ISSN 1814-9758. Ukr. Bioorg. Acta, 2022, Vol. 17, N 2
UDC 547.7/.8, 579.22, 615.281: 577.1
DOI: https://doi.org/10.15407/bioorganica2022.02.023

Ukrainica Bioorganica Acta
www.bioorganica.org.ua

UBA

RESEARCH ARTICLE

Isoxazole-containing sulfonylamides as new antibacterial agents: *in silico* study, synthesis and *in vitro* evaluation

Diana M. Hodyna*, Oleksandr V. Pavliuk, Maria M. Baran, Vitaliy O. Yevdokymenko, Vasyl V. Kovalishyn, Larysa O. Metelytsia

V.P. Kukhar Institute of Bioorganic Chemistry and Petrochemistry of the NAS of Ukraine, Kyiv, Ukraine

Abstract: The QSAR models previously created by the OCHEM web platform were used for the research and design of novel isoxazole derivatives as antimicrobial agents. Based on the created virtual set of promising isoxazole derivatives, a number of potential antibacterial agents were selected for synthesis and further research. A convenient synthetic sequence for obtaining initial isoxazole-containing sulfonylamides and preparative methods for the synthesis of target sulfonylamides of the isoxazole series, in particular, using ring-closing metathesis reactions, were worked out. The results of *in vitro* antimicrobial activity evaluation of synthesized compounds with predicted high activity showed that a series of isoxazole-containing sulfonylamides are promising antimicrobial agents with a wide spectrum of antibacterial action, especially against multidrug-resistant *E. coli*, *A. baumannii* and *S. aureus* bacterial pathogens. *In vivo* assessment of the acute toxicity of the studied compounds on the *D. magna* as a known biosensor proved that most of the studied isoxazole derivatives can be attributed to the class of slightly toxic substances according to the classification developed by Passino and Smith for hydrobionts.

Keywords: QSAR; antibacterial activity; isoxazole; sulfonylamide; toxicity.

Introduction

The wide range of pharmacological activity of sulfo-containing derivatives in organic chemistry makes them a convenient option for inclusion in a significant number of available drugs on the marketplace [1-2]. Sulfonylamides are a well-known class of broad-spectrum synthetic antibiotics. Thus, in particular, they are quite effective against gram-positive and some gram-negative bacteria such as *Salmonella*, *Escherichia coli,* and *Enterobacter,* but are ineffective against *Pseudomonas aeruginosa* and *Serratia species* [3-4]. It is known about the effectiveness of the use of sulfonylamides in the treatment of tonsillitis, hypoglycemia, thyroiditis, inflammation, glaucoma, bacillary dysentery, septicemia, and urinary tract diseases [5-6].

Received:	18.08.2021
Revised:	13.09.2021
Accepted:	25.10.2021
Published online:	30.12.2021

* Corresponding author. Tel.: +380-44-296-0409;
e-mail: dianahodyna@gmail.com (D.M. Hodyna)
ORCID: 0000-0001-6161-9833

There is evidence of the effectiveness of sulfonylamides against some fungal and bacterial infections caused by *Nocardia* species, *Staphylococcus aureus,* and *Escherichia coli* [7-9].

Isoxazole-containing derivatives are one of the interesting, promising but little-studied representatives of nitrogen-containing heterocycles with an Oxygen atom, which, due to a number of practically useful properties, are considered one of the most important classes of substances for use in medical chemistry and are an important source of valuable drugs with a diverse spectrum of action [10-12].

Sulfo-derivatives are one of the most intensively studied derivatives among the numerous isoxazole derivatives, a significant number of which are a variety of biologically active substances and even commercially available drugs. They are quite actively used in the development of synthetic bacteriostatic antibiotics of various spectrums of action [13-16]. In this study, we evaluated the use of isoxazole-containing sulfonylamides as a broad spectrum of action using *in vitro* studies and QSAR modeling as method for studying the quantitative relationship between the structure of substances and biological activity based on the

description of the structure of a chemical compound using a set of numerical descriptors and machine learning methods [17-19].

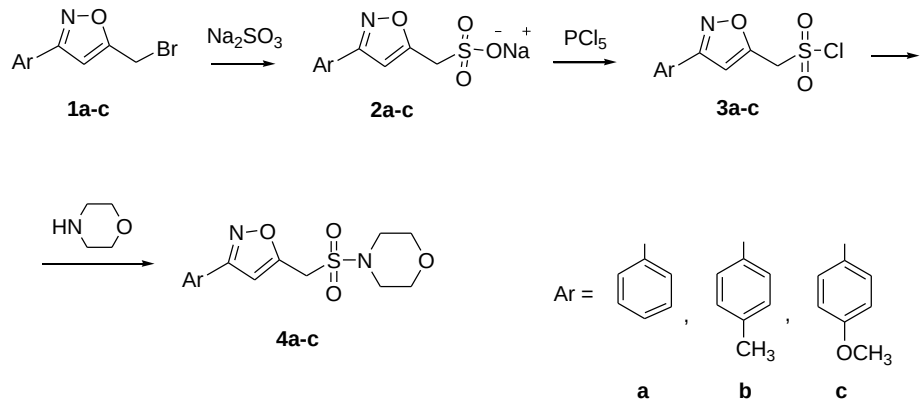
Results and Discussion

In this study the antibacterial activity of isoxazole-containing sulfonylamides as *E. coli* inhibitors was predicted using previously developed and published on the OCHEM server regression QSAR models [20]. The dataset included 5143 compounds active against *E. coli* ATCC 25922 which was randomly divided into training (3780 compounds) and test (1363 compounds) sets. Three regression QSAR models built by the Trans-CNN, ASNN, and RFR methods and the consensus model as an average of all three models were created using descriptor packages such as E-state, ALOGPS and CDK2, with the best performances. The results are summarized in Table 1 and the consensus model performance is shown in Figure 1.

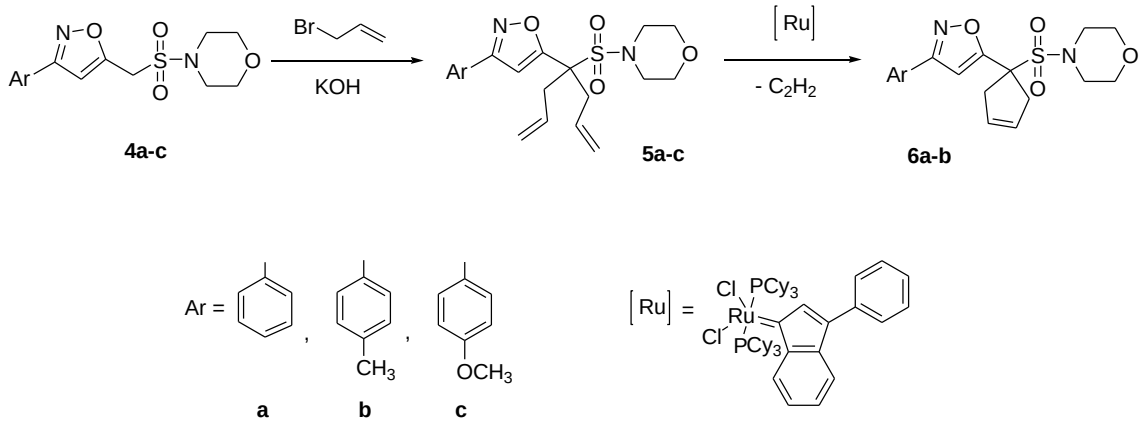
Table 1. Statistical coefficients calculated for QSAR models obtained from data with activity against *E. coli*

	MLM	Training Set			Test Set		
		R^2	q^2	RMSE	R^2	q^2	RMSE
1	Trans-CNN	0.80 ± 0.01	0.80 ± 0.01	0.48 ± 0.01	0.8 ± 0.02	0.8 ± 0.02	0.48 ± 0.02
2	ASNN	0.73 ± 0.01	0.72 ± 0.01	0.58 ± 0.01	0.74 ± 0.02	0.74 ± 0.02	0.57 ± 0.03
3	RFR	0.76 ± 0.01	0.75 ± 0.01	0.55 ± 0.01	0.78 ± 0.02	0.77 ± 0.02	0.53 ± 0.02
4	Consensus model	0.79 ± 0.01	0.79 ± 0.01	0.34 ± 0.01	0.80 ± 0.02	0.79 ± 0.01	0.33 ± 0.01

MLM – machine learning method; R^2 – square of correlation coefficient; q^2 – coefficient of determination; RMSE – Root Mean Squared Error.



Scheme 1. Synthesis of isoxazole-containing sulfonyl chlorides and corresponding sulfonylamides.



Scheme 2. Synthesis of isoxazole-containing target sulfonylamides.

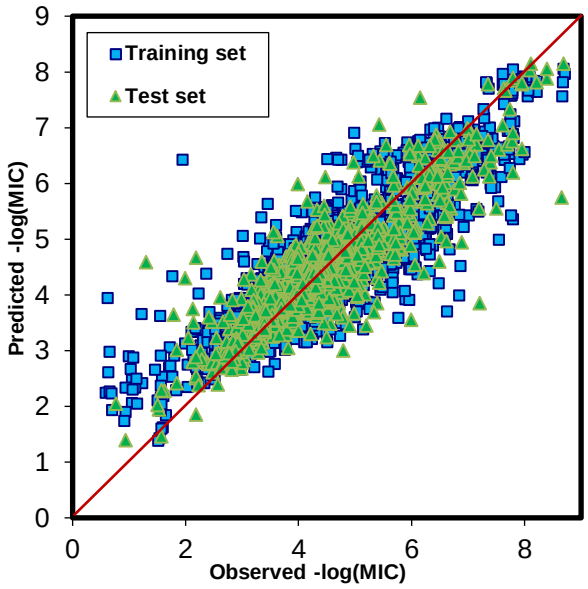


Figure 1. Plots of experimental versus predicted values for the consensus QSAR model for *E. coli* inhibitors; minimum inhibitory concentration (MIC) is the lowest concentration of a chemical which prevents the visible growth of a bacteria. Please refer to Table 1 for the goodness of fit parameters.

Figure 1 shows the regression line plotting the values predicted by the consensus QSAR model for *E. coli* inhibitors. Most predictions do not differ from the experimental values by more than 1 log unit. Only 5.6% of molecules in the training set have residuals between the experimental and predicted - log(MIC) higher than one log unit. In the test set, 5% of molecules have residuals between the experimental and predicted - log(MIC) that are higher than one log unit, but lower than two log units. This result supports the robustness of the developed consensus model (Figure 1, Table 1).

The antibacterial activity of virtual compounds was predicted by the developed consensus QSAR model. In order to select compounds for synthesis and biological testing, attention was paid to the anti-*E. coli* inhibitors with predicted activity (- log(MIC) as 100 μM including the structural features of isoxazole-containing sulfonylamides (Table 2).

As we previously reported, one of the convenient ways of obtaining isoxazole-containing sulfonyl chlorides is the interaction of sulfinic acid salts with phosphorus pentachloride. Sulfonyl chlorides of the isoxazole series are obtained by boiling water-alcohol solutions of the corresponding halides with equimolar amounts of sodium sulfite, followed by the interaction of thoroughly dried salts of sulfinic acids with phosphorus pentachloride (Scheme 1) [21].

As a result of the interaction of isoxazole-containing sulfonyl chlorides with the corresponding amines in the presence of triethylamine in the solutions of dichloromethane at a temperature of about 5-10 ºC for 0.5-1 hours a series of isoxazole-containing sulfonylamides were obtained (Scheme 1). Target products **3a-c** were obtained with 82-85% yields. The structure of obtained compounds is confirmed by the data of chromatomas spectra, ^{1}H, ^{13}C NMR spectra and elemental analysis.

Sulfonylamides **4a-c** were alkylated on active methylene group with 3 equivalents of allyl bromide (50% excess) in DMF solutions at a temperature of 65-70 ºC for 4-5 hours in the presence of 3 equivalents of potassium hydroxide (50% excess) (Scheme 2). Diallyl containing sulfonylamides **5a-c** were isolated with 63-67% yields. Their structure is confirmed by chromatomass spectra, elemental analysis and data of ^{1}H, ^{13}C NMR spectra.

Ring-closing metathesis reactions of the diallyl derivatives **5a-b** were carried out in solutions of dry degassed dichloromethane in the atmosphere of dry argon at a temperature of 25-30 ºC for 10-12 hours using a ruthenium-carbene catalyst ([Ru]) (Scheme 2). Cycloalkenil products **6a-b** were isolated after chromatographic purification with 71-75% yields. Their structure is confirmed by chromatomass spectra, elemental analysis and data of ^{1}H, ^{13}C NMR spectra, elemental analysis.

The obtained results of antibacterial activity of synthesized isoxazole-containing sulfonylamides with predicted anti-*E. coli* activity against standard and MDR bacterial strains are presented in Table 2.

The results presented in Table 2 demonstrated *in vitro* high antibacterial potential of almost all studied isoxazole-containing sulfonylamides (except compound **5b**) against Gram-negative *E. coli* ATCC strain, which confirmed the results of QSAR prediction of anti-*E. coli* activity. Zones diameters of growth inhibition of the indicated ATCC culture strain were 15-25 mm for seven tested compounds. It is also worth noting that all compounds except compound **5b** showed activity in the range of 11 to 24 mm against the *E. coli* HMR strain. The least sensitive strain of *E. coli* MDR showed sensitivity only to compound **4b** with an activity of 14 mm by the diameter of the growth inhibition zone. Thus, it was experimentally proven that the isoxazole-containing sulfanilamide **4b** is the most active against all studied *E. coli* strains (zone diameters of growth inhibition were in the range from 14 mm to 25 mm).

Considering the established high antibacterial potential of the studied isoxazole-containing sulfonylamides against *E. coli* strains, the futures studies were focusing on the compounds activity against a wider range of standard and antibiotic-resistant bacterial strains.

It was found that studied isoxazole-containing sulfonylamides **4a-b** and **6a-b** possessed a high antibacterial effect against all studied *A. baumannii* strains (zones diameters of growth inhibition were in the range from 8 mm to 22 mm). Also, compounds **4a-b**, **5b**, and **6a-b** were effective against the gram-positive *S. aureus* ATCC 25923 strain with a range of zones diameter of growth inhibition of 14-17 mm and against MDR *S. aureus* strain with a range of zones diameter of growth inhibition of 12-15 mm.

The prospects of the studied isoxazole-containing sulfonylamides as antibacterials were confirmed by the *in vivo* results of their toxic profile.

Table 2. *In vitro* antibacterial activity of isoxazole-containing sulfonylamides with predicted activity.

Compd	Zone diameter of growth inhibition of bacterial strains, mm									
	E. coli					*A. baumannii*			*S. aureus*	
	Predicted activity -log(MIC)	ATCC 25922	HMR[a]	MDR[b]	MDR 1355	MDR 1536	MDR 871	MDR 725	ATCC 25923	MDR[c]
4a	3.7 ± 0.8	16	15	na*	11	14	13	8	14	12
4b	3.7 ± 0.8	25	24	14	22	15	12	11	17	13
4c	3.7 ± 0.8	15	11	na	11	9	8	na	na	na
5a	3.9 ± 0.8	15	14	na	11	na	na	na	na	na
5b	4.0 ± 1.2	na	na	na	na	na	na	na	15	15
5c	4.0 ± 1.2	18	12	na	10	8	12	na	na	na
6a	3.8 ± 0.8	16	13	na	16	12	11	10	15	12
6b	3.8 ± 0.8	17	11	na	15	12	10	9	15	13

na* - no activity.
[a]Clinical isolate of hemolytic *E. coli* resistant to carbenicillin, ceftazidime, cefoxitin, oxacillin.
[b]Clinical isolate of *E. coli* resistant to ampicillin, doxycycline, carbenicillin, cefoxitin, ceftazidime, ceftriaxone, kanamycin, oxacillin, ofloxacin, tetracycline.
[c]Clinical isolate of *S. aureus* resistant to ampicillin, carbenicillin, ceftazidime, oxacillin, tetracycline.

Table 3. Acute toxicity of isoxazole-containing sulfonylamides as potential antibacterial agents using biomarker *D. magna*.

Compd	LC_{50} (mg/L)	95% confidence intervals	Toxicity level classification (by D.R. Passino and S.B. Smith)*
4a	> 100	-	+
4b	45.33 ± 13.40	17.18-73.48	+ +
4c	37.32 ± 10.59	15.69-58.95	+ +
5a	25.69 ± 6.08	12.91-38.47	+ +
5b	21.84 ± 5.12	10.36-32.13	+ +
5c	33.23 ± 10.04	13.67-52.45	+ +
6a	44.45 ± 14.36	18.25-74.49	+ +
6b	41.08 ± 13.97	11.73-70.42	+ +

«-» not determined. *Toxicity classification by LC_{50} range: « + » practically harmless (100-1000 mg/L); « + + » slightly toxic (10-100 mg/L); « + + + » moderately toxic (1-10 mg/L); « + + + +» highly toxic (0.1-1 mg/L).

One of the most widely used international biological tests for screening the toxicity of chemical substances is the acute toxicity test using invertebrate hydrobionts such as cyclops, amphipods, artemia and daphnia [22-23]. *Daphnia magna* is one of the most common planktonic crustaceans from the *Cladocera* suborder [24-25]. Due to the high reproduction rate, sizes sufficient for visual observation, availability in nature, laboratory maintenance and high sensitivity to the toxicity of chemical compounds, *D. magna* was used as a test organism in determining the acute toxicity of the studied isoxazole-containing sulfonylamides as antibacterials.

The results of the acute toxicity evaluation (48 h LC_{50} values) of the tested isoxazole-containing sulfonylamides as potential antibacterial agents are presented in Table 3.

Presented in Table 3 the obtained results of *in vivo* testing using the hydrobiont *D. magna* demonstrated two levels of acute toxicity of the studied isoxazole-containing sulfonylamides. In general, most of the investigated isoxazole-containing derivatives **4b-c**, **5a-c** and **6a-b** according to the classification of Passino and Smith can be attributed to the class of slightly toxic substances with a range of LC_{50} values from 21.84 mg/L to 45.33 mg/L [26]. The least toxic compound **4a** can be classified as practically harmless with LC_{50} > 100 mg/L.

Conclusions

The antibacterial activity of new isoxazole derivatives was predicted by a number of developed and published earlier regression QSAR models using the OCHEM web platform. Eight new isoxazole-containing sulfonylamides were identified for biological testing, the anti-*E. coli* activity of which was predicted up to 100 μM. Convenient and effective strategies for obtaining the corresponding new

sulfonylamides of the isoxazole series with an active methylene group were developed, which were subsequently transformed into the corresponding dialkenyl derivatives. Using ring-closing metathesis reactions, a number of new cyclopentenyl-containing arylsulfonylamides of the isoxazole series were synthesized. The obtained *in silico* and *in vitro* studies results of the antibacterial activity of synthesized isoxazole-containing sulfanilamides proved the high antibacterial potential of a number of tested compounds against both gram-positive and gram-negative bacterial pathogens of *E. coli*, *A. baumannii* and *S. aureus*, including multiresistant strains and their positive toxicity profile (LC_{50} values from 41.08 mg/L to > 100 mg/L, which corresponds to the class of practically harmless substances).

Experimental section

Chemistry

General information: ^{1}H (500 MHz) and ^{13}C (125 MHz) NMR spectra were recorded on Bruker Avance DRX 500 spectrometer in $CDCl_3$. Chromatomass spectra were recorded using a liquid chromatography-mass spectrometric system using an Agilent 1100 Series high- performance liquid chromatograph equipped with a diode array with an Agilent LC/MSD SL mass-selective detector (ionization method - is the chemical ionization at atmospheric pressure, APCI). Parameters of chromatography-mass analysis: column-Zorbax SB-C18, 1.8 µm, 4.6 x 15 mm; solvents A) MeCN-H_2O 95 : 5, 0.1% aqueous CF_3COOH, B) 0.1% aqueous CF_3COOH; flow eluent – 3 ml / min; Injection volume – 1 µm; UV detectors – 215, 254, 285 nm; Cl at atmospheric pressure. Elemental analysis was carried out in the laboratory of analytical chemistry of the Institute of Bioorganic Chemistry and Petrochemistry of the National Academy of Sciences of Ukraine: the carbon and hydrogen content was determined by the weight method of Pregle, nitrogen by the Dumas gas meterind method, and chlorine by the mercuric method. For column chromatography, was used silica gel Merck Grade 9385, 60 A, 230-400. The commercially available reagents and solvents were used for this work, sulfonyl chloride of isoxazoles series (**3a-c)** were synthesized from corresponding bromo derivatives by the procedure [21].

General procedure for the synthesis of 4-(3-arylisoxazol-5-yl-methyl) sulfonylamides

To a solution of 0,01 mol of the corresponding sulfonyl chloride (**3a-c**) in dichloromethane in the presence of 0.01 mol of triethylamine, 0.01 mol of morpholine in 30 ml of dichloromethane was added dropwise over 0,5-1 hour at 5-10 °C. After addition, the reaction mixture was stirred for 1 hour at room temperature. After completion of the reaction, the mixture was filtered, the solvent was evaporated in vacuo, and the residue was washed with water (2 x 20 ml). Target products were purified by recrystallization from 96% ethanol.

*4-(3-Phenyl-isoxazol-5-ylmethanesulfonyl)-morpholine (**4a**).*

Yield 82%; mp 135-136 °C. ^{1}H NMR (500 MHz, $CDCl_3$) *δ 7.87-7.79* (m, 2H), 7.53-7.45(m, 3H), 6.82(s, 1H), 4.49 (s, 2H), 3.70 (d, *J* 5.5 Hz, 4H), 3.25(d, *J* 5.5 Hz, 4H). ^{13}C NMR (125 MHz, $CDCl_3$) *δ* 162.65, 160.94, 130.04, 128.61, 127.75, 126.38, 103.70, 66.10, 47.16, 45.41. MS (Cl): *m/z* 309 (MH^+ 100). Anal. calcld. for $C_{14}H_{16}N_2O_4S$: C, 54.53; H, 5.23; N, 9.08; S, 10.40. Found: C, 54.55; H, 5.20; N, 9.05; S, 10.37.

*4-(3-p-Tolyl-isoxazol-5-ylmethanesulfonyl)-morpholine (**4b**).*

Yield 85%; mp 140-141 °C. ^{1}H NMR (500 MHz, $CDCl_3$) *δ 7.73-7.75* (m, 2H), 7.32-7.27 (m, 2H), 6.79 (s, 1H), 4.48 (s, 2H), 3.71(d, *J* 5.5 Hz, 4H), 3.26 (d, *J* 5.5 Hz, 4H), 2.42 (s, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) *δ* 162.55, 160.65, 140.22, 129.25, 126.22, 124.84, 103.60, 66.06, 47.15, 45.36, 20.95. MS (Cl): *m/z* 323 (MH^+ 100). Anal. calcld. for $C_{15}H_{18}N_2O_4S$: C, 55.88; H, 5.63; N, 8.69; S, 9.95. Found: C, 55.85; H, 5.66; N, 8.71; S, 9.93.

*4-[3-(4-Methoxy-phenyl)-isoxazol-5-ylmethanesulfonyl]-morpholine (**4c**).*

Yield 83%; mp 149-150 °C. ^{1}H NMR (500 MHz, $CDCl_3$) *δ 7.75* (d, *J* 10.0 Hz, 2H), 7.00(d, *J* 10.0 Hz, 2H), 6.76 (s, 1H), 4.47 (s, 2H), 3.87 (s, 3H), 3.69 (d, *J* 5.5 Hz, 4H), 3.2869 (d, *J* 5.5 Hz, 4H). ^{13}C NMR (125 MHz, $CDCl_3$) *δ* 162.19, 160.86, 160.56, 127.77, 120.15, 113.96, 103.44, 66.05, 54.91, 47.10, 45.35. MS (Cl): *m/z* 339 (MH^+ 100). Anal. calcld. for $C_{15}H_{18}N_2O_5S$: C, 53.24; H, 5.36; N, 8.28; S, 9.48. Found: C, 53.20; H, 5.40; N, 8.25; S, 9.5

General procedure for the synthesis of for 4-(4-(3-Aryl-isoxazol-5-yl))-hepta-1,6-diene-4- sulfonylamides

To a mixture of 0.005 mol of the corresponding sulfonylamide (**4a-c**) and 0.015 mol potassium hydroxide in 20 ml of DMF, 0.015 mol of allyl bromide was added. The reaction mixture was stirred at a temperature of 65-70 °C for 4-5 hours. The solvent was removed in vacuo, the residue was washed with water, and the product was extracted with 2 x 15 ml of dichloromethane. The extract was dried with anhydrous sodium sulfate and after purification by chromatography (silica gel Merck Grade 9385, 60 A, 230-400, eluent dichloromethane) the product was isolated by evaporation of the solvent and subsequent recrystallization from 70% aqueous ethanol.

*4-[4-(3-Phenyl-isoxazol-5-yl)-hepta-1,6-diene-4-sulfonyl]-morpholine (**5a**).*

Yield 67%; mp 87-88 °C. ^{1}H NMR (500 MHz, $CDCl_3$) *δ 7.87-7.79* (m, 2H), 7.53-7.46 (m, 3H), 6.77 (s, 1H), 5.80-5.93 (m, 2H), 5.18-5.31 (m, 4H), 3.64 (s, 4H), 3.16 (s, 4H), 3.08-3.14 (m, 2H), 2.98-3.07 (m, 2H). ^{13}C NMR (125 MHz, $CDCl_3$) *δ* 168.60, 162.18, 130.34, 130.01, 128.61, 127.84, 126.32, 120.06, 103.55, 68.56, 66.62, 46.58, 35.75. MS (Cl): *m/z* 389 (MH^+ 100). Anal. calcld. for $C_{20}H_{24}N_2O_4S$: C, 61.83; H, 6.23; N, 7.21; S, 8.25. Found: C, 61.80; H, 6.25; N, 7.18; S, 8.30.

*4-[4-(3-p-Tolyl-isoxazol-5-yl)-hepta-1,6-diene-4-sulfonyl]-morpholine (**5b**).*

Yield 63%; mp 99-100 °C. ^{1}H NMR (500 MHz, $CDCl_3$) *δ 7.72* (d, *J* 10.5 Hz, 2H), *7.30* (d, *J* 10.5 Hz, 2H), 6,74 (s, 1H), 5.80-5.94 (m, 2H), 5.17-5.30 (m, 4H), 3.64 (s, 4H), 3.13-3.23 (m, 4H), 3.10-3.12 (m, 2H), 2.99-3.05 (m, 2H), 2.43 (s, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) *δ* 168.35, 162.11, 140.25, 130.36, 129.27, 126.19, 124.97, 120.01, 68.52, 66.60, 45.54, 35.72, 29.22, 20.96. MS (Cl): *m/z* 403 (MH^+ 100). Anal. calcld. for $C_{21}H_{26}N_2O_4S$: C, 62.66; H, 6.51; N, 6.96; S, 7.97. Found: C, 62.62; H, 6.55; N, 6.93; S, 8.00.

*4-{4-[3-(4-Methoxy-phenyl)-isoxazol-5-yl]-hepta-1,6-diene-4-sulfonyl}-morpholine (**5c**).*

Yield 65%; light yellow oil. ^{1}H NMR (500 MHz, $CDCl_3$) *δ 7.75* (d, *J* 5.0 Hz, 2H), *6.97* (d, *J* 5.0 Hz, 2H), 6.69 (s, 1H), 5.90-5.79 (m, 2H), 5.27-5.16 (m, 4H), 3.86 (s, 3H), 3.62 (s, 4H), 3.22-3.11 (m, 4H), 3.12-3.06 (m, 2H), 3.04-2.96 (m, 2H). ^{13}C NMR (125 MHz, $CDCl_3$) *δ* 168.68, 162.19, 161.31, 130.80, 128.19, 120.71, 120.47, 114.43, 103.75, 68.94, 67.06, 55.39, 46.97, 36.17. MS (Cl): *m/z* 419 (MH^+ 100). Anal. calcld. for $C_{21}H_{26}N_2O_5S$: C, 60.27; H, 6.26; N, 6.69; S, 7.66. Found: C, 60.30; H, 6.23; N, 6.70; S, 7.65.

General procedure for the synthesis of for 4-[1-(3-Aryl-isoxazol-5-yl)-cyclopent-3-enyl]sulfonylamides

The [Ru] catalyst (0.028-0.03 mmol, 5% mol) was added to a solution 0.6 mmol of the corresponding diallyl derivatives **5a-b** in 15 ml of dry degassed dichloromethane under a dry argon atmosphere. The mixture was kept at room temperature for 10-12 hours. After completion of the reaction, the target products were isolated from the reaction mixture by column chromatography (Merck Grade 9385, 60 A, 230-400, dichloromethane) followed by evaporation and recrystallization of the products from 70% aqueous ethanol.

*4-[1-(3-Phenyl-isoxazol-5-yl)-cyclopent-3-enesulfonyl]-morpholine (**6a**).*

Yield 75%; mp 109-110 °C. ^{1}H NMR (500 MHz, $CDCl_3$) *δ 7.79-7.87* (m, 2H), 7.44-7.53 (m, 3H), 6.84 (s, 1H), 5.75 (s, 2H), 3.65 (t, *J* 6.0 Hz, 4H), 3.46 (d, *J* 20.0 Hz, 2H), 3.28 (d, *J* 20.0 Hz, 2H), 3.19 (t, *J* 6.0 Hz, 4H). ^{13}C NMR (125 MHz, $CDCl_3$) *δ* 169.97, 162.42, 129.64, 128.57, 127.91, 127.13, 126.31, 103.03, 70.56, 66.48, 46.35, 40.77. MS (Cl): *m/z* 361 (MH^+ 100). Anal. calcld. for $C_{18}H_{20}N_2O_4S$: C, 59.98; H, 5.59; N, 7.77; S, 8.90. Found: C, 59.95; H, 5.55; N, 7.75; S, 8.90.

*4-[1-(3-p-Tolyl-isoxazol-5-yl)-cyclopent-3-enesulfonyl]-morpholine (**6b**).*

Yield 71%; mp 103-104 °C. ^{1}H NMR (500 MHz, $CDCl_3$) *δ 7.70* (d, *J* 8.0 Hz, 2H), *7.27* (d, *J* 8.0 Hz, 2H), 6.79 (s, 1H), 5.73 (s, 2H), 3.68-3.59 (m, 4H), 3.43 (d, *J* 15.5 Hz, 2H), 3.27 (d, *J* 15.5 Hz, 2H), 3.21-3.12 (m, 4H), 2.40 (s, 3H).^{13}C NMR (125 MHz, $CDCl_3$) *δ* 170.15, 162.79, 140.64, 129.70, 127.58, 126.64, 125.47, 103.42, 70.96, 66.93, 46.77, 41.21, 21.43. MS (Cl): *m/z* 375 (MH^+ 100). Anal. calcld. for $C_{19}H_{22}N_2O_4S$: C, 60.94; H, 5.92; N, 7.48; S, 8.56. Found: C, 60.90; H, 5.95; N, 7.50; S, 8.54.

Antibacterial study

The antibacterial activity of the synthesized isoxazole-containing sulfonylamides was determined by the disk-diffusion method [27] using the Mueller-Hinton agar against a number of standard (American Type Culture Collection, ATCC) and multidrug-resistant strains of gram-negative bacteria, such as *E. coli* and *A. baumannii*, and gram-positive bacterial strains of *S. aureus*. All culture strains were obtained from the collection of the Museum of Microbial Culture Collection of the Shupyk National Healthcare University of Ukraine. The microbial inoculum concentration was 1 x 10^5 colony-forming units in 1 ml (CFU/ml) of the culture liquid and was monitored by the 0.5 McFarland optical standard. The studied compounds were applied to standard paper discs (6 mm in diameter) in a volume of 0.02 ml. The inoculum was applied to Petri dishes with the appropriate nutrient medium in a volume of 0.2 ml. Incubation was carried out for 24 hours at a temperature of + 37 °C. The compound content on the disc was 10 µM. All compounds were dissolved in dimethylsulfoxide (DMSO). The studied microbial cultures were not sensitive to this solvent. The antibacterial activity of the studied compounds was determined by the diameters of the growth inhibition zones of microorganisms in mm. The experiment was repeated three times.

Acute toxicity study

The acute toxicity of the investigated compounds was evaluated by the LC_{50} indicator (the lowest concentration of compounds associated with a 50% death of test organisms within 48 hours) on the freshwater *D. magna* model according to OECD 202 for testing chemical compounds [28]. *D. magna* was kept in ventilated aquariums (pH=7.3 ± 0.3) at a temperature of 20-22 °C and a concentration of dissolved oxygen > 6.0 mg/l. Cultivation illumination was 400-600 lux with a light period of 16 ± 1 h, and 8 ± 1 h of darkness. Compounds concentrations from 0.01 mg/L to 1000 mg/L were used to determine the acute toxicity with *D. magna* neonates aged < 24 hours obtained by cultivation. Five neonates were placed in a 50 mL glass beaker containing 30 mL of the test solution according to the corresponding concentration of the studied compound. Two hours before the experiment, *D. magna* was fed with *Chlorella vulgaris* or baker's yeast suspension and was not fed during the experiment. Mortality of individuals in each glass was assessed within 48 hours. The experiment was repeated three times and the average value of LC_{50} was taken. Statistical analysis of the obtained results was performed using the Statistica 7 program. The degree of toxicity of the compound was determined according to the D.R. Passino and co-authors classification [26]. In addition, the sensitivity of *D. magna* to the reference model toxicant potassium dichromate ($K_2Cr_2O_7$) was determined.

Notes

The authors declare no conflict of interest.

Author contributions. **D. M. H.**: conceptualization, supervision, bioactivity investigation, results analysis, writing-original draft. **O. V. P.**: conceptualization, synthesis of compounds, analysis. **M. M. B**: synthesis of compounds, writing experimental section. **V. O. Y**: conceptualization, analysis. **V. V. K**: analysis results, conceptualization, writing and editing. **L. O. M**: supervision, conceptualization, results analysis, editing.

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Ізооксазоловмісні сульфонаміди як нові антибактеріальні агенти: *in silico* дослідження, синтез та *in vitro* оцінка

Д.М. Година*, О.В. Павлюк, М.М. Баран, В.О. Євдокименко, В.В. Ковалішин, Л.О. Метелиця

Інститут біоорганічної хімії та нафтохімії ім. В.П. Кухаря НАН України, Київ, Україна

Резюме: Розроблені за допомогою веб-платформи OCHEM та раніше опубліковані QSAR-моделі були використані для дослідження та розробки нових похідних ізооксазолу як антимікробних агентів. На основі створеного віртуального набору перспективних похідних ізооксазолу відібрано низку потенційних антибактеріальних агентів для синтезу та подальших досліджень. Розроблено зручну послідовність синтезу вихідних ізооксазоловмісних сульфоніламідів та препаративні методи синтезу цільових сульфоніламідів ізоксазолового ряду, зокрема з використанням реакцій метатезису із закриттям циклу. Результати *in vitro* оцінки антимікробної активності синтезованих сполук із прогнозованою високою активністю показали, що ряд ізооксазоловмісних сульфоніламідів є перспективними антимікробними засобами з широким спектром антибактеріальної дії, особливо проти мультирезистентних бактеріальних патогенів *E. coli*, *A. baumannii* та *S. aureus*. Дослідження *in vivo* гострої токсичності синтезованих сполук на біомоделі *D. magna* як відомому біосенсорі засвідчили, що більшість ізооксазоловмісних сульфоніламідів можна віднести до класу малотоксичних речовин згідно з класифікацією, розробленою Passino та Smith для гідробіонтів.

Ключові слова: QSAR; антибактеріальна активність; ізооксазол; сульфаніламід; токсичність.