents practically did not affect the level of probable interaction with this target (62.43% and 53.85%, respectively).

SAR analysis showed that the key structural factor determining direct antiradical activity in this series is the presence of a phenolic OH group in the 5-ylidene moiety. Therefore, compound 2, bearing a 4-hydroxy-3-methoxybenzylidene substituent, demonstrated the most favorable direct radical-scavenging profile via the HAT mechanism and the second stage of SPLET. This can be attributed to the ability of the phenolic OH group to act as an efficient H-donor center and to stabilize the resulting phenoxyl radical. Compound 3, containing a 2,3-dichlorobenzylidene moiety, should also be highlighted: although the absence of a phenolic OH group limits its direct HAT activity, the electron-withdrawing chlorine atoms may contribute to increased lipophilicity, membrane affinity, and indirect redox modulation. Thus, compound 2 appears to be the most promising candidate for direct radical-scavenging activity, whereas compound 3 may be considered a promising compound with a potential multitarget or membrane-associated antioxidant mechanism.

Conclusions

The introduction of the 5-ylidene moiety enhanced the predicted antioxidant potential of rhodamine-benzothiazole hybrids, as confirmed by high SR-ARE values for the derivatives of *N*-(4-oxo-2-thioxothiazolidin-3-yl)-2-(2-oxobenzo[*d*]thiazol-3(2*H*)-yl)acetamide (>0.800; maximum values were observed for compound 1 – 0.978, compound 5 – 0.959, compound 7 – 0.953, and compound 2 – 0.927), an optimal

Summary of quantum-chemical descriptors of the antioxidant potential of compounds 1–7

Criterion	Best variant	Justification
Most favorable primary ionization / SET component	Compound 1	ΔE_{ion}=114.38 kcal·mol^{-1} in H_2O and 115.42 kcal·mol^{-1} in MeOH
Best HAT center	Compound 2, OH	$\Delta E_{H\text{-}loss}$=405.90 kcal·mol^{-1} in H_2O and 407.07 kcal·mol^{-1} in MeOH
Best second stage of SPLET	Compound 2, OH	$\Delta E_{from\ anion}$=107.62 kcal·mol^{-1} in H_2O and 106.84 kcal·mol^{-1} in MeOH
Easiest deprotonation in H_2O	Compound 7, NH_1	ΔE_{deprot}=295.03 kcal·mol^{-1}; similar values were observed for the NH/NH_2 centers of compounds 2, 3, and 5
Leading direct radical scavenger of the series	Compound 2	Combination of the phenolic OH group, methoxy stabilization, and the lowest HAT/SPLET-II values
Predominantly indirect antioxidant profile	Compounds 3–7	Absence of a phenolic OH group or Predominance of N–H centers; possible contribution of multitarget /Nrf2/ARE mechanisms

Log *P* range of 1.576–3.446, and potential interactions with redox-regulatory targets such as GPCR, NF-κB, APE1, AKR1B1, and Nrf2. The SwissTargetPrediction and SuperPred 3.0 results suggest that the studied compounds may exert antioxidant effects both through direct radical-scavenging and through indirect receptor- and enzyme-mediated regulation of the cellular response to oxidative stress. Quantum-chemical calculations identified compound 2 as the most promising direct radical scavenger: for its phenolic OH group, $\Delta E_{H\text{-}loss}$ values were 405.90 kcal·mol^{-1} in water and 407.07 kcal·mol^{-1} in methanol, while $\Delta E_{from\ anion}$ values were 107.62 and 106.84 kcal·mol^{-1}, respectively, confirming the predominance of HAT and SPLET mechanisms. At the same time, compound 3, despite having the highest ΔE_{ion} values of 143.09–143.73 kcal·mol^{-1} and, consequently, less favorable direct electron transfer, remains important as a potential multitarget candidate due to its 2,3-dichlorobenzylidene moiety, increased lipophilicity, possible membrane affinity, and indirect redox-modulating properties. Thus, compound 2 can be considered the leading candidate for direct antiradical activity, whereas compound 3 may be promising for further study as a compound with a predominantly indirect antioxidant mechanism.

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Conflicts of interest

The authors declare no conflict of interest.

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ОЦІНЮВАННЯ БІОФАРМАЦЕВТИЧНИХ ВЛАСТИВОСТЕЙ ТА IN SILICO МОДЕЛЮВАННЯ АНТИОКСИДАНТНОГО ПОТЕНЦІАЛУ ГІБРИДІВ РОДАНІН-БЕНЗОТІАЗОЛУ

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Оксидативний стрес залучений до патогенезу багатьох системних захворювань, тому пошук нових мультифункціональних антиоксидантів залишається актуальним завданням. У цій роботі сім гібридів роданін-бензотіазолу, а саме 5-іліденпохідних *N*-(4-оксо-2-тіоксотіазолідин-3-іл)-2-(2-оксобензо[*d*]тіазол-3(2*H*)-іл)ацетаміду, було оцінено за допомогою прогнозування ADMET-параметрів, профілювання потенційних біомішеней та DFT-орієнтованих квантово-хімічних розрахунків. Досліджувані сполуки характеризувалися помірною ліпофільністю з прогнозованими значеннями Log *P* у межах 1,576–3,446, а також високою ймовірністю активації елемента антиоксидантної відповіді; значення SR-ARE для 5-іліденпохідних перевищували 0,800 і досягали 0,978 для сполуки 1 та 0,927 для сполуки 2. Сполука 2, що містить 4-гідрокси-3-метоксибензиліденовий фрагмент, продемонструвала найсприятливіший профіль прямої радикал-поглинаючої дії: $\Delta E_{H\text{-}loss}$ для фенольного OH становила 405,90 ккал·моль$^{-1}$ у воді та 407,07 ккал·моль$^{-1}$ у метанолі, тоді як $\Delta E_{from\ anion}$ – 107,62 і 106,84 ккал·моль$^{-1}$, відповідно. Сполука 3, яка містить 2,3-дихлоробензиліденовий фрагмент, вирізнялася підвищеною ліпофільністю та перспективним опосередкованим антиоксидантним/мультитаргетним профілем, що може сприяти мембраноасоційованій редокс-протекції. Загалом сполуки 2 і 3 можна вважати найбільш перспективними представниками для подальшої оптимізації як мультифункціональних антиоксидантних агентів.

Ключові слова: роданін, бензотіазол, 5-іліденпохідні, ADMET властивості, квантово-хімічні розрахунки, SAR аналіз, антиоксидантна активність.

EVALUATION OF BIOPHARMACEUTICAL PROPERTIES AND IN SILICO MODELING OF THE ANTIOXIDANT POTENTIAL OF RHODANINE-BENZOTHIAZOLE HYBRIDS

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Oxidative stress is involved in the pathogenesis of many systemic disorders; therefore, the search for new multifunctional antioxidants remains a relevant task. In this study, seven rhodanine–benzothiazole hybrids, 5-ylidene derivatives of N-(4-oxo-2-thioxothiazolidin-3-yl)-2-(2-oxobenzo[d]thiazol-3(2H)-yl)acetamide, were evaluated using ADMET prediction, target profiling, and DFT-based quantum-chemical calculations. The compounds showed moderate lipophilicity, with predicted Log P values of 1.576–3.446, and a high probability of antioxidant response element activation; SR-ARE values for 5-ylidene derivatives exceeded 0.800 and reached 0.978 for compound 1, 0.927 for compound 2, and high values for other active derivatives. Compound 2, bearing a 4-hydroxy-3-methoxybenzylidene moiety, demonstrated the most favorable direct radical-scavenging profile: $\Delta E_{H\text{-}loss}$ for the phenolic OH was 405.90 kcal·mol^{-1} in water and 407.07 kcal·mol^{-1} in methanol, while $\Delta E_{from\ anion}$ values were 107.62 and 106.84 kcal·mol^{-1}, respectively. Compound 3, containing a 2,3-dichlorobenzylidene moiety, was distinguished by increased lipophilicity and a promising indirect antioxidant/multitarget profile, which may support membrane-associated redox protection. Overall, compounds 2 and 3 can be considered the most promising representatives for further optimization as multifunctional antioxidant agents.

Keywords: rhodanine; benzothiazole; 5-ylidene derivatives; ADMET properties; quantum-chemical calculations; SAR analysis; antioxidant activity.

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