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Evaluation of antioxidant activity of derivatives with 6,7-dihydro-5*H*-imidazo[2,1-*b*][1,3]thiazine scaffold

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Aim. Study of antioxidant (antiradical) activity of 6,7-dihydro-5*H*-imidazo[2,1-*b*][1,3]thiazine derivatives. **Methods.** *In vitro* study of antiradical/scavenging activity using 2,2-diphenyl-1-picrylhydrazyl (DPPH) radicals inhibition assay; IC_{50} values determination. **Results.** The series of 29 modified derivatives of 6,7-dihydro-5*H*-imidazo[2,1-*b*][1,3]thiazine were evaluated for their ability to scavenge DPPH radicals in conditions close to physiological at 5 mM concentration, and the IC_{50} values were determined for the most promising compounds using the serial dilutions method. The structure - antiradical activity correlations were performed and possible mechanisms of action were discussed. **Conclusions.** Tested 6,7-dihydro-5*H*-imidazo[2,1-*b*][1,3]thiazine derivatives possess a moderate level of antiradical/scavenging activity.

K e y w o r d s: 6,7-dihydro-5*H*-imidazo[2,1-*b*][1,3]thiazine; antiradical/scavenging activity; DPPH; IC_{50}; SAR

Introduction

The last few decades brought enormous progress in our understanding of the role of redox balance and reactive oxygen species (ROS) in the regulation of physiological functions as well as [in] the development and progression of multiple diseases [1–3]. The oxidative stress (OS), defined as redox imbalance with an absolute or relative deficit of antioxidant power to prevent cells/macromolecules from the damage by ROS, plays important role in diseases, such as cancer, diabetes, psychiatric diseases [4], inflammatory diseases [5], neurodegen-

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erative diseases, and aging [6]. Thus, there is no wonder that the potential correction of this imbalance in cells and tissues is considered as a massive unmet need and could be attractive as a therapeutic intervention for either treatment or prevention of related conditions [7–9]. Several alternative approaches, suggested to address the modulation of redox balance, vary widely from the introducing of exogenous antioxidants to the modulation of endogenous redox-regulation processes/pathways. This field, therefore, attracts ever-growing interest in the modern drug discovery experts [10]. So far, the free radical scavengers [11], "multipotent" antioxidants [12], "sensor/effector agents" [13] and "redox cyclers" [14] have been designed and are currently studied as new opportunities and strategies for the treatment of OS-associated disorders as well as potential prevention/delay of aging. {{Since redox dysregulations are widely spread among multiple types of diseases and are not limited to cancer, inflammation and infectious diseases, there are many reasons to believe those novel drug molecules capable to selectively modulating the intensity of ROS production enhance the utilization or activation/inhibition of redox-sensitive enzymes may provide new avenues to tackle OS-related diseases and improve their treatments.

The organic sulfur-containing molecules present a large group of derivatives from different chemotypes and sources (natural, synthetic) as potential regulators of formation/neutralization of ROS and reactive nitrogen species (RNS). For instance, the natural organic sulfur-containing molecules, such as glutathione, *S*-adenosylmethionine, cysteine, taurine, play a key role in antioxidant protection and redox-regulation in cells [15–17]. On the other hand, numerous heterocyclic molecules with sulfur atoms are successfully studied due to their impact on free radicals, cells' antioxidant mechanism as well as other possible molecular pathways [18, 19]. That is why, the design of potential antioxidants and redox-modulators using sulfur-containing heterocyclic matrix/scaffolds is a prospective area in the modern bioorganic and medicinal chemistry.

As a part of our ongoing interest in developing new and efficient pharmacological agents, we recently reported the synthesis of new derivatives with 6,7-dihydro-5*H*-imidazo[2,1-*b*][1,3]thiazine scaffold that possess promising anti-inflammatory activity in the *in vivo* experiments as well as satisfactory drug-like properties [20-22]. Thus, considering the relation between pathophysiological pathways of inflammation and oxidative stress/redox imbalance [1-3] the main objective of the present study was the *in vitro* evaluation of the antioxidant (DPPH free radical scavenging) activity of the mentioned derivatives.

Materials and methods

Synthesis and characterization of the compounds The synthesis and physico-chemical properties of tested 6,7-dihydro-5*H*-imidazo[2,1-b][1,3]thiazine derivatives were described in [20-22].

Anti-oxidant activity (DPPH assay). The DPPH radical inhibition assay was used for evaluation of anti-oxidant properties following the protocol described in [23] with some changes. The stock solutions of the compounds were prepared in Tris-HCl methanolic buffer (pH = 7.40). Then 1 mL of DPPH (8 mg/100 mL

of methanol) solution was added to the sample and the blank. This setup was left at room temperature for 60 min (vortexed in between). The absorbance value was taken at 517 nm against the ethanol by using a UV-1800 spectrophotometer (Shimadzu, Japan). Each sample was analyzed in triplicate. The percentage of inhibition was calculated against blank:

$$I\% = (A_{blank} - (A_{sample + DPPH} - A_{sample}))/A_{blank} \times 100\ \%,$$

where A_{blank} is the absorbance of the control reaction (containing all reagents except the tested compounds); $A_{sample+DPPH}$ is the absorbance of the tested compounds after 60 min incubation with DPPH solution; A_{sample} is the absorbance of the tested compounds without DPPH solution.

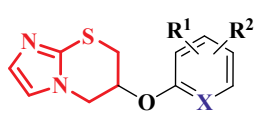
Statistical analysis. All values were expressed as mean values ± SEM (standard error of mean) and data were analyzed by applying an analysis of variance (ANOVA) followed by Student's t-test. The results were considered statistically significant if $P < 0.05$.

Results and Discussion

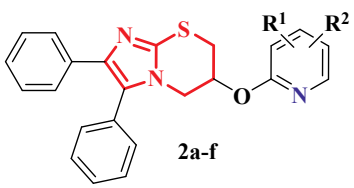
Three sub-types of derivatives with 6,7-dihydro-5*H*-imidazo[2,1-*b*][1,3]thiazine scaffold **1a-l**, **2a-f**, **3a-k** were *in vitro* screened for their ability to scavenge DPPH (1,1-diphenyl-2-picrylhydrazyl) radicals [23] and the compounds' structure are presented in Fig. 1.

In the experimental studies design, the initial stage included evaluation of the DPPH radical scavenging activity of all derivatives **1a-l**, **2a-f**, **3a-k** in conditions close to physiological (methanol stock solution + Tris-HCl buffer pH = 7.40, measurements after 60 min) at 5.0 mM concentration. Such an approach allows fast identifying of potential hit-compounds as well as provides time and compounds amount economy. Ascorbic acid was used as a reference compound (standard). The results of screening of radical scavenging activity at 5.0 mM concentration of compounds **1a-l**, **2a-f**, **3a-k** are presented in the Figure 2.

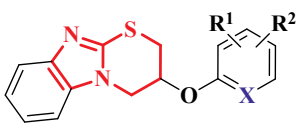
Overall, all tested compounds were found active at 5 mM and exhibited a wide-ranging



1a-l



2a-f



3a-k

X=CH

1a R^1=H, R^2=4-NO_2; **1b** R^1=2-NO_2, R^2=4-NO_2;
1c R^1=2-CN, R^2=3-Cl; **1d** R^1=2-Cl, R^2=5-CN;
1e R^1=2-CN, R^2=4-CF_3;
1f R^1=3-Cl, R^2=4-CH_2CONH_2

X=N

1g R^1=H, R^2=4-Cl; **1h** R^1=H, R^2=4-CF_3;
1i R^1=H, R^2=3-CN; **1j** R^1=H, R^2=4-CN;
1k R^1=2-Cl, R^2=4-Cl; **1l** R^1=2-Cl, R^2=4-CF_3

2a R^1=H, R^2=4-Cl;
2b R^1=H, R^2=4-CF_3;
2c R^1=H, R^2=3-CN;
2d R^1=H, R^2=4-CN;
2e R^1=2-Cl, R^2=4-Cl;
2f R^1=2-Cl, R^2=4-CF_3

X=CH

3a R^1=H, R^2=4-NO_2; **3b** R^1=2-NO_2, R^2=4-NO_2;
3c R^1=2-CN, R^2=3-Cl; **3d** R^1=2-Cl, R^2=4-CN;
3e R^1=2-CN, R^2=4-CF_3

X=N

3f R^1=H, R^2=4-Cl; **3g** R^1=H, R^2=4-CF_3;
3h R^1=H, R^2=3-CN; **3i** R^1=H, R^2=4-CN;
3j R^1=2-Cl, R^2=4-Cl; **3k** R^1=2-Cl, R^2=4-CF_3

Fig. 1. Structures of the tested 6,7-dihydro-5*H*-imidazo[2,1-b][1,3]thiazine derivatives **1a-l**, **2a-f**, **3a-k**.

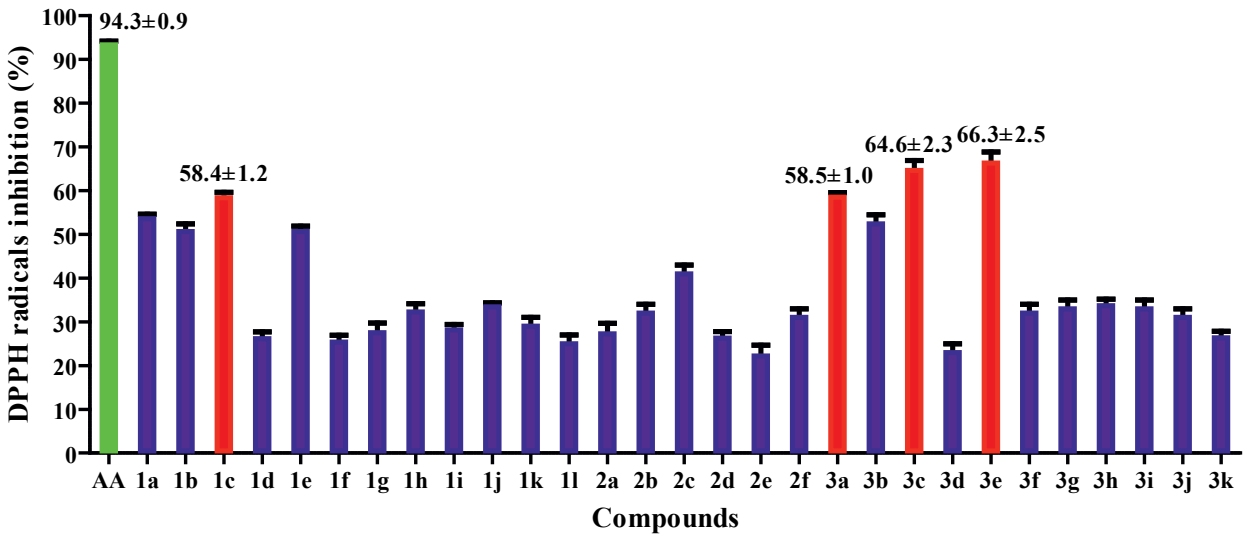


Fig. 2. The inhibition of DPPH radicals by the 6,7-dihydro-5*H*-imidazo[2,1-b][1,3]thiazine derivatives **1a-l**, **2a-f**, **3a-k** at 5 mM concentration. Ascorbic acid (AA) was employed as a positive control (green). The highest activity was observed for compounds **1c**, **3a**, **3c**, and **3e** (red).

of radical scavenging activity in DPPH assay in the range 25.0 to 66.3 %. Such results suggest that anti-radical activity is mostly determined by to electron nature of 6,7-dihydro-5*H*-imidazo[2,1-*b*][1,3]thiazine core stabilized by the hyper-conjugative effect and the electron-withdrawing groups. The IC_{50} values were determined in the next experimental stage for the most active derivatives **1c**, **3a**, **3c**, and **3e** that demonstrated the most promising behaviour at 5 mM concentration. The IC_{50} value corresponds to the concentration of a sample, which has ability to scavenge 50 % of the free radicals present in the reaction mixture. Low IC_{50} values indicate a high antioxidant activity of the sample compound. The serial dilutions of stock methanol solutions at five or six concentrations (1.0, 2.0, 4.0, 6.0, 8.0, 10.0 mM + Tris-HCl buffer pH = 7.40, measurements after 60 min) were used for studies. The same experiment for the determination of the ascorbic acid IC_{50} value was performed as well. The results of DPPH free radicals inhibition at different concentrations and IC_{50} values are presented in the Figure 3.

As a result, tested compounds **1a, 3a, 3c** and **3e** possess moderate activity in DPPH assay and the established IC_{50} values for compounds were: 3.67 mM (**1a**), 4.13 mM (**3a**), 3.63 mM (**3c**), 3.06 mM (**3e**), whereas for ascorbic acid IC_{50} = 0.056 mM. So, in the context of the structure-radical scavenging activity relationship (SAR) for screened compounds it should be noted that pyridine-substituted derivatives (**1g-l**, **3f-k**) possess low activity compared to the appropriate benzene analogues (Figure 4). At the same time, benzene-condensed derivatives (**3a-k**) were more active than diphenyl- (**2a-f**) or unsubstituted (**1a-l**) ones. The presence of NO_2-group was

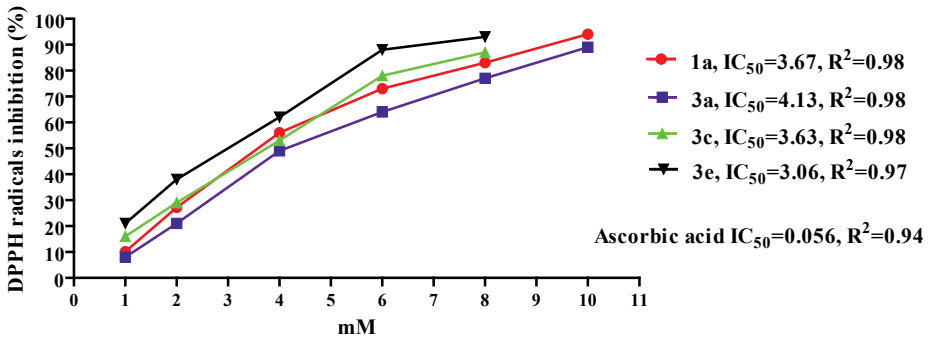


Fig. 3. DPPH free radicals inhibition at different concentrations and IC_{50} values of compounds **1a, 3a, 3c** and **3e.**

crucial for high activity level for compounds **1a**, **3a**. Interestingly, the introduction of next one NO2-group (**1b, 3b**) leads to a lower activity level compared to **1a, 3a**.The highest activity level was observed for derivatives with *o*-CN-group combined with *p*-CF_3 (**3e**) or *m*-Cl (**3c**) whereas the change of the CN-group position leads to the activity decrease (**1d**, **1i**, **1j**, **2c**, **2d**, **3d**, **3h**, **3i**) compared to compounds **3c** and **3e**.

Taking into account that the antiradical mechanism of DPPH assay is based on the HAT (hydrogen-atom transfer) and SET (single-electron transfer) it is possible to assume that both mechanisms are acceptable for derivatives with 6,7-dihydro-5*H*-imidazo[2,1-*b*][1,3]thiazine scaffold. Nitrogen and sulfur atoms with undivided electron pairs in heterorings (imidazo[2,1-*b*][1,3]thiazine moiety, pyridine ring) together with a strong electron-withdrawing groups could play a role of possible centres for stabilizing free radicals. Also noteworthy, no significant correlation between anti-radical and anti-inflammatory activity was found. However, antioxidant activity/properties of 6,7-dihydro-5*H*-imidazo[2,1-*b*][1,3] thiazines are an important point in the pharmacological features of these heterocyclic compounds. Further in-depth studies should be conducted using alternative *in vitro* (ABTS, ORAC *etc.*) as well as *in vivo* screening models.

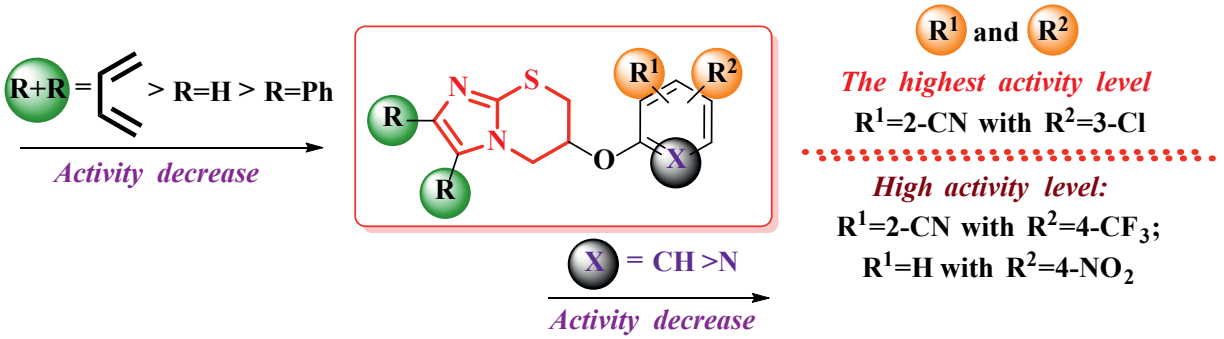


Fig. 4. Structure–anti-radical activity relationships for derivatives with 6,7-dihydro-5*H*-imidazo[2,1-*b*][1,3]thiazine scaffold **1a-l**, **2a-f**, **3a-k**

Conclusions

In the present paper, the results of *in vitro* evaluation of the antioxidant activity using DPPH assay of a series of 6,7-dihydro-5*H*-imidazo[2,1-*b*][1,3]thiazines are described. The derivatives with a moderate level of radical scavenging activity and satisfactory IC_{50} values were identified. The SAR correlations were performed and possible mechanisms of action were discussed. The obtained results are promising for the search and design of new potential antioxidants among 6,7-dihydro-5*H*-imidazo[2,1-b][1,3]thiazine derivatives as well as related hybrid molecules subtype.

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Оцінка антиоксидантної активності похідних з 6,7-дигідро-5H-імідазо[2,1-b][1,3]тіазиновим каркасом

Н. Ю. Сливка, С. М. Голота, Л. М. Салієва, Е. М. Кадикало, М. А. Колішецька, М. В. Вовк

Мета. Вивчення антиоксидантної (антирадикальної) активності похідних 6,7-дигідро-5H-імідазо[2,1-*b*][1,3] тіазину. **Методи.** Дослідження *in vitro* антиоксидантної/ антирадикальної активності з використанням методу інгібування радикалів 2,2-дифеніл-1-пікрилгідразилу (DPPH); визначення значень IC_{50}. **Результати.** Проведено дослідження 29 модифікованих похідних 6,7-дигідро-5H-імідазо[2,1-b][1,3]тіазину на предмет їх здатності поглинати радикали DPPH в умовах, близьких до фізіологічних при концентрації 5 мМ, а також для найбільш перспективних сполук визначено значення IC_{50} методом серійних розведень. Проведено кореляції між структурою та антирадикальною активністю та обговорено можливі механізми дії. **Висновки.** Досліджені похідні 6,7-дигідро-5H-імідазо[2,1-*b*][1,3] тіазину мають помірний рівень антирадикальної активності.

Ключові слова: 6,7-дигідро-5H-імідазо[2,1-*b*][1,3] тіазини; антирадикальна активність; DPPH; IC_{50}; залежність структура-активність

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