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RESEARCH ARTICLE

Synthesis of novel pyrazoline-thiazolidin-4-one hybrids and evaluation of their biological activity

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Abstract: In the present work, the synthesis of pyrazoline-thiazolidin-4-one hybrids and their pharmacological properties are described. The structure of compounds is characterized using ^{1}H, ^{13}C NMR, and LC-MS spectra. The antioxidant (DPPH assay), antimicrobial (Gram-positive bacterium *Lactobacillus plantarum*, Gram-negative bacterium *Escherichia coli*, and yeasts *Candida albicans*, MIC determination), redox (cyclic voltammetry) as well as herbicidal activity (against grass species *Agrostis stolonifera*) of compounds have been studied. All derivatives have demonstrated radical scavenging activity with IC_{50} values in the range of 4.67-7.12 mM that were measured by the DPPH test. The tested compounds showed very low antimicrobial and herbicidal activity and no redox peaks were observed in the cyclic voltammetry studies.

Keywords: pyrazoline-thiazolidin-4-ones hybrids; DPPH assay; antimicrobial/herbicidal activity; cyclic voltammetry.

Introduction

The last decade has witnessed a growing interest in the development of redox modulating agents as effective tool in therapy oxidative-stress associated processes: cancers, diabetes, inflammatory diseases, neurological disorders, and others [1-4]. In this context, the structure modified thiazolidin-4-one and pyrazoline nucleus are prospective molecular platforms for design antioxidants and redox-modulating agent design [5-8]. For example, the application of the mentioned scaffolds is an attractive direction for the development of selective modulators of Nrf2 and NF-kB transcription factors, that play a key role in the regulation of cellular responses to oxidative-stress factors and are potential drug targets [9-11].

In our early-described researches some types thiazolidin-4-one hybrids linked through “enamine” linker at C-5 has been synthesized and several compounds been have been identified with a high level of antibacterial and antifungal [12-14], anticancer and trypanocidal [15], and anti-inflammatory activity [16] (Figure 1). In our opinion, the 5-aminomethylidene derivatives have several important advantages in synthetic variability and structure optimization processes compared to 5-ylidene analogous.

On the other hand, the pyrazolines possess a wide range of biological activities and belong to unsaturated heterocycles that can be oxidized to the corresponding pyrazoles [17]. These properties are of great interest in the design and development of potential redox-active compounds as possible pharmacological agents.

Taking into account the above reasons, the main goal of the present work was the design and synthesis of novel “enamine”-bearing pyrazoline-thiazolidin-4-one hybrid molecules and further evaluation of their antioxidant, antimicrobial, herbicidal, and redox activities.

Results and Discussion

The synthetic design included two key routes (Scheme 1). Initially, the derivatives **2a, b** were easily obtained using Holmberg’s protocol (*i*) [18] from corres-

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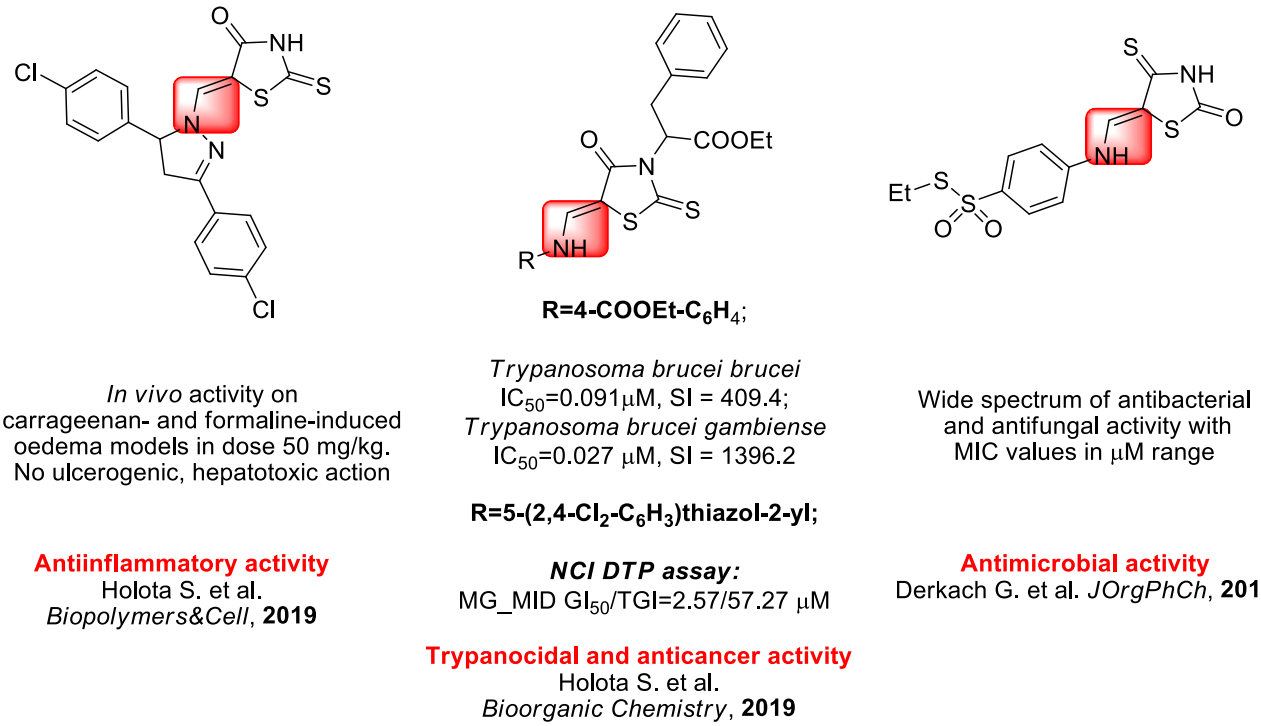


Figure 1. The "enamine"-bearing thiazolidin-4-one hybrids as potential pharmacological agents.

ponding aminobenzoic acids **1a, b**. The procedure (*ii*) [15] was used for synthesis **3a, b** from obtained derivatives **2a, b**. The convenient synthetic approach [19] starting from aromatic aldehyde **4a, b**, and acetophenone (*iii* and *iv*) was used for the synthesis of diarylpyrazolines **6a, b**. The target pyrazoline-thiazolidin-4-one hybrids **7a-d** were synthesized in satisfactory yields and purity by reacting compounds **3a, b** and **6a, b** under reflux in ethanol for 30-45 min.

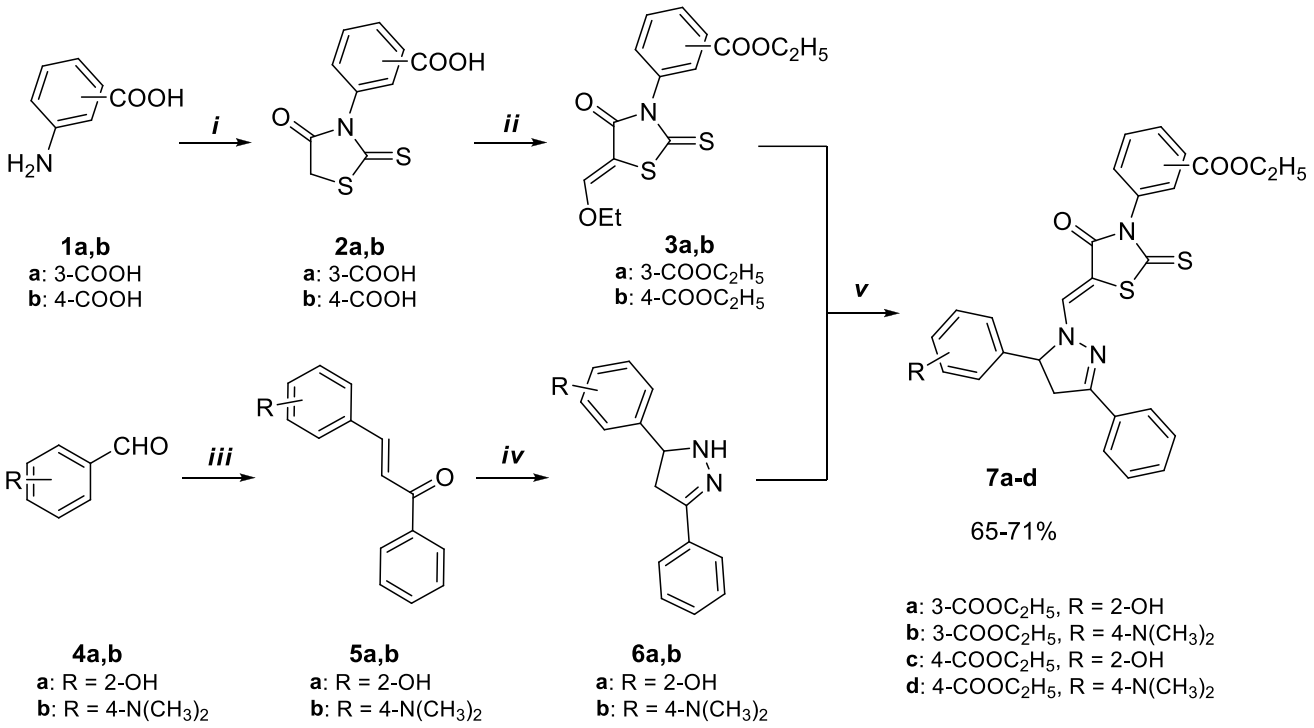
The structures of all synthesized compounds were confirmed by ^{1}H, ^{13}C NMR spectroscopy, and LC-MS-spectrometry. Esterification of the carboxylic group of compounds **3a, b** under condition *ii* was observed by the appearance of signals from the protons of the ethyl group at ~ 4.31 (q, J = 6.3 Hz) and ~ 1.31 (t, J = 6.3 Hz) ppm in the ^{1}H NMR spectra. In the ^{1}H NMR spectra of derivatives **3a, b** and **7a-d** the proton signal at C-5 double bond appears mainly in the field of aromatic protons, and only for derivative **7b** it was observed as a singlet at 7.60 ppm. The pyrazoline fragment of compounds **7a-d** shows the characteristic patterns of the AMX system for CH_2-CH protons.

The synthesized compounds **7a-d** have been evaluated for their antioxidant activity in vitro in the DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging assay [20] in the conditions close to physiological (serial dilutions of stock methanol solutions at six concentrations of 1.0, 2.0, 4.0, 6.0, 8.0, 10.0 mM + Tris-HCl buffer pH = 7.40, measurements after 60 min). Ascorbic acid was used as a reference compound (standard). The IC_{50} values have been determined for compounds **7a-d** as well as ascorbic acid to characterize their antioxidant activity (Figure 2).

As a result, the tested compounds **7a-d** have low-moderate activity in DPPH assay, and the established IC_{50} values of the synthesized compounds were: 4.67 mM (**7a**), 5.90 mM (**7b**), 6.05 mM (**7c**), 7.12 mM (**7d**), and for ascorbic acid IC_{50} = 0.045 mM. It should be noted that this level of antioxidant activity may be more likely associated with the presence of phenolic (-OH), and dimethylamino groups (-$N(CH_3)_2$) in compounds **7a-d** than with other molecular fragments. Nevertheless, all tested derivatives show activity from 8.38 to 13.43 mg/mL that is promising for searching for new potential antioxidants among this subtype of hybrid molecules.

Compounds **7a-d** were preliminary screened for their potential antimicrobial activity against Gram-positive bacteria as *Lactobacillus plantarum*, Gram-negative bacteria as *Escherichia coli*, and yeasts (*Candida albicans).* Antimicrobial activity was evaluated in terms of minimum inhibitory concentrations (MICs), and the values were compared with standard reference antimicrobial agents [21-22]. Overall, the tested compounds showed very low antimicrobial activity against the *E. coli* and *C. albicans* compared to the reference drugs (36.5 μM for ampicillin and 38.96 μM for fluconazole), Table 1. Only derivative **7c** showed activity with MIC value of 1.25 mM against *E. coli*, and derivative **7d** showed antifungal activity against *C. albicans* with MIC value of 1.25 mM. It is also worth noting that compounds **7a-d** were inactive against *L. plantarum.*

The herbicidal activity of the compounds **7a-d** was tested against the monocot grass species Creeping bentgrass (*Agrostis stolonifera*). Methanol solutions at the concentration of 1mg/ml of all compounds were added to



Scheme 1. Synthesis of target pyrazoline-thiazolidin-4-one hybrids **7a-d.** Reagents and conditions: *i*) **1a, b** (10 mmole), $CS(SCH_2COOH)_2$ (10 mmole), C_2H_5OH:H_2O, reflux, 5h; *ii*) **2a, b** (10 mmole), $HC(OC_2H_5)_3$ (10 mmole), Ac_2O, reflux, 3h; *iii*) **4a, b** (10 mmole), acetophenone (10 mmole), NaOH (10 mmole); *iv*) **5a, b** (10 mmole), NH_2-NH_2 (10 mmole), KOH (10 mmole), C_2H_5OH; *v*) **3a, b** (10 mmole), **6a, b** (10 mmole), C_2H_5OH, reflux, 2h.

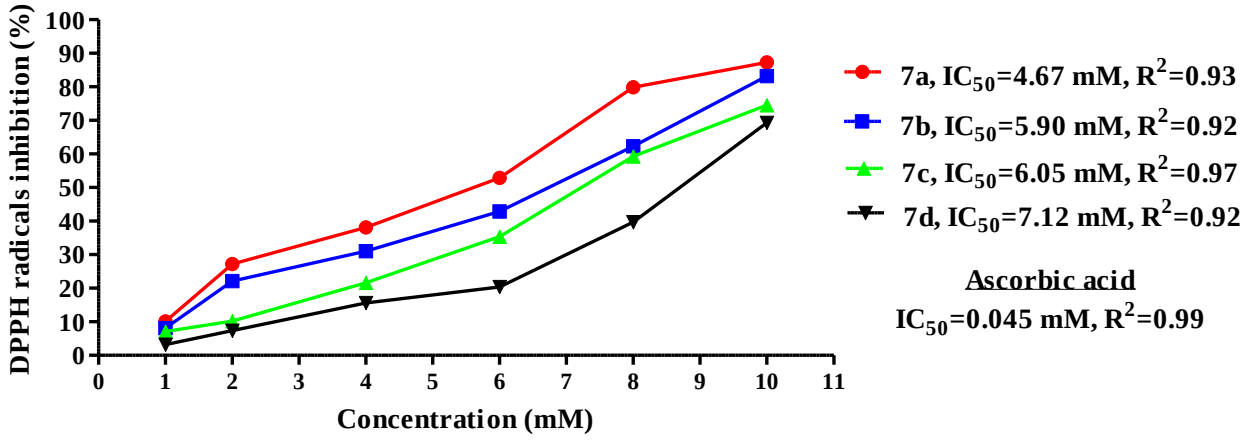


Figure 2. The dose-depending DPPH radical inhibition and IC_{50} values for compounds **7a-d**.

the plant seeds and incubated in a minimal medium. Seed germination was observed after 3 days, and only compound **7b** inhibited of grass growth by 15 %. No inhibitory effect on *A. stolonifera* was observed in the case of compounds **7a**, **7c**, and **7d**.

The redox activity of **7a-d** was evaluated by cyclic voltammetry technique using stock solutions of compounds in methanol (C = 5 mM) with the addition of phosphate buffer solution (pH = 6.40). The glassy carbon working electrode, a platinum wire counter, and a saturated calomel electrode were used, and the measurements were performed at 0 min and after 60 min in the potential range from -1500 mV to 1500 mV with scan rates between 10 and 100 mV/s. No redox peaks were observed under mentioned experimental conditions in cyclic voltammetry studies for tested compounds **7a-d**.

Table 1. Antimicrobial properties of compounds **7a-d** (MICs values)

Compounds/ Microorganisms	*E.coli*	*L. plantarum*	*C. albicans*
7a	2.5 mM	>2.5 mM	>2.5 mM
7b	>2.5 mM	>2.5 mM	2.5 mM
7c	1.25 mM	>2.5 mM	2.5 mM
7d	2.5 mM	>2.5 mM	1.25 mM
References[a,b]	36.5 μM[a]	39.8 μM[a]	38.96 μM[b]

- [a] – ampicillin
- [b] – fluconazole

Conclusions

In the present paper, a synthesis of the series of new pyrazoline-thiazolidin-4-one hybrids has been reported. The structure of the compounds was confirmed using ^{1}H, ^{13}C NMR, and LC-MS spectra. All synthesized compounds were evaluated for their antioxidant, antibacterial, antifungal, herbicidal, and redox properties. The synthesized hybrid compounds have promising free radical scavenging activities, and obtained results argue to the next development of antioxidant agents among these types of molecules.

Experimental section

General

Commercial reagents were purchased from Merck and used without purification. Melting points were measured in open capillary tubes on a BŰCHI B-545 melting point apparatus and are uncorrected. The elemental analyses (C, H, N) was performed using the Perkin-Elmer 2400 CHN analyzer and was within ±0.4% of the theoretical values. The ^{1}H and ^{13}C NMR spectra were recorded on a Bruker-500 spectrometer at 500 MHz and 126 MHz using a mixture of DMSO-d6+CCl4 as a solvent and TMS as an internal standard. Chemical shift values are reported in ppm units with use of δ scale. Mass spectra were obtained using electrospray ionization (ESI) techniques on an Agilent 1100 Series LCMS. The purity of the compounds was checked by thin-layer chromatography performed with Merck Silica Gel 60 F254 aluminum sheets. Spots were detected by their absorption under UV light.

Synthesis

*General procedure for the synthesis derivatives **7a-d.***

In a round bottom flask is placed by 0.01 mole of **3a** or **3b** and **6a** or **6b**, add 10 ml of ethanol. The mixture was heated at reflux for 2 hours. After cooling, the precipitate formed is filtered off and recrystallized from DMF-ethanol.

*Ethyl (Z)-3-(5-(ethoxymethylene)-4-oxo-2-thioxothiazolidin-3-yl)benzoate (**3a**)*

Yield 52%, mp 163-165 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.99 (s, 1H), 7.90 (s, 1H), 7.47-7.55 (m, 2H), 4.35 (q, *J* 6.2 Hz, 2H), 4.15 (q, *J* 6.3 Hz, 2H), 1.35 (t, *J* 6.2 Hz, 3H), 1.15 (t, *J* 6.3 Hz, 3H). LC/MS m/z 338 $(M+H)^+$. Anal. Calcd. for $C_{15}H_{15}NO_4S_2$: C, 53.40; H, 4.48; N, 4.15. Found: C, 53.50; H, 4.60; N, 4.20.

*Ethyl (Z)-4-(5-(ethoxymethylene)-4-oxo-2-thioxothiazolidin-3-yl)benzoate (**3b**)*

Yield 63%, mp 187-189 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.90 (s, 1H), 7.84 (d, *J* 8.6 Hz, 2H), 7.69 (d, *J* 8.6 Hz, 2H), 4.35 (q, *J* 6.2 Hz, 2H), 4.15 (q, *J* 6.3 Hz, 2H), 1.35 (t, *J* 6.2 Hz, 3H), 1.15 (t, *J* 6.3 Hz, 3H). LC/MS m/z 338 $(M+H)^+$. Anal. Calcd. for $C_{15}H_{15}NO_4S_2$: C, 53.40; H, 4.48; N, 4.15. Found: C, 53.60; H, 4.50; N, 4.30.

*Ethyl (Z)-3-(5-((5-(2-hydroxyphenyl)-3-phenyl-4,5-dihydro-1H-pyrazol-1-yl)methylene)-4-oxo-2-thioxothiazolidin-3-yl)benzoate (**7a**)*

Yield 65%, mp 212-214 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.53 (s, 1H), 8.10-8.00 (m, 2H), 7.99-7.91 (m, 2H), 7.69-7.57 (m, 3H), 7.49-7.39 (m, 3H), 7.28-7.22 (m, 2H), 6.81-6.75 (m, 2H), 5.56 (dd, *J* 11.3, 7.0 Hz, 1H), 4.31 (q, *J* 6.3 Hz, 2H), 4.00 (dd, *J* 18.4, 11.3 Hz, 1H), 3.51 (dd,
J 18.4, 7.0 Hz, 1H), 1.00 (t, *J* 6.3 Hz, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 186.5, 179.3, 167.2, 164.1, 161.4, 159.4, 157.2, 154.0, 151.1, 149.4, 142.2, 139.0, 137.4, 129.7, 128.3, 127.0, 126.2, 121.2, 118.4, 113.9, 92.4, 88.7, 62.5, 13.4. LC/MS m/z 530 $(M+H)^+$. Anal. Calcd. for $C_{28}H_{23}N_3O_4S_2$: C, 63.50; H, 4.38; N, 7.93. Found: C, 63.70; H, 4.50; N, 8.00.

*Ethyl (Z)-3-(5-((5-(4-(dimethylamino)phenyl)-3-phenyl-4,5-dihydro-1H-pyrazol-1-yl)methylene)-4-oxo-2-thioxothiazolidin-3-yl)benzoate (**7b**)*

Yield 67%, mp 228-231 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.00 (dt, *J* 7.8, 1.4 Hz, 1H), 7.94-7.89 (m,

2H), 7.78 (t, *J* 1.9 Hz, 1H), 7.70-7.59 (m, 2H), 7.60 (s, 1H), 7.62-7.56 (m, 1H), 7.58-7.51 (m, 1H), 7.41 (s, 1H), 7.26-7.21 (m, 2H), 6.79-6.73 (m, 2H), 5.57 (dd, *J* 11.3, 7.1 Hz, 1H), 4.31 (q, *J* 6.3 Hz, 2H), 4.00 (dd, *J* 18.4, 11.3 Hz, 1H), 3.51 (dd, *J* 18.5, 7.1 Hz, 1H), 2.90 (s, 6H), 1.31 (t, *J* 6.3 Hz, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 184.4, 179.5, 166.5, 163.6, 160.9, 159.5, 156.8, 154.1, 150.5, 148.5, 141.7, 138.5, 137.1, 129.2, 128.1, 127.3, 125.8, 120.6, 116.3, 112.6, 91.5, 88.7, 64.4, 35.2, 13.1. LC/MS m/z 557 $(M+H)^+$. Anal. Calcd. for $C_{30}H_{28}N_4O_3S_2$: C, 64.73; H, 5.07; N, 10.06. Found: C, 64.90; H, 5.20; N, 10.20.

*Ethyl (Z)-4-(5-((5-(2-hydroxyphenyl)-3-phenyl-4,5-dihydro-1H-pyrazol-1-yl)methylene)-4-oxo-2-thioxothiazolidin-3-yl)benzoate (**7c**)*

Yield 68%, mp 230-232 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.50 (s, 1H), 8.11-8.02 (m, 2H), 7.96-7.89 (m, 2H), 7.64-7.53 (m, 3H), 7.48-7.38 (m, 3H), 7.25-7.20 (m, 2H), 6.81-6.74 (m, 2H), 5.56 (dd, *J* 11.3, 7.0 Hz, 1H), 4.31 (q, *J* 6.3 Hz, 2H), 4.00 (dd, *J* 18.4, 11.3 Hz, 1H), 3.51 (dd, *J* 18.4, 7.0 Hz, 1H), 1.05 (t, *J* 6.3 Hz, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 184.0, 179.1, 166.8, 162.9, 160.7, 159.2, 156.8, 153.3, 150.4, 141.5, 138.3, 137.2, 129.7, 129.4, 128.3, 127.5, 126.9, 113.1, 91.1, 86.5, 64.0, 13.2. LC/MS m/z 530 $(M+H)^+$. Anal. Calcd. for $C_{28}H_{23}N_3O_4S_2$: C, 63.50; H, 4.38; N, 7.93. Found: C, 63.80; H, 4.60; N, 8.10.

*Ethyl (Z)-4-(5-((5-(4-(dimethylamino)phenyl)-3-phenyl-4,5-dihydro-1H-pyrazol-1-yl)methylene)-4-oxo-2-thioxothiazolidin-3-yl)benzoate (**7d**)*

Yield 71%, mp 244-246 °C. ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.09-8.00 (m, 2H), 7.94-7.88 (m, 2H), 7.65-7.55 (m, 3H), 7.48-7.38 (m, 3H), 7.26-7.20 (m, 2H), 6.79-6.72 (m, 2H), 5.56 (dd, *J* 11.3, 7.0 Hz, 1H), 4.31 (q, *J* 6.3 Hz, 2H), 4.00 (dd, *J* 18.4, 11.3 Hz, 1H), 3.51 (dd, *J* 18.4, 7.0 Hz, 1H), 2.90 (s, 6H), 1.05 (t, *J* 6.3 Hz, 3H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 183.7, 178.6, 165.7, 162.7, 160.1, 158.8, 156.2, 153.7, 150.0, 141.3, 138.1, 137.0, 129.9, 129.2, 128.1, 127.3, 126.4, 112.6, 90.8, 86.3, 63.7, 39.0, 13.0. LC/MS m/z 557 $(M+H)^+$. Anal. Calcd. for $C_{30}H_{28}N_4O_3S_2$: C, 64.73; H, 5.07; N, 10.06. Found: C, 65.00; H, 5.10; N, 10.30.

Antioxidant activity (DPPH assay)

DPPH inhibition was determined by using the protocol [20]. The DPPH radical is stable due to the delocalization of a spare electron over the molecule, thus preventing dimer formation. This radical is used in the DPPH radical scavenging capacity assay to quantify the ability of antioxidants to quench the DPPH radical. The dark purple color of DPPH will be lost when it is reduced to its non-radical form stable organic nitrogen centered free radical with a dark purple color which when reduced to its non-radical form by antioxidants becomes colorless. DPPH radicals are widely used in the model system to investigate the scavenging activities of several natural compounds. When the DPPH radical is scavenged, the color of the reaction mixture changes from purple to yellow with decreasing of absorbance at wavelength 517 nm. The stock solutions of compounds were prepared in mixture methanol + Tris-HCl 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 30 min (vortexed in between). Absorbance was taken at 517 nm against the ethanol by using 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.

Antimicrobial activity

The minimal inhibitory concentrations (MICs) were determined by the standard microdilution method in cation-adjusted Mueller-Hinton II Broth (MHB, Becton-Dickinson, Germany) according to the recommendations of the Clinical and Laboratory Standard Institute. The tested compounds were evaluated for their antimicrobial activity against Gram-positive bacteria (*L. plantarum*), Gram-negative bacteria (*E. coli*), and yeasts (*C. albicans*). Ampicillin was used as a reference antibacterial agent and fluconazole as antifungal one. A representative colony was lifted off with a wire loop and placed in 5 mL of nutrient broth medium, which was then incubated with shaking at 37 °C for 5 h. Then, 1×10^6 cells/mL were suspended in a nutrient broth medium to generate the working suspension. Different concentrations of peptides were prepared in a 96-well plate using nutrient broth medium, and each well contained 100 µL compound solutions. A 100-µL cell working suspension was then added to each well. The plate was incubated at 37 °C for 24 h, and the optical density (OD) of each well was then measured at 600 nm after gently shaking the plate for 10s using a Hybrid Multi-Mode Microplate reader (BioTek, Synergy H4). Wells containing medium only (blank) and wells containing cells in medium without peptides (positive control) were included on the same plate. The values of MIC were recorded after 20 h and 24 h of incubation with the compounds for bacteria and yeasts, respectively. Experiments were performed in triplicate and on three different occasions (i.e., a total of nine repeats for each individual measurement).

Herbicidal activity - Herbicidal Pre-emergence Test

Seeds of *A. stolonifera* (JuliwaHESA, Heidelberg, Germany) were placed into the wells of a 96-well microtiter plate (Sarstedt, Nümbrecht, Germany). A solution containing 2.2 g/l Murashige & Skoog plant salts (Serva, Heidelberg, Germany) and 1.6 g/l Gamborg's B5

plant medium (Serva, Heidelberg, Germany) was added to the wells. The stock solutions in concentration 1 mg/ml in methanol were prepared for compounds **7a-d** and were added to the wells. Identical volumes of methanol without compounds were used as a toxicity test of the organic solvent. The solution containing the plant medium was used as a negative control. The plate was closed and incubated at room temperature under constant light (Osram Fluora lamp) in a humidity chamber. After 3 days of incubation, the plate lid was removed and a container with tap water was placed inside the chamber for increasing the air humidity. The plate was incubated up to 6 days. Three technical replicates were performed.

Voltammetric parameters and electrochemical cells

Voltammetric experiments were performed using BAS 100W Potentiostat.A glassy carbon (GC) (A = 0.07 cm^2) was used as working electrode. Pt wire and saturated calomel electrode (SCE) were used as counter and reference electrodes. Before each experiment, the surface of GCE was polished with diamond spray (particle size 1 μm) followed by thorough rinsing with distilled water. All the voltammetric experiments were conducted in a high purity nitrogen atmosphere at room temperature (25 ± 1 °C) potential range from -1500 mV to 1500 mV, scan rates between 10 and 100 mV/s; stock solutions in methanol
C = 5 mM, PBS pH = 6.40; measurements at 0 and after 60 min. For reproducible experimental results, the polished working electrode was used to place in the desired electrolyte solution followed by recording of various voltammograms until the achievement of steady state baseline.

Notes

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The author declare no conflict of interest.

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Синтез нових піразолін-тіазолідин-4-онових гібридних молекул та оцінка їх біологічної активності

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Резюме: Протягом останніх десятиріч гібридні молекули на основі піразолінових та тіазолідин-4-онових каркасів є об'єктом інтенсивних досліджень в медичній хімії як джерело потенційних біологічно активних сполук із широким фармакологічним профілем. В даній роботі запропонований та представлений ефективний підхід до синтезу піразолін-тіазолідин-4-онових гібридних молекул з єнаміновим лінкером у молекулах. Структура синтезованих сполук підтверджена з використанням методів ^{1}H-, ^{13}C-ЯМР спектроскопії та РХ-МС-спектрометрії. Для всіх сполук досліджена антиоксидантна (DPPH метод), протимікробна (по відношенню до грам-позитивних *Lactobacillus plantarum*, грам-негативних *Escherichia coli* та грибів *Candida albicans*, визначення МІК), редокс (метод циклічної вольтметрії) та гербіцидна активності (по відношенню до *Agrostis stolonifera*). Всі тестовані сполуки продемонстрували здатність інгібувати радикали в умовах DPPH-тесту з IC_{50} в межах 4.67-7.12 mM. Отримані результати скринінгу антирадикальної активності є аргументом для поглиблених досліджень із застосуванням додаткових/альтернативних експериментальних моделей, а також оптимізації молекулярної структури. Всі тестовані сполуки проявили низьку протимікробну та гербіцидну активності, а також не володіють редокс-властивостями.

Ключові слова: піразолін-тіазолідин-4-онові гібриди; метод DPPH; протимікробна/гербіцидна активність; циклічна вольтметрія.