Letters in Applied NanoBioScience
Open-Access Journal (ISSN: 2284-6808)

Article
Volume 13, Issue 2, 2024, 89
https://doi.org/10.33263/LIANBS132.089

Synthesis, Characterization, and Study of Anti-HPV Activity and Cell Cytotoxicity of Novel 1,3-Oxazole-4-Carbonitrile and 4-Sulfonylamide-5-Phenyl-1,3-Thiazole Derivatives *in Vitro*

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Scopus Author ID 6603929049
Received: 27.03.2023; Accepted: 30.05.2023; Published: 30.06.2024

Abstract: Five new 1,3-oxazole-4-carbonitrile and two 4-sulfonylamide-5-phenyl-1,3-thiazole derivatives were synthesized, and their activity against human papillomavirus (HPV) types 11, 16, and 18 was evaluated in C33-A cells *in vitro*. Bioassays showed that compounds 1 and 5-7 were inactive, while compounds 2 and 3 showed moderate activity against low-risk HPV11 and low cellular toxicity but were slightly selective. Only compound 4 (5-(4-benzenesulfonylpiperazine-1-sulfonyl)-2-phenyl-1,3-oxazole-4-carbonitrile) showed high activity against HPV11 similar to the reference drug (9-[2-phosphonomethoxy)ethyl]guanine) (EC_{50}=3.68 and 1.77 μM, respectively), but had worse toxicity and selectivity However, the activity and selectivity of compound 4 exceeded that of the reference drug in concentration that caused 90% inhibition of virus replication, but was more toxic. Compound 4 also showed moderate activity against high-risk HPV16 and HPV18 in half maximal effective concentration. Thus, we have shown that compound 4 is a promising structure for in-depth study in order to develop a novel drug against HPV lesions.

Keywords: 1,3-oxazole derivatives; 1,3-thiazole derivatives; anti-HPV activity; C33-A cells

1. Introduction

Human papillomaviruses (HPV) are so-called opportunistic viruses [1] that develop latent or subclinical viral infections in immunosuppressive and immunodeficient hosts, increasing the risk of various dysplasias [2]. More than 200 different genotypes of HPV have been identified that induce hyperproliferative lesions in the differentiating epithelium of the skin and mucous membranes, causing various anomalies ranging from genital warts to invasive cancer [3,4]. Of these, HPV16 and HPV18 belong to high-risk HPV types involved in developing malignant neoplasms of the genital organs, anal canal, and oropharynx [5-7]. Low-risk HPV types such as HPV11 are also associated with specific pathological conditions, including anogenital condyloma or benign genital warts, as well as recurrent laryngeal papillomatosis [8,9]. Most HPV infections resolve spontaneously over time, although in some

cases, it may persist and be a risk factor for developing malignant tumors. Despite the widespread HPV infection and the risk of developing associated malignant tumors, there is no effective drug for HPV therapy yet. Recently, Walhart *et al*. (2020) screened 1,906 FDA-approved drugs that could potentially be repurposed for HPV prevention. Pentamidine, producing inhibition of the synthesis of DNA, RNA, phospholipids, and proteins, and the GABA antagonist securinin have been shown to inhibit HPV16 ≥90.0% with <10.0% cellular cytotoxicity, which could potentially be repurposed to reduce oncogenic HPV infections [10]. However, we did not find data on their use in HPV infection in the available literature. One of the frequently used methods is the destructive surgical removal and radiation treatment of HPV foci [11,12]. The effectiveness of such treatments is limited by the high recurrence rate, especially in immunocompromised patients. To date, the development of prophylactic vaccines against HPV is one of the perspective directions in preventing human cancer induced by HPV [13]. Yet the effectiveness of prophylactic vaccines is limited by the duration of the worldwide vaccination campaign and the herd immunity period. In addition, prophylactic vaccines can only prevent HPV-associated cancers for limited types of HPV and do not work against pre-existing infections. Therefore, the clinical availability of drugs effective against HPV remains relevant [10, 14-16].

We have previously shown that some 1,3-oxazole-4-carbonitriles exhibited potent antiviral activity against HPV-11 (IC_{50} = 2-8 μM) [17]. However, these compounds were moderately and highly active against HPV11 but had no antiviral activity against HPV18. This was the basis for their further modification in order to increase activity against high-risk HPV.

In order to study the anti-HPV activity of new azole derivatives, we applied *in silico* modeling, virtual screening, synthesis, and experimental trials. The present study is a preliminary study of the anti-HPV activity of novel 1,3-oxazole and 1,3-thiazole derivatives synthesized at the Department of Chemistry of Bioactive Nitrogen-Containing Heterocyclic Bases of the V. P. Kukhar Institute of Bioorganic Chemistry and Petrochemistry, NAS of Ukraine. The antiviral activity of the compounds was evaluated at the UAB Department of Biochemistry and Molecular Genetics (USA).

2. Materials and Methods

2.1. In silico modeling.

A set of 98 compounds and their bioactivities against HPV-16 were taken from the ChEMBL database [18]. The activity of compounds was expressed as half-maximal inhibitory concentration (IC_{50}) ranging from 0.005 to 100 μM. The data were divided into high active (50 compounds with IC_{50} < 1 μM) and low active molecules (48 compounds with $IC_{50} \geq 1$ μM). The 16 compounds from the initial dataset were randomly selected to form an external independent test set, while the remaining 80 compounds were used as the training set.

2.1.1. Machine learning methods. Optimization of the number of descriptors.

In this work, we performed data analysis and selection of descriptors using our Batch Pruning Algorithm (BPA) [19]. This algorithm is a combination of the Associative Neural Network (ASNN) method [20] and the Self-Organized Map (SOM) of Kohonen [21]. The use of a combination of these algorithms results in the identification of an optimum set of descriptors in a fraction of the time compared to linear selection methods for QSAR modeling [19]. The final model was built by ASNN. An Associative Neural Network unites an ensemble

of feed-forward neural networks trained with a backpropagation algorithm (BPNN) [22] and *k*-nearest neighbors method (*k*-NN) [23]. ASNN uses the *k*-NN approach in the space of ensemble residuals.

The neural network ensemble represents All compounds as vectors of neural network predictions. Correlation between such vectors is used by the nearest neighbor method as a measure of distance between the analyzed cases. Therefore, this approach improves prediction by correcting the bias of the neural network ensemble [20]. The individual BPNN models had five neurons in the hidden layer and were trained by SuperSAB [24]. The number of input neurons corresponded to the number of analyzed descriptors. The neural network weight coefficients were initialized with random values within [-0.5; +0.5] for each network in the ensemble. A bias neuron was also presented in both the input and hidden layers of nodes. The ensemble included 100 neural networks. The possibility of over-fitting the data has been rigorously controlled through cross-validation techniques [25].

2.1.2. Descriptors.

For this study, we used the MOPAC [26] package embedded in the OCHEM server for descriptor calculation [27]. MOPAC2016B™ is a semi-empirical software package for predicting chemical properties and modeling chemical reactions. MOPAC-derived descriptors include molecule-based descriptors (molecular descriptors) and atom-based descriptors (atomic descriptors). More details about the descriptors can be found elsewhere [26]. A simple Pearson's pairwise correlation method was used as a filtering method for the descriptor set before it was used as an input for the machine-learning method.

2.1.3. Statistical coefficients.

The performance of classification QSAR models can be evaluated on the basis of many statistical coefficients such as sensitivity (*SN*), specificity (*SP*), and overall accuracy (*Ac*). Sensitivity and specificity (or the true positive/negative rates) are calculated as:

$$SN = TP / (TP + FN) \quad (1)$$

$$SP = TN / (TN + FP) \quad (2)$$

Here *TP*, *FP*, *TN* and *FN* denote the number of true positives, false positives, true negatives and false negatives, respectively.

Overall accuracy Ac is the percentage of correctly classified samples. For binary classification accuracy can be calculated as follows:

$$Ac=(TP+TN)/(TP+FP+TN+FN) \quad (3)$$

Since the number of active and inactive compounds was approximately the same in data set, we have used Ac as to measure the predictive performance of classification models.

2.2. Chemistry.

All chemicals and solvents for the synthetic work were acquired from commercial sources and used without further purification. The reaction progress was monitored using the TLC method. Melting points were determined using a FisherJohns apparatus. IR spectra were recorded on a Vertex-70 spectrometer from KBr pellets. ^{1}H and ^{13}C NMR spectra were recorded on Bruker Avance DRX 500 spectrometer (400 and 126 MHz, respectively) in DMSO-d_6 or $CF_3C(O)OD$, taking its residual protons signal as a standard.Chromato–mass spectra were recorded using an Agilent 1100 Series liquid chromatography-mass spectrometry

system equipped with a diode array and an Agilent LC\MSD SL mass-selective detector. Parameters of chromatography-mass spectral analysis: column Zorbax SB-C18, 1.8 μm, 4.6×15 mm; solvents: A, MeCN–H_2O, 95:5, 0.1% CF_3COOH; B, Combustion elemental analysis was performed by hand in the V.P. Kukhar Institute of Bioorganic Chemistry and Petrochemistry analytical laboratory. The carbon and hydrogen contents were determined using the Pregl gravimetric method, nitrogen – using Duma's gasometrical micromethod, and sulfur – by the Scheininger titrimetric method.

Chemicals and reagents were purchased from commercially available sources. Boc-substituted piperazine IV, phenylsulfonyl chloride, Lawesson's reagent were purchased from Aldrich. 3-Phenyl-5-(piperidin-4-yl)-1,2,4-oxadiazoles Ia,b were synthesized by the previously described method [28].

5-(4-(3-(4-Methoxyphenyl)-1,2,4-oxadiazol-5-yl)piperidin-1-yl)-2-(p-tolyl)-1,3-oxazole-4-carbonitrile 1. A mixture of 0.001 mol of N-(2,2-dichloro-1-cyanovinyl)-4-methylbenzamide IIa [29], 0.0011 mol of 3-(4-methoxyphenyl)-5-(piperidin-4-yl)-1,2,4-oxadiazole Ia and 0.002 mol of triethylamine in 10 ml of anhydrous tetrahydrofuran was stirred on a magnetic stirrer at 20-25 °C for 48 hours. The precipitate was filtered, the solvent was removed in vacuo, and the residue was treated with water, filtered, dried, and purified by recrystallization from ethanol. Yield 63% (0.31 g). Light brown powder, mp 180-182 °C. IR (KBr, ν_{max}, cm^{-1}): 844, 1173, 1254, 1363, 1585, 1610, 2207 (CN), 2939. ^{1}H NMR (400 MHz, DMSO-d_6), δ: 1.98 (q, J_{HH} = 8 Hz, 2H), 2.26 (d, J_{HH} = 8 Hz, 2H), 2.36 (s, 3H), 3.45 (t, J_{HH} = 12 Hz, 3H), 3.83 (s, 3H), 4.06 (d, J_{HH} = 16 Hz, 2H), 7.10 (d, J_{HH} = 8 Hz, 2H), 7.32 (d, J_{HH} = 8 Hz, 2H), 7.77 (d, J_{HH} = 8 Hz, 2H), 7.95 (d, J_{HH} = 8 Hz, 2H). ^{13}C NMR (126 MHz, DMSO-d_6), δ: 181.0, 167.1, 161.6, 160.0, 150.5, 140.3, 129.6, 128.6, 125.3, 122.9, 118.4, 116.2, 114.6, 85.7, 55.3, 45.5, 32.5, 27.8, 21.0. MS (ES), m/z : 442.2 $[M+H]^+$. Anal. calcd for $C_{25}H_{23}N_5O_3$, %: C, 68.01; H, 5.25; N, 15.86. Found, %: C, 68.11; H, 5.29; N, 16.12.

5-((4-(3-Phenyl-1,2,4-oxadiazol-5-yl)piperidin-1-yl)sulfonyl)-2-(p-tolyl)-1,3-oxazole-4-carbonitrile 2. A mixture of 0.001 mol of 4-cyano-2-(p-tolyl)-1,3-oxazole-5-sulfonyl chloride IIIa [30], 0.001 mol of 3-phenyl-5-(piperidin-4-yl)-1,2,4-oxadiazole Ib, and 0.001 mol of triethylamine in 50 ml of anhydrous dioxane was refluxed for 2 hours then left for 12 hours at 20-25 °C. The mixture was treated with water, formed a precipitate, filtered, dried, and purified by recrystallization from acetonitrile. Yield 67% (0.35 g). White powder, mp 224-226 °C. IR (KBr, ν_{max}, cm^{-1}): 573, 598, 723, 933, 1163, 1383, 1497, 1566, 1615, 2252 (CN), 2934. ^{1}H NMR (400 MHz, DMSO-d_6), δ: 1.90 (q, J_{HH} = 8 Hz, 2H), 2.29 (d, J_{HH} = 12 Hz, 2H), 2.41 (s, 3H), 3.22-3.28 (m, 2H), 3.84 (d, J_{HH} = 12 Hz, 2H), 7.44 (d, J_{HH} = 8 Hz, 2H), 7.51-7.60 (m, 3H), 7.92-7.98 (m, 4H). ^{13}C NMR (126 MHz, DMSO-d_6), δ: 181.2, 167.4, 163.7, 150.4, 143.6, 131.5, 130.1, 129.2, 127.4, 126.9, 126.1, 121.5, 117.5, 110.8, 44.6, 32.0, 28.0, 21.2. MS (ES), m/z : 476.2 $[M+H]^+$. Anal. calcd for $C_{24}H_{21}N_5O_4S$, %: C, 60.62; H, 4.45; N, 14.73; S, 6.74. Found, %: C, 60.51; H, 4.38; N, 14.97; S, 6.94.

Methyl 5-((4-(4-cyano-2-phenyloxazol-5-yl)piperazin-1-yl)sulfonyl)-2-(p-tolyl)-1,3-oxazole-4-carboxylate 3. A mixture of 0.01 mol of N-(2,2-dichloro-1-cyanovinyl)benzamide IIb [29], 0.011 mol of tert-butyl piperazine-1-carboxylate IV, and 0.02 mol of triethylamine in 50 ml of anhydrous tetrahydrofuran was stirred on a magnetic stirrer at 20-25 °C for 48 hours. The mixture was treated with water, fprmed precipitate was filtered off, dried, and purified by recrystallization from ethanol to obtain tert-butyl 4-(4-cyano-2-phenyl-1,3-oxazol-5-yl)piperazine-1-carboxylate V.

Gaseous HCl was passed through a solution of 0.005 mol of tert-butyl 4-(4-cyano-2-phenyl-1,3-oxazole-5-yl)piperazine-1-carboxylate V for 0.5 hours. The mixture was left at 20-25 °C for 12 hours. The precipitate was filtered, washed with anhydrous hexane, dried, and the formed 2-phenyl-5-(piperazin-1-yl)-1,3-oxazole-4-carbonitrile hydrochloride was used for the next stage.

The mixture of 0.001 mol of methyl 5-(chlorosulfonyl)-2-(p-tolyl)-1,3-oxazole-4-carboxylate VI [31], 0.001 mol of 2-phenyl-5-(piperazin-1-yl)-1,3-oxazole-4-carbonitrile hydrochloride formed at the previous stage, and 0.002 mol of triethylamine in anhydrous dioxane (30 ml) was boiled for 4 hours, left at 20-25 °C for 12 hours, the formed residue was treated with water, filtered, dried, and formed compound 3 was purified by recrystallization from acetonitrile. Yield 69% (0.37 g). White powder, mp 160-165 °C. IR (KBr, ν_{max}, cm^{-1}): 602.73, 704.95, 956.65, 1148.55 (SO_2), 1178.45, 1371.32 (SO_2), 1634.59, 1733.92 (C=O), 2209.35 (CN), 2922.02, 3455.31. ^{1}H NMR (400 MHz, DMSO-d_6), δ: 1.07 (s, 3H), 2.42 (s, 3H), 3.59 (s, 2H), 3.78 (s, 2H), 3.94 (s, 2H), 4.35 (br s, 2H), 7.43-7.51 (m, 6H), 7.88-7.95 (m, 4H). ^{13}C NMR (126 MHz, DMSO-d_6), δ: 162.3, 160.3, 160.2, 150.9, 145.8, 143.3, 135.4, 130.9, 130.4, 129.5, 127.5, 125.8, 122.7, 116.2, 86.7, 53.3, 46.1, 45.0, 21.6. MS (ES), m/z : 534.2 $[M+H]^+$. Anal. calcd for $C_{26}H_{23}N_5O_6S$, %: C, 58.53; H, 4.35; N, 13.13; S, 6.01. Found, %: C, 58.50; H, 4.30; N, 13.43; S, 6.34.

Synthesis of bissulfonylamides 4 and 5: Synthesis of tert-butyl 4-((4-cyano-2-aryl-1,3-oxazol-5-yl)sulfonyl)piperazine-1-carboxylates VIIa,b. A mixture of 0.01 mol of 4-cyano-2-aryl-1,3-oxazole-5-sulfonyl chloride IIIa or IIIb [30], 0.01 mol of triethylamine and 0.01 mol of tert-butyl piperazine-1-carboxylate IV was refluxed for 4 hours, left at 20-25 °C for 12 hours, the formed residue was treated with water, filtered, dried, and formed tert-butyl 4-((4-cyano-2-aryl-1,3-oxazol-5-yl)sulfonyl)piperazine-1-carboxylate VIIa or VIIb was purified by recrystallization from ethanol.

Gaseous HCl was passed through a solution of 0.005 mol of tert-butyl 4-((4-cyano-2-aryl-1,3-oxazol-5-yl)sulfonyl)piperazine-1-carboxylate VIIa or VIIb for 0.5 hours and left at 20-25 °C for 12 hours. The formed precipitate was filtered off, washed with anhydrous hexane, dried, and the resulting 2-aryl-5-(piperazin-1-ylsulfonyl)-1,3-oxazole-4-carbonitrile hydrochloride was used for the next stage.

2-Phenyl-5-((4-(phenylsulfonyl)piperazin-1-yl)sulfonyl)-1,3-oxazole-4-carbonitrile 4. To a solution of 0.001 mol of 2-phenyl-5-(piperazin-1-ylsulfonyl)-1,3-oxazole-4-carbonitrile hydrochloride, formed in the previous step, in anhydrous dioxane (30 ml) 0.002 mol of triethylamine and 0.001 mol of the corresponding sulfonyl chloride RSO_2Cl were added, the mixture was boiled for 2 hours, left at 20-25 °C for 12 hours, the residue was treated with water, filtered, dried, and compound 4 was purified by recrystallization from ethanol. Yield 60% (0.28 g). White powder, mp > 250 °C. IR (KBr, ν_{max}, cm^{-1}): 639.80, 762.02, 944.10, 1091.94, 1166.94 (SO_2), 1267.10, 1380.90 (SO_2), 1498.68, 1548.52, 1618.38, 2254.60 (CN), 2924.80. ^{1}H NMR (400 MHz, DMSO-d_6), δ: 3.07 (s, 4H), 3.48 (s, 4H), 7.64-7.71 (m, 8H), 8.04 (s, 2H). ^{13}C NMR (126 MHz, DMSO-d_6), δ: 163.7, 158.5, 150.1, 134.8, 133.4, 133.1, 129.5, 129.4, 127.4, 127.3, 124.3, 117.8, 110.6, 45.3, 44.7. MS (ES), m/z : 459.4 $[M+H]^+$. Anal. calcd for $C_{20}H_{18}N_4O_5S_2$, %: C, 52.39; H, 3.96; N, 12.22; S, 13.98. Found, %: C, 52.30; H, 3.87; N, 12.45; S, 13.67.

5,5'-(Piperazine-1,4-disulfonyl)bis(2-(p-tolyl)oxazole-4-carbonitrile) 5. To a mixture of 0.001 mol of 5-(piperazin-1-ylsulfonyl)-2-(p-tolyl)-1,3-oxazole-4-carbonitrile hydrochloride, formed in the previous step, in anhydrous dioxane (30 ml) 0.002 mol of

triethylamine and 0.001 mol of 4-cyano-2-(p-tolyl)-1,3-oxazole-5-sulfonyl chloride IIIa [30] were added, the mixture was boiled for 2 hours, left at 20-25 °C for 12 hours, the residue was treated with water, filtered, dried, and compound 5 was purified by recrystallization from MeCN/DMFA (1:1). Yield 65% (0.38 g). White powder, mp > 250 °C. IR (KBr, ν_{max}, cm^{-1}): 600.8, 730.02, 944.11, 1166.88 (SO_2), 1178.45, 1380.97 (SO_2), 1497.65, 1555.52, 1615.31, 2254.68 (CN), 2926.84, 3436.99. ^{1}H NMR (400 MHz, $CF_3C(O)OD$), δ: 0.36 (s, 3H), 1.23 (s, 3H), 2.39 (s, 3H), 3.66 (s, 3H), 7.32 (d, J_{HH} = 8 Hz, 2H), 7.85 (d, J_{HH} = 8 Hz, 2H). MS (ES), m/z : 577.0 $[M+H]^+$. Anal. calcd for $C_{26}H_{22}N_6O_6S_2$, %: C, 53.97; H, 3.83; N, 14.52; S, 11.08. Found, %: C, 53.90; H, 3.97; N, 14.67; S, 11.35.

General procedure for the synthesis of 5-phenyl-1,3-thiazole-4-sulfonamide derivatives 6,7 [32]. A mixture of 89.2 g (0.25 mol) of ethyl 2-((1-(benzylthio)-2-oxo-2-phenylethyl)amino)-2-oxoacetate VIII and 101 g (0.25 mol) of Lawesson's reagent (RL) in 200 ml of anhydrous dioxane was refluxed for 8 h. Then the mixture was kept at 20-25 °C for 12 h. The solvent was removed in a vacuum. To the residue, 100 ml of 5% NaOH aqueous solution was added. The reaction product was extracted from the resulting oil with chloroform (2×100 ml), dried, the solvent was removed in a vacuum, and the residue was dissolved in 100 ml of ethanol.

To ethanol solution containing ethyl 4-(benzylsulfanyl)-5-phenyl-1,3-thiazole-2-carboxylate (0.2 mol) formed at the previous stage was added 100 ml of 10% NaOH aqueous solution, the mixture boiled for 1 h and left at 20-25 °C for 2 h. Hydrochloric acid (conc.) was added to the solution to pH 2, and the reaction mixture was boiled for 1 h. After cooling, the solvent was removed in a vacuum, and 200 ml of water was added to the residue. The product was extracted with chloroform (2×100 ml), and the extract was dried.

The solvent was removed in a vacuum, the residue was dissolved in 80 ml of acetic acid (95%), and gaseous Cl_2 was bubbled through the solution containing benzyl-5-phenyl-1,3-thiazole-4-ylsulfide for 0.5 h, maintaining the temperature of the reaction mixture within 0-5 °C. Next, the solution was kept at 0-5 °C for 2 h and poured into ice (500 g). The formed precipitate was filtered off and dried in a vacuum. 5-phenyl-1,3-thiazole-4-sulfonyl chloride IX was purified by recrystallization from toluene.

To a solution of 0.259 g (0.001 mol) of 1,3-thiazole-4-sulfonyl chloride IX in 30 ml of anhydrous dioxane 0.001 mol of the corresponding dialkylamine and 0.14 ml (0.001 mol) of triethylamine were added. The mixture was boiled for 2 h and left at 20-25 °C for 12 h. The solvent was removed in a vacuum, and 50 ml of water was added to the residue. The solid was filtered off, washed with water, and purified by crystallization from ethanol.

5-Phenyl-4-((4-phenylpiperazin-1-yl)sulfonyl)-1,3-thiazole 6. Yield 81% (0.312 g). Colorless crystals, mp 145-147 °C. IR (KBr, ν_{max}, cm^{-1}): 549.69, 585.37, 699.16, 754.13, 947, 1150.48 (SO_2), 1241.13, 1349.14 (SO_2), 1505.37, 1598.91, 2822.69, 3082.1. ^{1}H NMR (400 MHz, DMSO-d_6), δ: 3.19-3.20 (m, 4H), 3.36 (m, 4H), 6.82 (t, d, J_{HH} = 8 Hz, 1H), 6.94 (d, J_{HH} = 8 Hz, 2H), 7.22 (d, J_{HH} = 8 Hz, 2H), 7.48-7.49 (m, 3H), 7.57-7.59 (m, 2H). ^{13}C NMR (126 MHz, DMSO-d_6), δ: 154.7, 150.5, 145.6, 143.4, 130.2, 129.5, 129.0, 128.4, 128.2, 119.6, 116.1, 48.3, 46.0. MS (ES), m/z : 386.2 $[M+H]^+$. Anal. calcd for $C_{19}H_{19}N_3O_2S_2$, %: C, 59.20; H, 4.97; N, 10.90; S, 16.63. Found, %: C, 59.11; H, 4.89; N, 10.73; S, 16.83.

N-(1,1-Dioxidotetrahydrothiophen-3-yl)-N-(4-methoxybenzyl)-5-phenyl-1,3-thiazole-4-sulfonamide 7. Yield 74% (0.35 g). White powder, mp 174-176 °C. IR (KBr, ν_{max}, cm^{-1}): 543.9, 596.94, 755.1, 843.82, 1042.48, 1139.88 (SO_2), 1250.7, 1289.35, 1349.14 (SO_2), 1425.33, 1514.05, 1613.38, 2825.58, 2975.06, 3081.14. ^{1}H NMR (400 MHz, DMSO-d_6), δ:

1.06 (t, J_{HH} = 8 Hz, 2H), 1.30 (t, J_{HH} = 8 Hz, 3H), 2.01-2.16 (m, 2H), 3.45 (t, J_{HH} = 8 Hz, 2H), 4.00 (t, J_{HH} = 8 Hz, 2H), 4.37-4.58 (m, 2H), 4.76-4.78 (m, 1H), 6.87 (d, J_{HH} = 8 Hz, 2H), 7.24 (d, J_{HH} = 8 Hz, 2H), 7.48-7.54 (m, 5H), 9.28 (s, 1H). ^{13}C NMR (126 MHz, DMSO-d_6), δ: 157.8, 155.0, 146.5, 142.9, 130.1, 129.6, 129.6, 128.4, 128.3, 114.2, 62.9, 56.0, 54.0, 51.4, 50.4, 47.4, 27.5, 18.5, 14.6. MS (ES), m/z : 493.0 $[M–H]^-$. Anal. calcd for $C_{21}H_{22}N_2O_5S_3$, %: C, 52.70; H, 4.63; N, 5.85; S, 20.10. Found, %: C, 52.61; H, 4.54; N, 5.98; S, 20.23.

2.3. Antiviral and cytotoxicity assays.

Primary assay: An HPV11 replicon assay was developed and expressed the essential E1 and E2 proteins from the native promoter. The E2 origin binding protein interacts with the virus origin of replication and recruits the E1 replicative helicase, which unwinds the DNA and helps to recruit the cellular DNA replication machinery (including DNA polymerases, type I and type II topoisomerases, DNA ligase, single-stranded DNA binding proteins, proliferating cell nuclear antigen). The replication complex then drives the amplification of the replicon, which can be assessed by the expression of a destabilized NanoLuc reporter gene carried on the replicon. In this assay, the replicon (pMP619) is transfected into C-33 A cells grown as monolayers in 384-well plates. At 48 h post-transfection, the enzymatic activity of the destabilized NanoLuc reporter is assessed with the NanoGlo reagent. The reference compound for this assay is 9-[2-phosphonomethoxy)ethyl]guanine (PMEG) [33].

Secondary Assay: Secondary HPV genome replication assays are performed by methods similar to HPV11 but are done in a plasmid system utilizing additional HPV types (HPV16 and HPV18) with additional replicates to ensure estimates of EC_{50} and EC_{90} values are more precise [17,33].

In both analyzes, control and drug concentration ranges are 0.048-150 μM, and the final concentration of compound-derived DMSO was maintained at 1% (v/v) in all samples. In control samples (with no compound), an appropriate volume of pure DMSO was added for a final concentration of 1%.

3. Results and Discussion

3.1. QSAR modeling of Anti-HPV Activity for ChEMBL data.

Before creating QSAR models, the numerical values were discretized as described in Materials and Methods. The initial set of 98 compounds was split into training (80 compounds) and test (16 compounds) sets.

Table 1. Prediction accuracy of the obtained classification models.

Model	Set	Compounds	Descriptors	Sn [1]	Sp [2]	Correct [3] (Ac, %) [4]
1	Training	80	26	0.780	0.782	62 (77.5)
	Test	16		0.55	0.607	9 (56.3)
2	Training	80	7	0.8070	0.82	65 (81.2)
	Test	16		0.70	0.83	12 (75)

[1] Sn: the sensitivity.
[2] Sp: the specificity.
[3] Correct: the number of correctly predicted compounds.
[4] Ac: the overall prediction accuracy in %.

The initial number of descriptors was submitted to an additional reduction procedure, as follows: descriptors with constant values were removed, and a pairwise correlation analysis

was then performed, whereby a given descriptor was eliminated if its correlation coefficient with another descriptor was equal to or greater than 0.99. As a result of this process, 26 descriptors were selected.

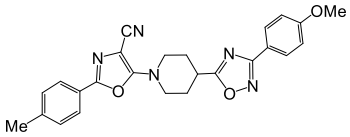
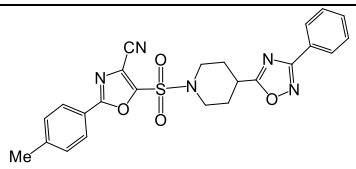
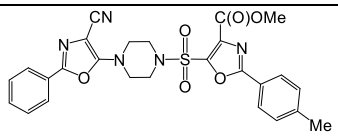
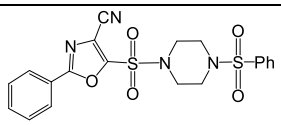
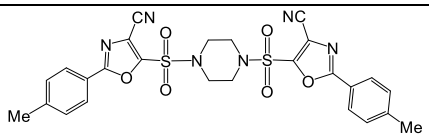
Data analysis was done using both a complete set of descriptors and BPA. Consequently, two different QSAR models were built for an initial dataset. Table 1 summarizes the statistical coefficients obtained for each model.

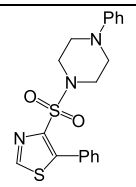
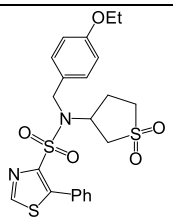
In the first step, the classification QSAR model was developed using all descriptors. The performances of the individual models for the validation set were used to compare the predictive ability of the developed models. The total accuracy (*Ac*) for the training set was 77.5%. The 16 compounds in the test set were predicted with an accuracy of 56.3% (see Table 1, model 1).

Next, the importance of the descriptors for the observed activity was evaluated by BPA software. Most descriptors were detected as non-significant and removed by BPA. As a result, only 7 descriptors were chosen from 26. New ASNN model was developed with the final selection of descriptors. The total accuracy (Ac) for the training set was 81.2%, and 75 % for the external test set (see Table 1, model 2). The final QSAR model was then applied to a virtual data set to assist in screening promising compounds against HPV-16.

The 2D structures of new compounds were built using MarvinSketch template libraries [34] and consisted of residues of phenyl**,** azoles, and others (Table 2). The activity of all virtual compounds was predicted using the proposed QSAR model. Five compounds were predicted as highly active, with the most confident predictions (>80%). The remaining two compounds (**1** and **3**) were predicted to be weakly active against HPV-16 (see Table 2).

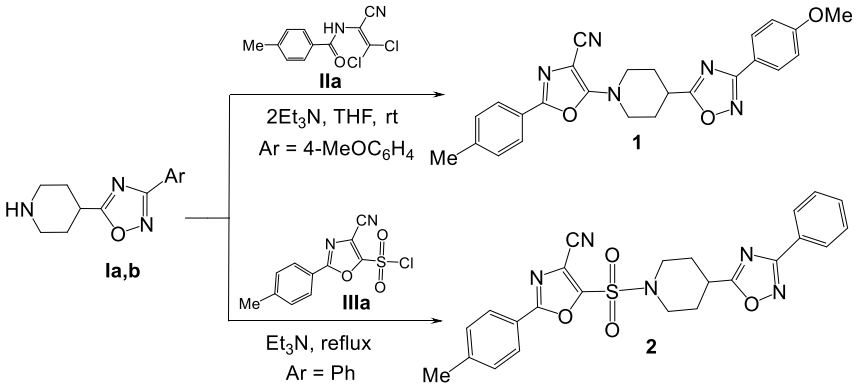
Table 2. The chemical structures of seven compounds and their anti-HPV-16 activity calculated by the classification models.

Number	Chemical name	Chemical structure	Predicted activity	Estimated accuracy (%)
1	5-(4-(3-(4-methoxyphenyl)-1,2,4-oxadiazol-5-yl)piperidin-1-yl)-2-(*p*-tolyl)-1,3-oxazole-4-carbonitrile		low	98
2	5-((4-(3-phenyl-1,2,4-oxadiazol-5-yl)piperidin-1-yl)sulfonyl)-2-(*p*-tolyl)-1,3-oxazole-4-carbonitrile		high	88
3	methyl 5-((4-(4-cyano-2-phenyloxazol-5-yl)piperazin-1-yl)sulfonyl)-2-(*p*-tolyl)-1,3-oxazole-4-carboxylate		low	89
4	2-phenyl-5-((4-(phenylsulfonyl)piperazin-1-yl)sulfonyl)-1,3-oxazole-4-carbonitrile		high	99
5	5,5'-(piperazine-1,4-disulfonyl)bis(2-(*p*-tolyl)-1,3-oxazole-4-carbonitrile)		high	81

Number	Chemical name	Chemical structure	Predicted activity	Estimated accuracy (%)
6	5-phenyl-4-((4-phenylpiperazin-1-yl)sulfonyl)-1,3-thiazole		high	99
7	*N*-(1,1-dioxidotetrahydrothiophen-3-yl)-*N*-(4-ethoxybenzyl)-5-phenyl-1,3-thiazole-4-sulfonamide		high	99

3.2. Chemistry.

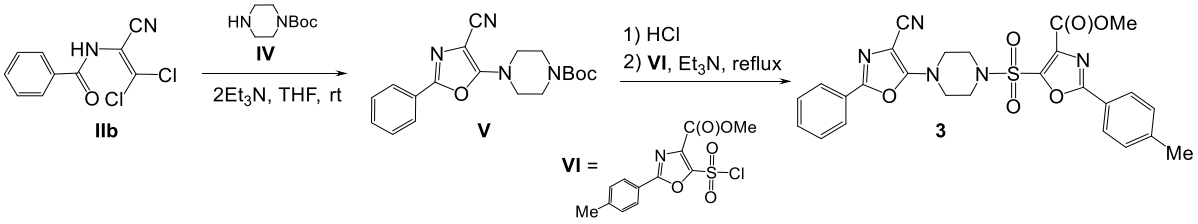
For the synthesis of 5-(1-(1,3-oxazol-5-yl)piperidin-4-yl)-1,2,4-oxadiazole 1 we used the reaction of 5-(piperidine-4-yl)-1,2,4-oxadiazole Ib with N-(2,2-dichloro-1-cyanovinyl)-4-methylbenzamide IIa [29]. This reaction occurs regioselectively at a temperature of 20-25 °C in anhydrous tetrahydrofuran in the presence of triethylamine. 5-((4-(3-Phenyl-1,2,4-oxadiazol-5-yl)piperidin-1-yl)sulfonyl)-2-(p-tolyl)-1,3-oxazole-4-carbonitrile 2 was obtained by the reaction of compound Ia with 4-cyano-2-(p-tolyl)-1,3-oxazole-5-sulfonyl chloride IIIa [30] (Scheme 1).



Ar = 4-$MeOC_6H_4$ (**Ia**), Ph (**Ib**)

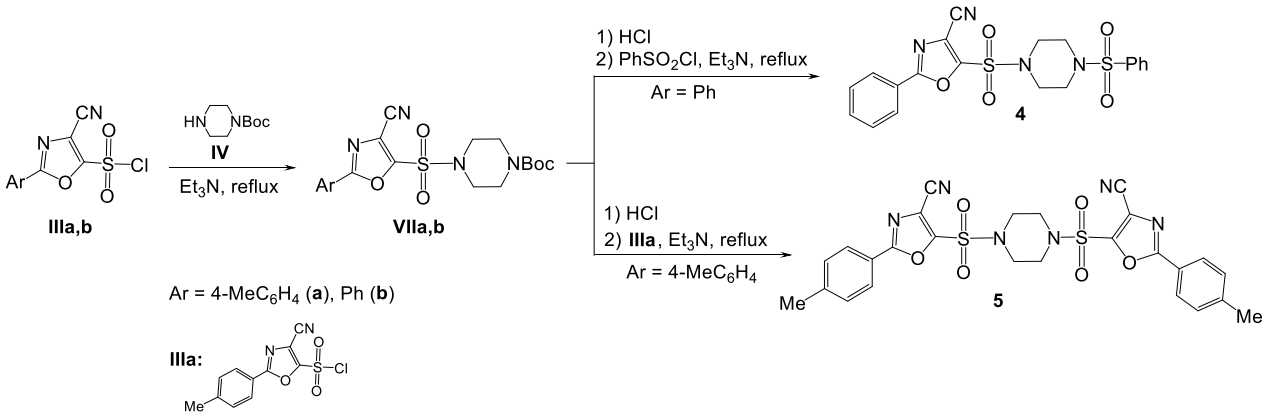
Scheme 1. Synthesis of 5-(1-(1,3-oxazol-5-yl)piperidin-4-yl)-1,2,4-oxadiazoles **1** and **2**.

The interaction of N-(2,2-dichloro-1-cyanovinyl)benzamide IIb with boc-substituted piperazine IV gave the tert-butyl 4-(4-cyano-2-phenyloxazol-5-yl)piperazine-1-carboxylate V. After removal of the boc-protection and reaction with methyl 5-(chlorosulfonyl)-2-(p-tolyl)-1,3-oxazole-4-carboxylate VI [31] it was obtained methyl 5-((4-(4-cyano-2-phenyl-1,3-oxazol-5-yl)piperazin-1-yl)sulfonyl)-2-(p-tolyl)-1,3-oxazole-4-carboxylate 3 (Scheme 2).



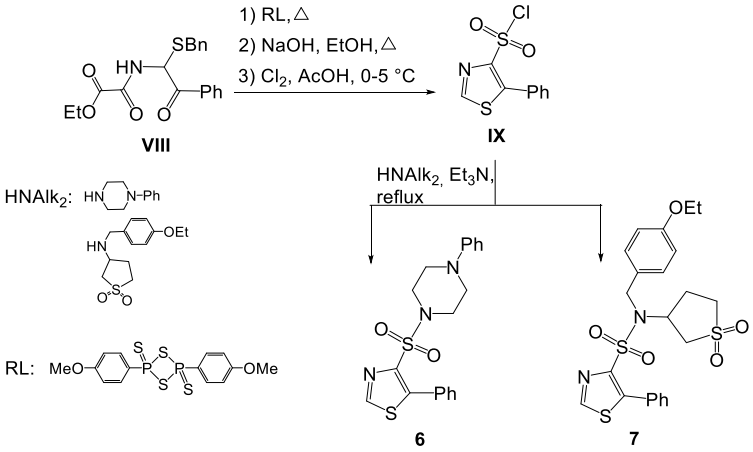
Scheme 2. Synthesis of methyl 5-((4-(4-cyano-2-phenyl-1,3-oxazol-5-yl)piperazin-1-yl)sulfonyl)-2-(*p*-tolyl)-1,3-oxazole-4-carboxylate **3**.

For the synthesis of bissulfonylamides 4 and 5 it was used tert-butyl 4-((4-cyano-2-aryloxazol-5-yl)sulfonyl)piperazine-1-carboxylates VIIa,b obtained from sulfonylchlorides IIIa,b [30] and Boc-substituted piperazine IV. Compounds VIIa,b after removal of the boc-protection were reacted with benzenesulfonyl chloride or 4-cyano-2-(p-tolyl)-1,3-oxazole-5-sulfonyl chloride IIIa [30] led to substances 4 and 5 (Scheme 3).



Scheme 3. Synthesis of bissulfonylamides **4** and **5**.

Thionization of ethyl 2-((1-(benzylthio)-2-oxo-2-phenylethyl)amino)-2-oxoacetate VIII with Lawesson's reagent resulted in cyclization to the 1,3-thiazole derivative, which was decarboxylated by the NaOH treatment. Further chlorination leads to 5-phenyl-1,3-thiazole-4-sulfonyl chloride IX, which is converted into corresponding sulfonylamides 6 and 7 [32] (Scheme 4).



Scheme 4. Synthesis of 1,3-thiazole-4-sulfonylamides 6 and 7.

Data of synthesized novel 1,3-oxazoles 1-5 and 1,3-thiazoles 6,7 are presented in the Materials and Methods section and Supplementary materials. IR, ^{1}H NMR, and ^{13}C NMR, chromato-mass spectra, and elemental analysis reliably confirm the structure of the obtained compounds 1-7.

3.3. Biology.

Bioassays showed (Table 3) that compounds 1 and 5-7 were inactive, while 2 and 3 demonstrated moderate and equal activity against low-risk HPV11, although 2, unlike 3, was non-toxic, and both were slightly selective. Only compound 4 (5-(4-Benzenesulfonyl-

piperazine-1-sulfonyl)-2-phenyl-1,3-oxazole-4-carbonitrile) showed anti-HPV11 activity near reference drug 9-[2-phosphono-methoxy)ethyl]guanine (EC_{50} = 3.68 and 1.77 μM, respectively) but worse to the latter in terms of toxicity and selectivity. However, the activity of compound 4 exceeded that of the reference drug in concentration, causing 90% inhibition of virus replication but was more toxic.

Table 3. The activity of synthesized derivatives against HPV amplification in C33-A cells.

Compound	EC_{50} [1]	EC_{90} [1]	CC_{50} [1]	SI_{50}	SI_{90}
HPV11, strain HE 611260.1					
1	>150.00	>150.00	>150.00	1	1
2	21.00	>150.00	>150.00	>7	1
3	17.21	>30.00	103.04	6	<3
4	3.68	20.44	88.10	24	4
5	>30.00	>30.00	105.44	<4	<4
6	>150.00	>150.00	>150.00	1	1
7	>150.00	>150.00	>150.00	1	1
9-[2-phosphono-methoxy)ethyl]guanine	**1.77**	**82.94**	**>150.00**	**>85**	**>2**
HPV16, strain KP212151.1					
4	9.24	60.40	>100.00	>11	>2
9-[2-phosphono-methoxy)ethyl]guanine	**1.59**	**8.28**	**>250.00**	**>158**	**>30**
HPV18, strain KC470230					
4	10.23	>100.00	>100.00	>10	>10
9-[2-phosphono-methoxy)ethyl]guanine	**1.77**	**11.43**	**>250.00**	**>141**	**>22**

[1] Data termed in μM. EC_{50} – compound concentration that reduces viral replication by 50%; EC_{90} – compound concentration that reduces viral replication by 90%; CC_{50} – compound concentration that reduces cell viability by 50%; SI_{90} – CC_{50}/EC_{90}; SI_{50} – CC_{50}/EC_{50}

It follows from the data presented that the replacement of the benzenesulfonylpiperazine-1-sulfonyl group in compound 4 at position 5 of 1,3-oxazole with piperazine-1-sulfonyl]-2-p-tolyl-1,3-oxazole-4-methylcarboxyl group (3) or with 3-phenyl-[1,2,4]oxadiazol-5-ylpiperidin-1-sulfonyl group with simultaneous methylation of 2-phenyl in the para-position (2) led to an increase in EC_{50} by about 5 times, and in addition, virus replication was not reduced by these derivatives in concentrations < 150 μM. Other modifications of compound 4 in the 5th position of 1,3-oxazole (1 and 5), as well as derivatives of 1,3-thiazole (6 and 7) gave inactive substances.

Compound 4, which demonstrated higher activity against HPV11 among the tested compounds, was taken to study its activity against high-risk HPV16 and HPV18. The results obtained showed that this compound exhibited equally effective moderate activity against HPV16 and HPV18.

Compound 4 in EC_{50} showed moderate and equal activity against both high-risk HPVs, but in EC_{90}, it showed higher activity against HPV16, and in both cases, it was lower than that of the reference drug. This compound is an analog of the previously synthesized 4-Cyano-2-phenyl-1,3-oxazole-5-sulfonamide carbonitrile [17], in which the sulfonamide in the 5th position of the 1,3-oxazole was replaced by 4-benzenesulfonylpiperazine-1-sulfonyl groups. As follows from the data presented in this work, this substitution led to an increase in the activity of the obtained derivative against HPV11 at a concentration that reduces virus replication by 50% (3.68 and 8.44 μM, respectively) with the same activity in EC_{90}, and the appearance of activity against HPV18 (the starting compound was not tested against HPV16), albeit with increased toxicity (CC_{50} = 88 and >100 μM, respectively). However, it should be

taken into account that the antiviral activity of these compounds was determined on different cell lines (normal HEK293 and cancerous C-33A), which could affect the data obtained.

Severson *et al*. (2008) classified compound selectivity with an SI_{50} value of < 4 as not selective, SI_{50} = 4-9 as slightly selective, SI_{50} = 10-49 as moderately selective, and a SI_{50} value >50 as highly selective. As a rule, only compounds with SI_{50} of ≥ 10 are considered promising for further study [35]. According to the above classification, compounds 2 and 3 showed weak selectivity against HPV11, and only compound 4 of the tested compounds demonstrated moderate selectivity both against low- and high-risk HPV types, meeting the requirements for an in-depth study of its effectiveness against HPV lesions. Thus, this compound, which has an acceptable SI_{50}, can serve as the basis for structural modification in reducing toxicity and increasing activity to create more effective drugs to combat HPV infection.

4. Conclusions

In this study, a QSAR modeling approach employing Artificial Neural Networks (ANN) was applied to make *in silico* predictions of the anti-HPV activity of newly synthesized compounds. The accuracy of this method was based on the statistical averaging of predictions over ensembles of ANN. The Bath Pruning Algorithm was used to select descriptors that determined the anti-HPV activity. Derived QSAR model resulted in a good predictive ability and robustness.

Five new 1,3-oxazole-4-carbonitrile derivatives and two 4-sulfanilamide-5-phenyl-1,3-thiazole derivatives have been synthesized, and their activity against HPV types 11, 16, and 18 have been evaluated in C33-A cells *in vitro*. From them, only compound 4 showed high activity and met the requirements for an in-depth study of its activity against HPV11, which allows us to consider this compound a promising compound for further functional modification and construction of new derivatives with an improved pharmacological profile against HPV lesions.

Funding

This research was funded by THE PROGRAM OF THE NAS OF UKRAINE NO. 6541230 “SUPPORT FOR THE DEVELOPMENT OF PRIORITY AREAS OF SCIENTIFIC RESEARCH” (project No. 6.4/2-2023).

Acknowledgments

These studies were funded in whole or in part with Federal funds from the National Institute of Allergy and Infectious Diseases, National Institutes of Health, Department of Health and Human Services, under Contract No. HHSN75N93019D00016 (SHJ). We would like to thank Enamine Ltd for the material and technical support. Y. V. is grateful to INSA Rouen Normandie and the Région Normandie for financial support.

Conflicts of Interest

The authors declare no conflict of interest

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Supplementary materials

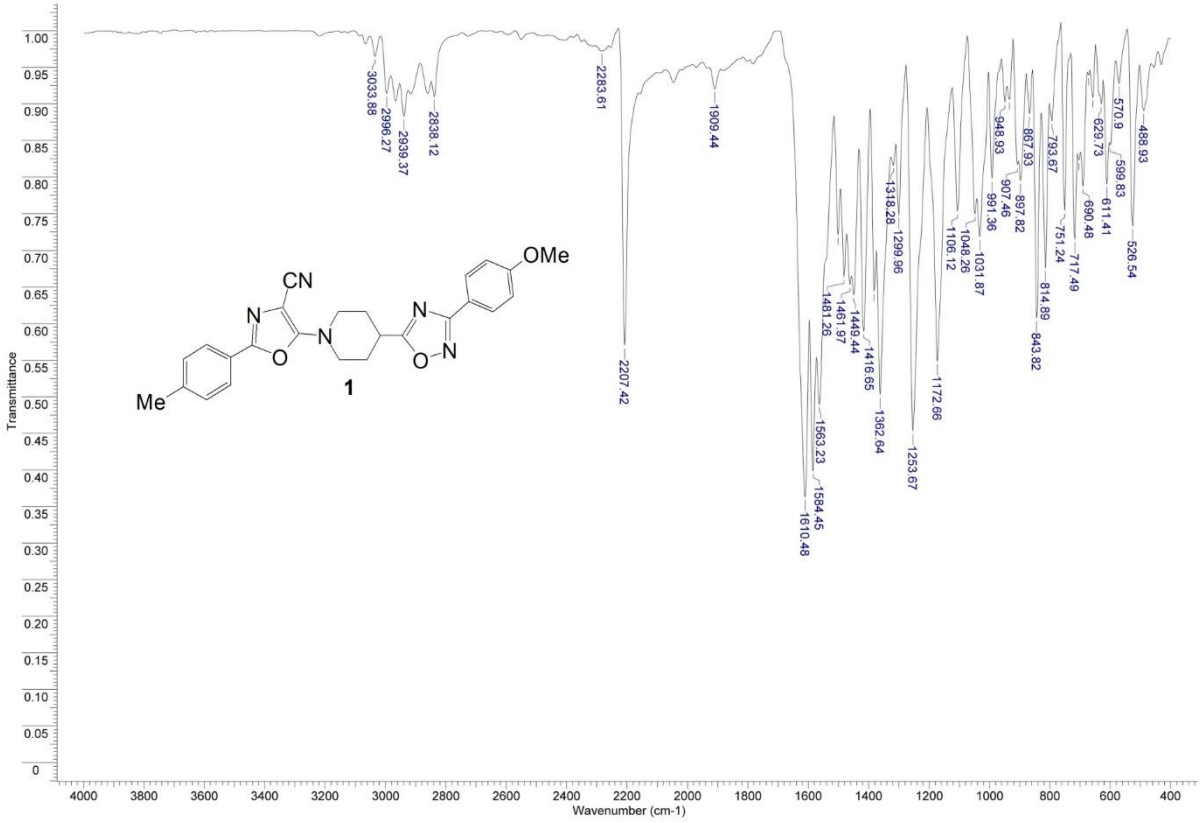

Fig. S1. IR spectrum of 5-(4-(3-(4-methoxyphenyl)-1,2,4-oxadiazol-5-yl)piperidin-1-yl)-2-(*p*-tolyl)-1,3-oxazole-4-carbonitrile **1**.

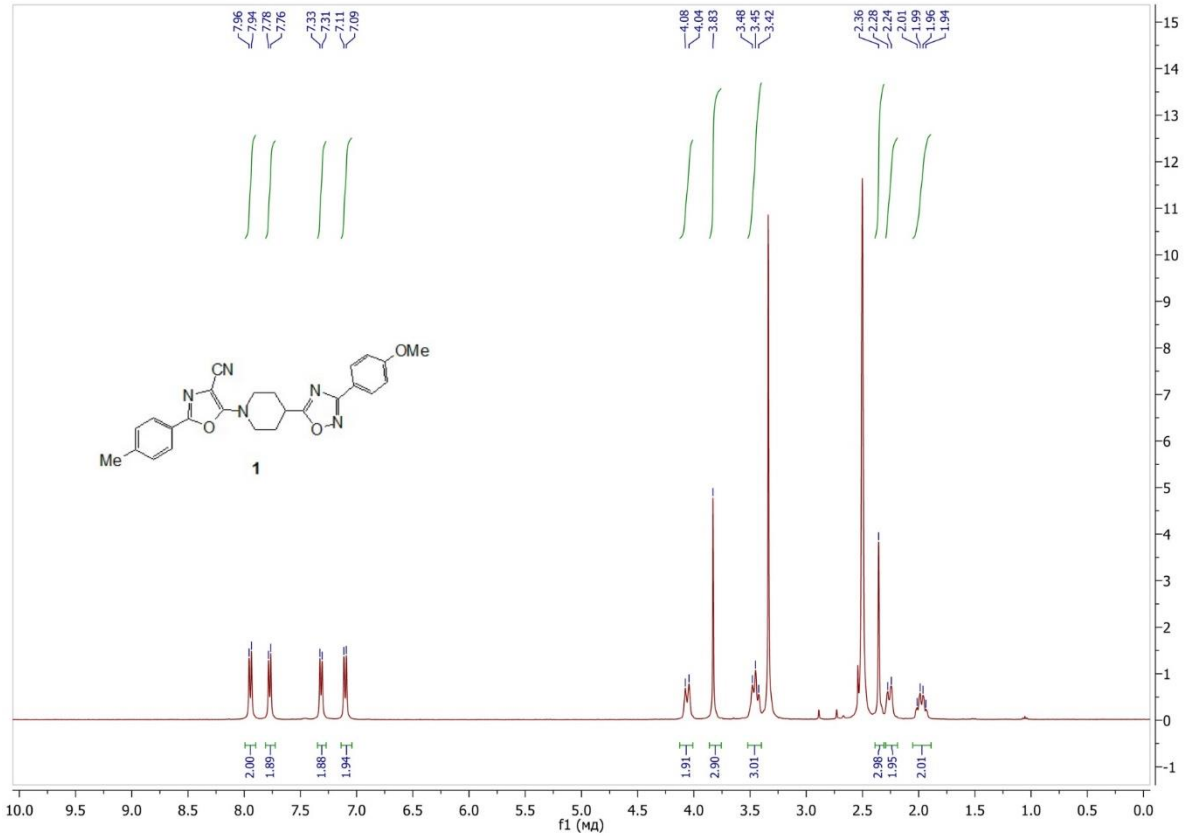

Fig. S2. [1]H NMR spectrum of 5-(4-(3-(4-methoxyphenyl)-1,2,4-oxadiazol-5-yl)piperidin-1-yl)-2-(*p*-tolyl)-1,3-oxazole-4-carbonitrile **1**.

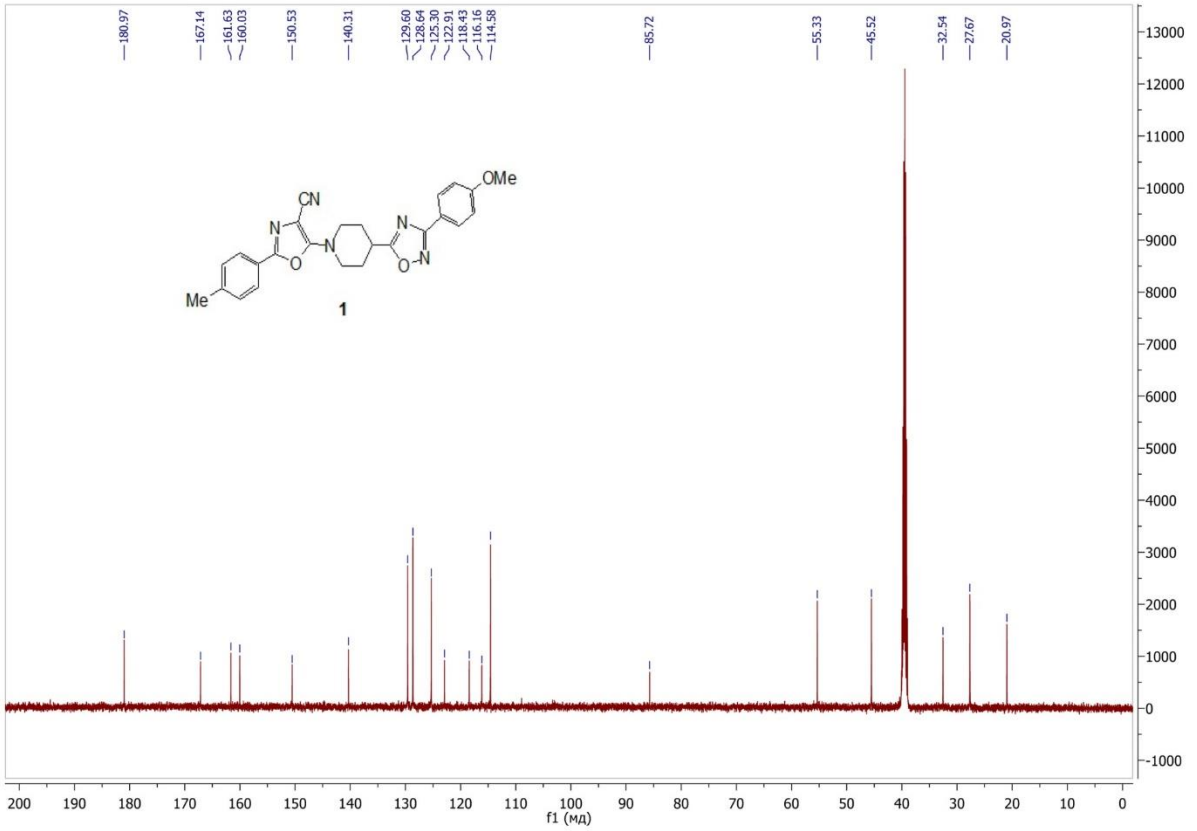

Fig. S3. [13]C NMR spectrum of 5-(4-(3-(4-methoxyphenyl)-1,2,4-oxadiazol-5-yl)piperidin-1-yl)-2-(*p*-tolyl)-1,3-oxazole-4-carbonitrile **1**.

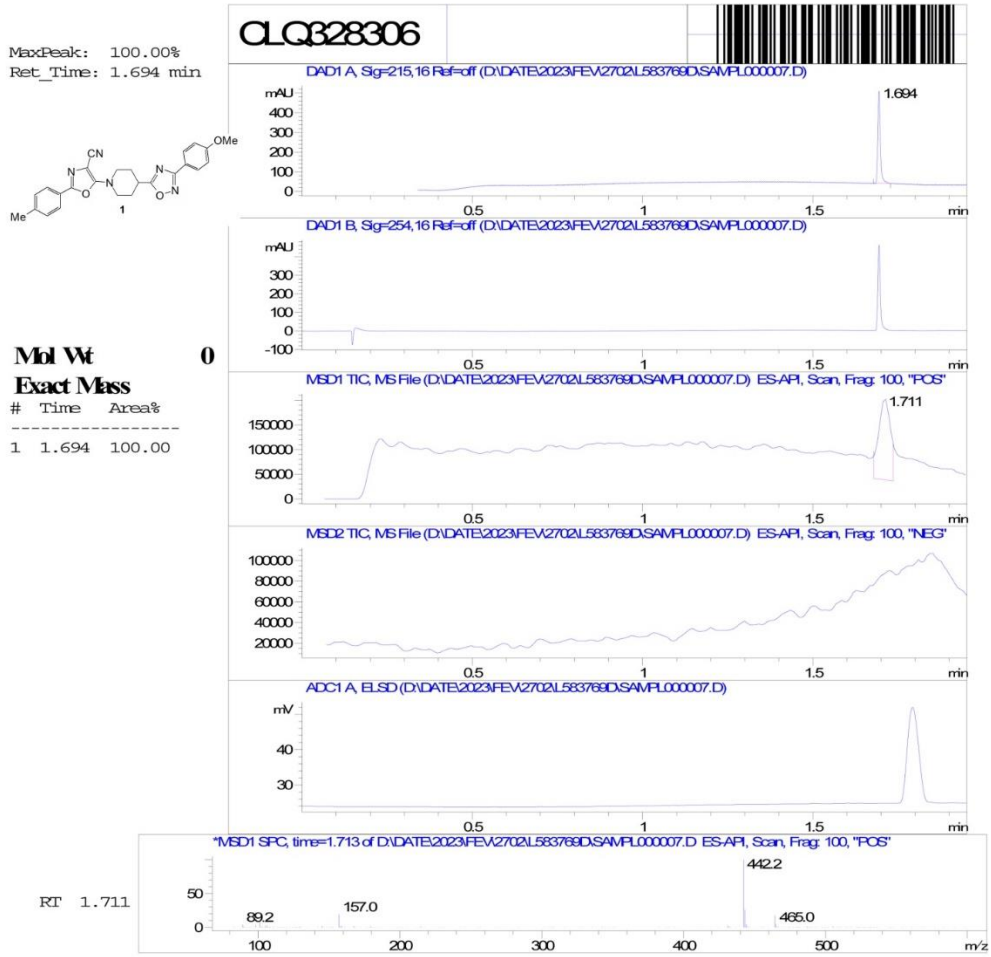

Fig. S4. LSMS spectrum of 5-(4-(3-(4-methoxyphenyl)-1,2,4-oxadiazol-5-yl)piperidin-1-yl)-2-(*p*-tolyl)-1,3-oxazole-4-carbonitrile **1**.

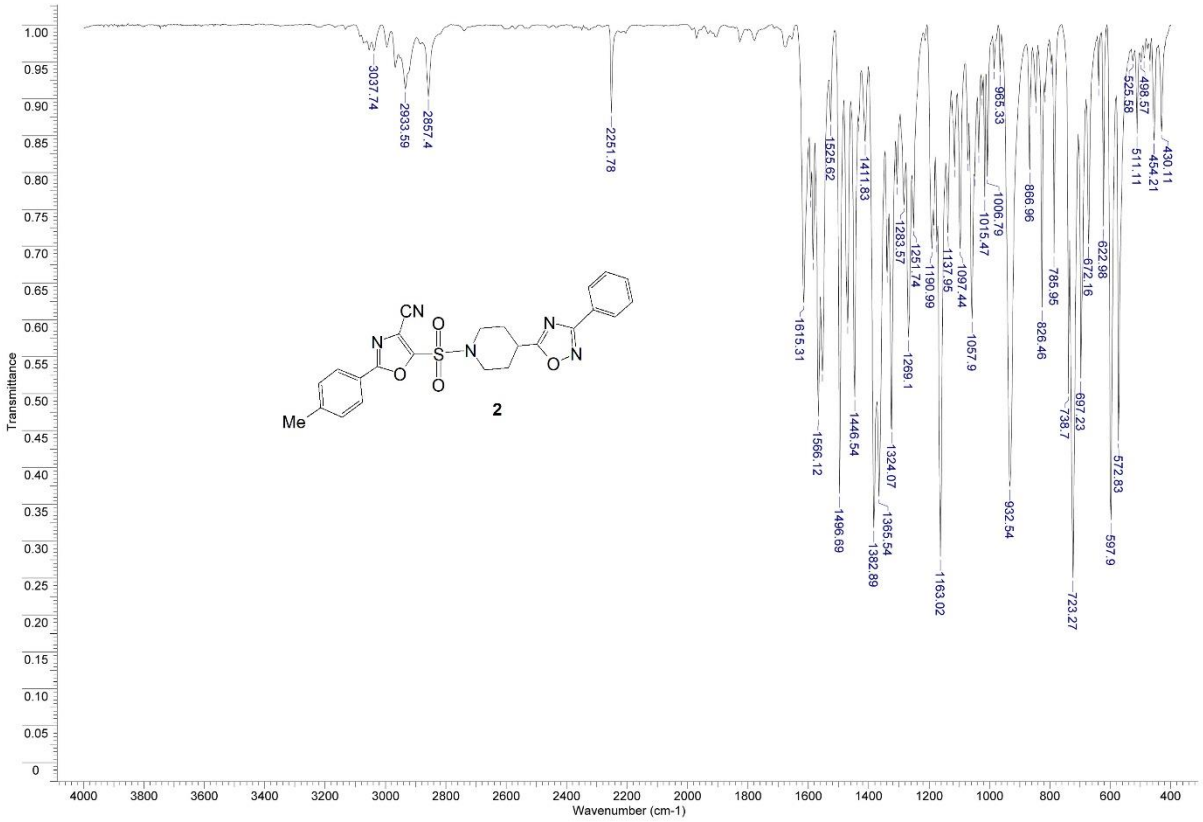

Fig. S5. IR spectrum of 5-((4-(3-phenyl-1,2,4-oxadiazol-5-yl)piperidin-1-yl)sulfonyl)-2-(*p*-tolyl)-1,3-oxazole-4-carbonitrile **2**.

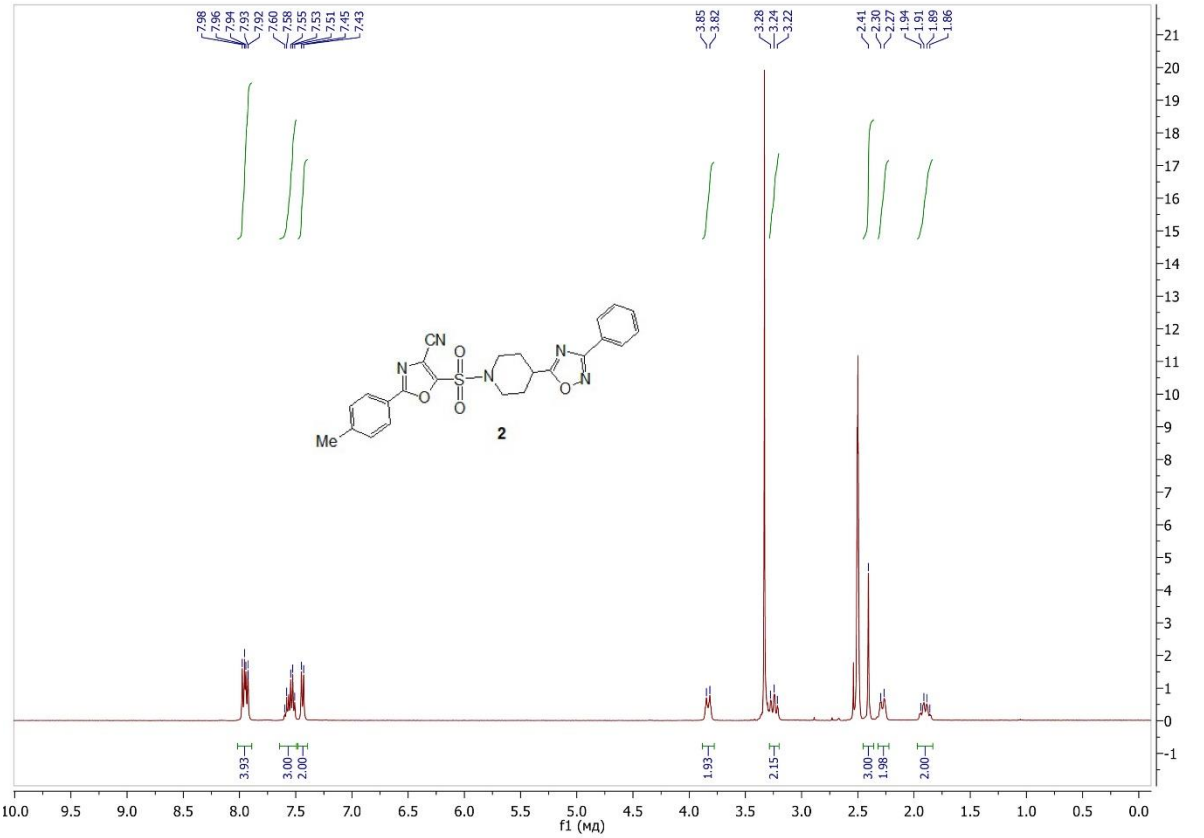

Fig. S6. ^{1}H NMR spectrum of 5-((4-(3-phenyl-1,2,4-oxadiazol-5-yl)piperidin-1-yl)sulfonyl)-2-(*p*-tolyl)-1,3-oxazole-4-carbonitrile **2**.

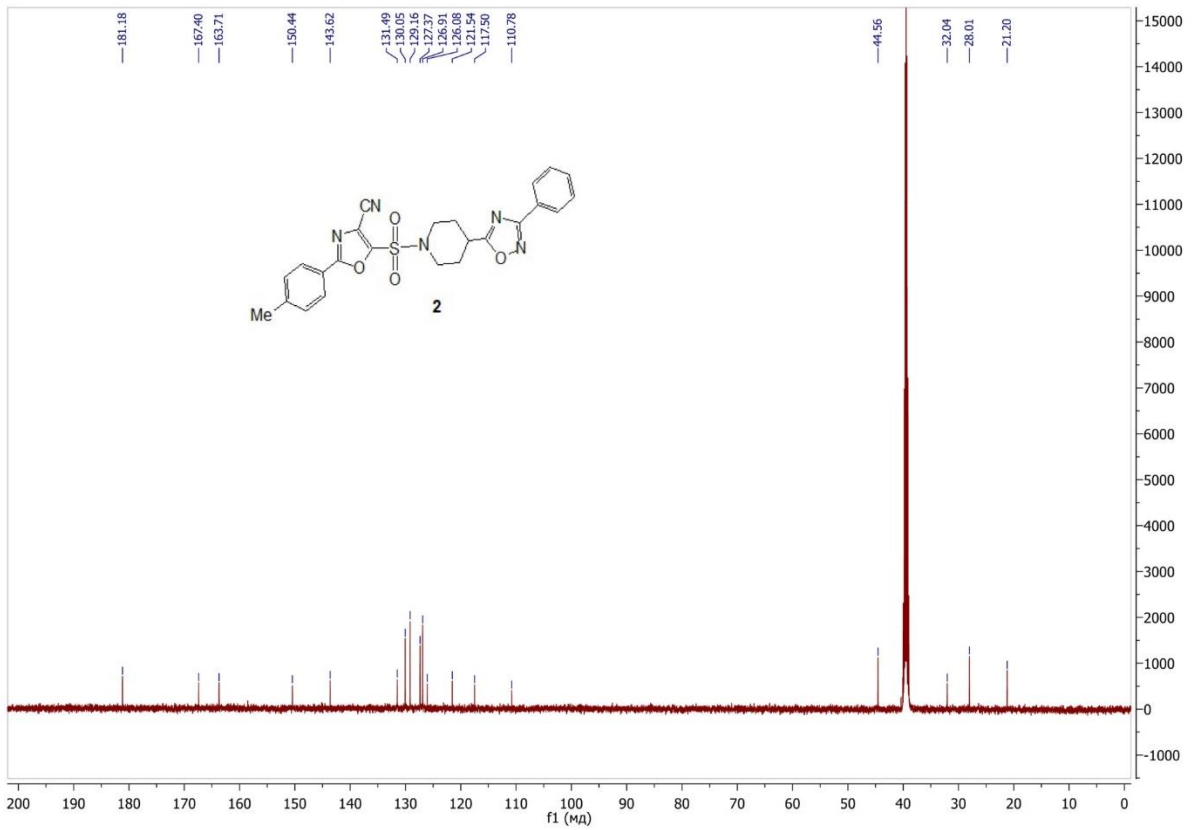

Fig. S7. ^{13}C NMR spectrum of 5-((4-(3-phenyl-1,2,4-oxadiazol-5-yl)piperidin-1-yl)sulfonyl)-2-(*p*-tolyl)-1,3-oxazole-4-carbonitrile **2**.

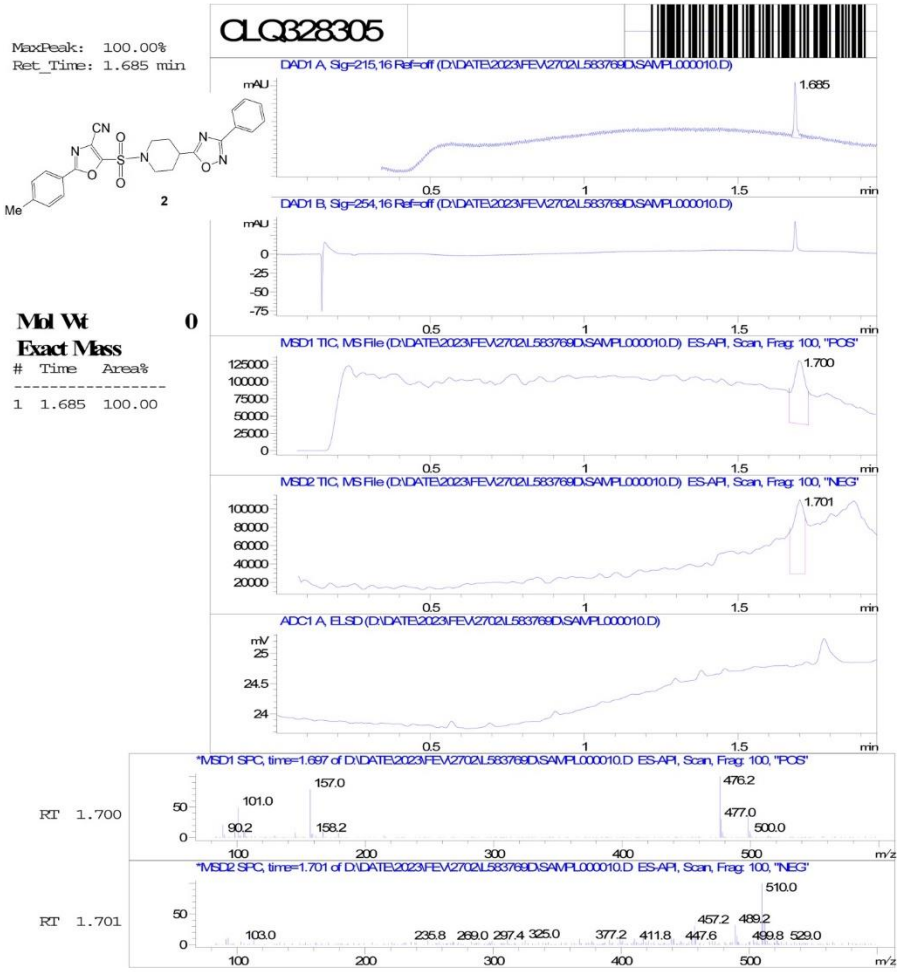

Fig. S8. LSMS spectrum of 5-((4-(3-phenyl-1,2,4-oxadiazol-5-yl)piperidin-1-yl)sulfonyl)-2-(*p*-tolyl)-1,3-oxazole-4-carbonitrile **2**.

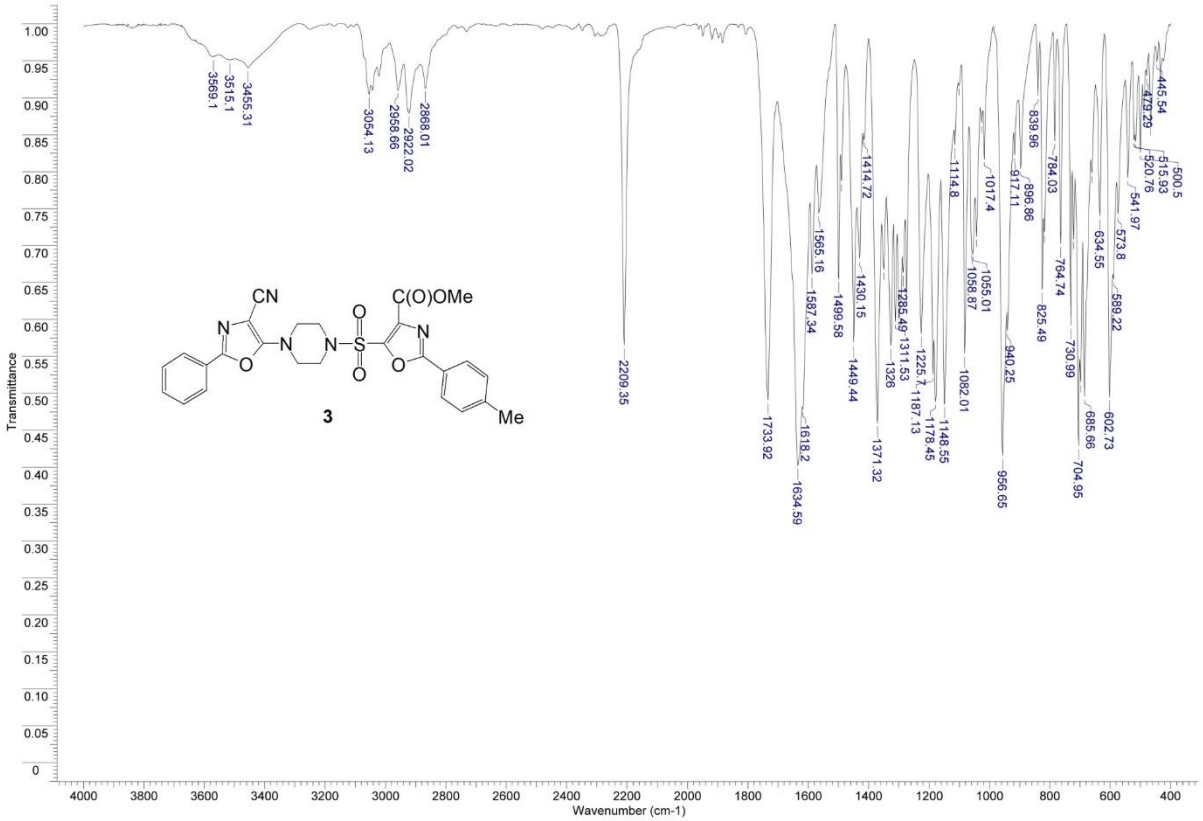

Fig. S9. IR spectrum of methyl 5-((4-(4-cyano-2-phenyloxazol-5-yl)piperazin-1-yl)sulfonyl)-2-(*p*-tolyl)-1,3-oxazole-4-carboxylate **3**.

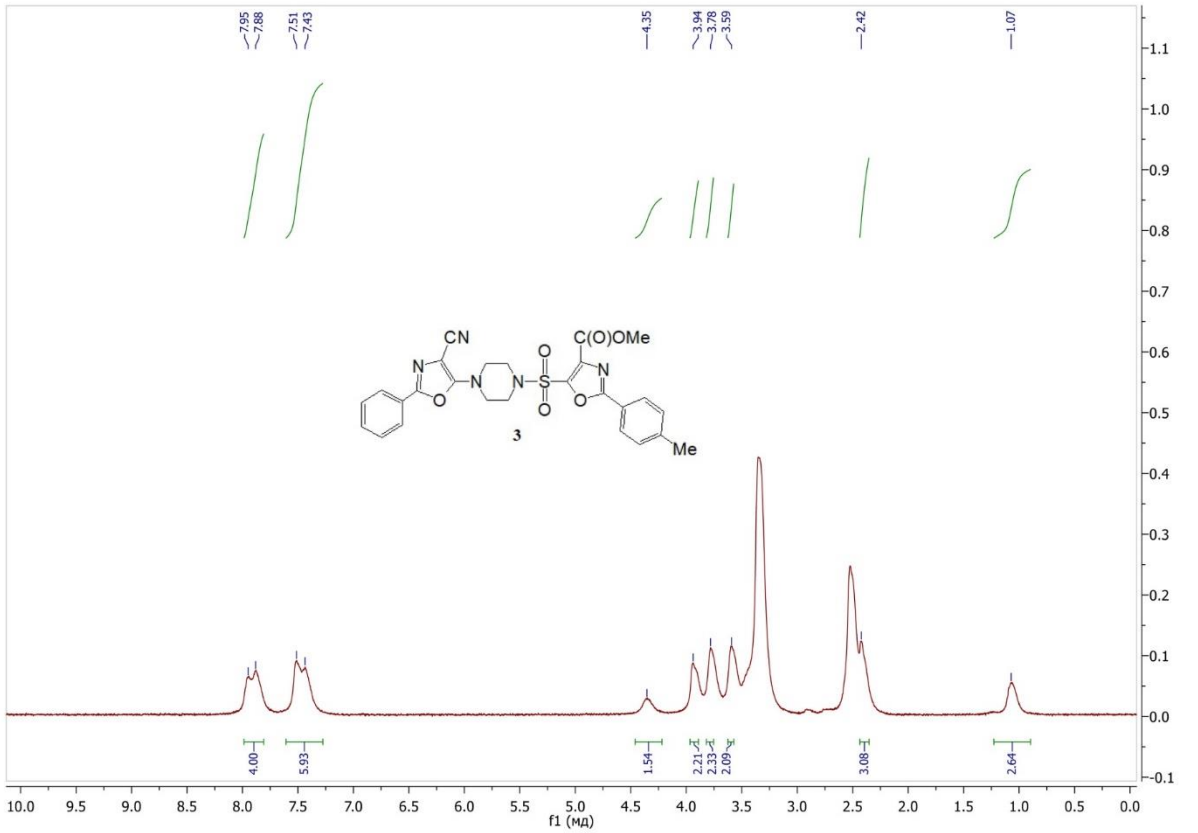

Fig. S10. ^{1}H NMR spectrum of methyl 5-((4-(4-cyano-2-phenyloxazol-5-yl)piperazin-1-yl)sulfonyl)-2-(*p*-tolyl)-1,3-oxazole-4-carboxylate **3**.

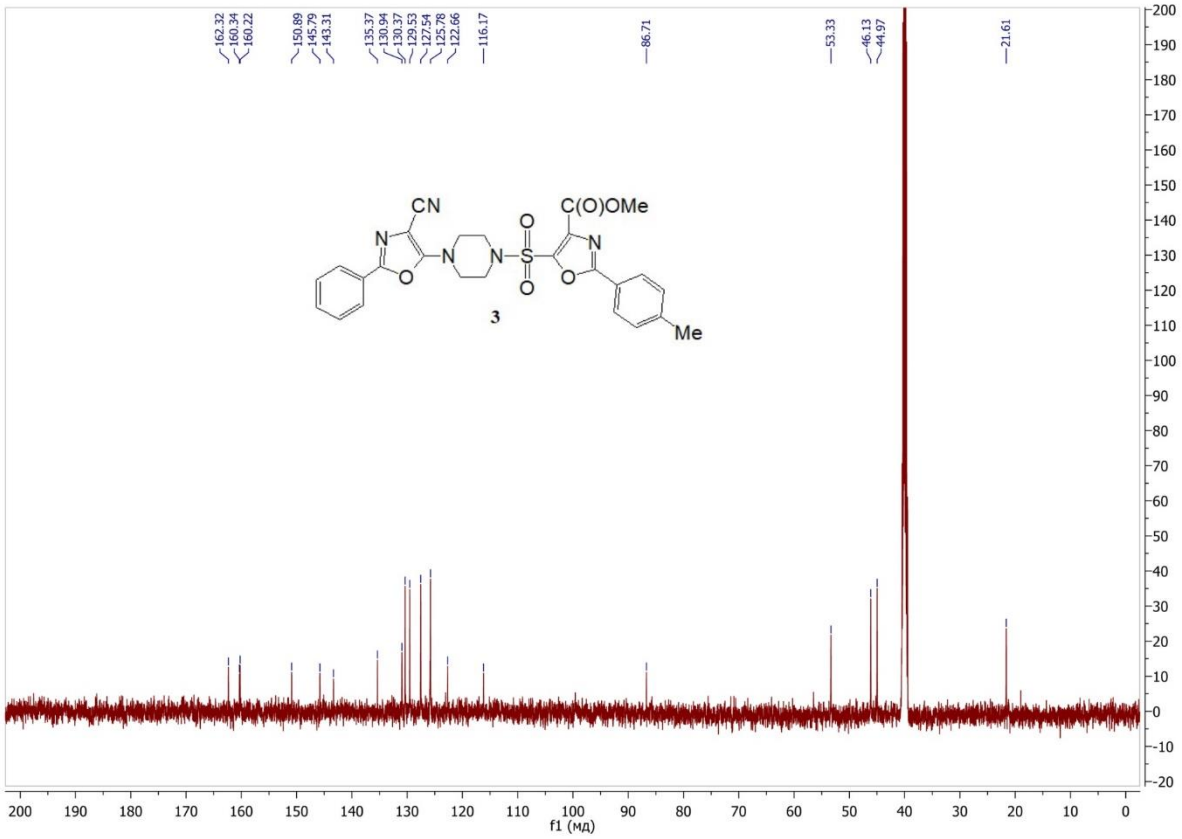

Fig. S11. ^{13}C NMR spectrum of methyl 5-((4-(4-cyano-2-phenyloxazol-5-yl)piperazin-1-yl)sulfonyl)-2-(*p*-tolyl)-1,3-oxazole-4-carboxylate **3**.

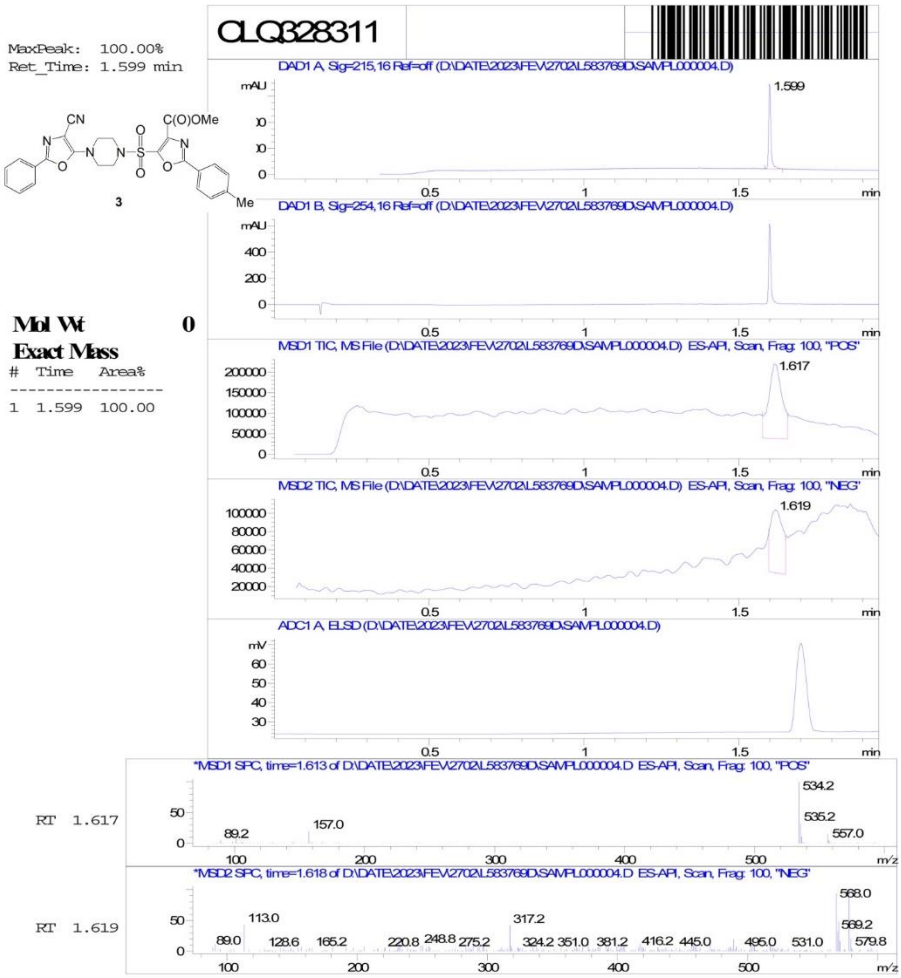

Fig. S12. LSMS spectrum of methyl 5-((4-(4-cyano-2-phenyloxazol-5-yl)piperazin-1-yl)sulfonyl)-2-(*p*-tolyl)-1,3-oxazole-4-carboxylate **3**.

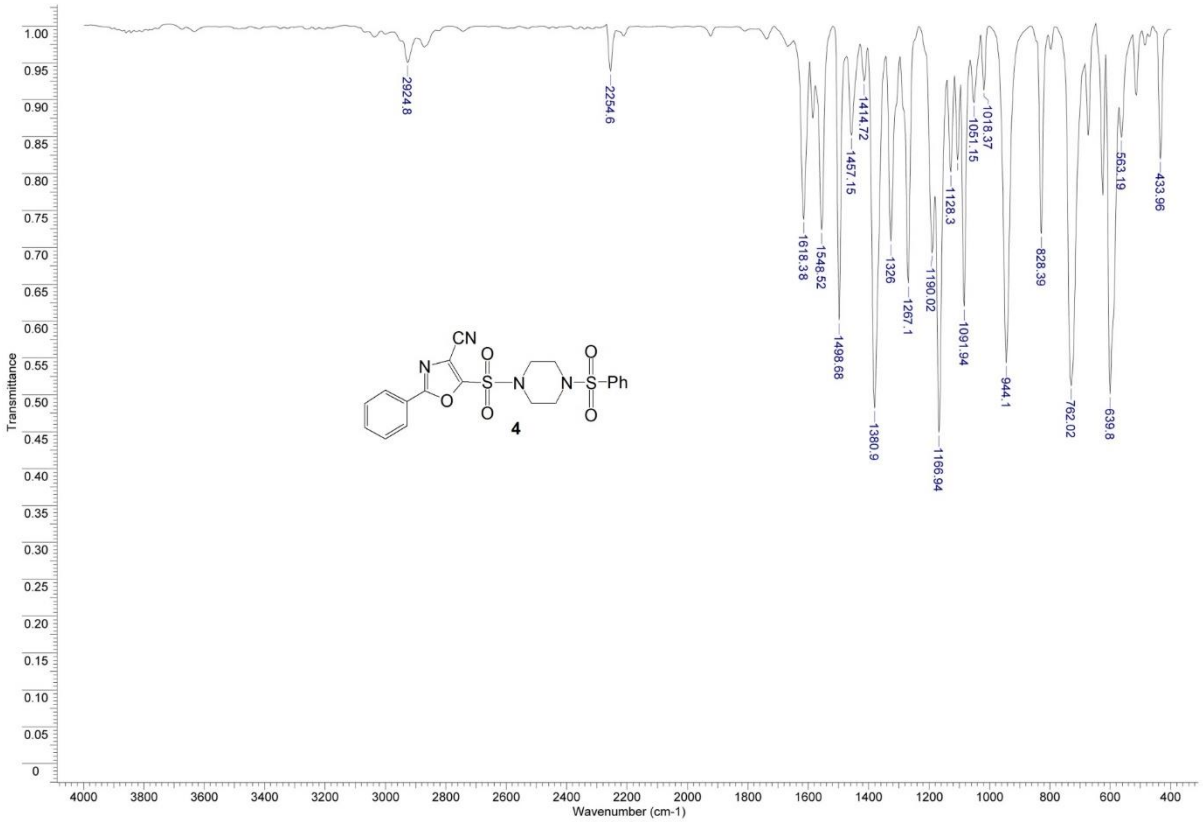

Fig. S13. IR spectrum of 2-phenyl-5-((4-(phenylsulfonyl)piperazin-1-yl)sulfonyl)-1,3-oxazole-4-carbonitrile **4**.

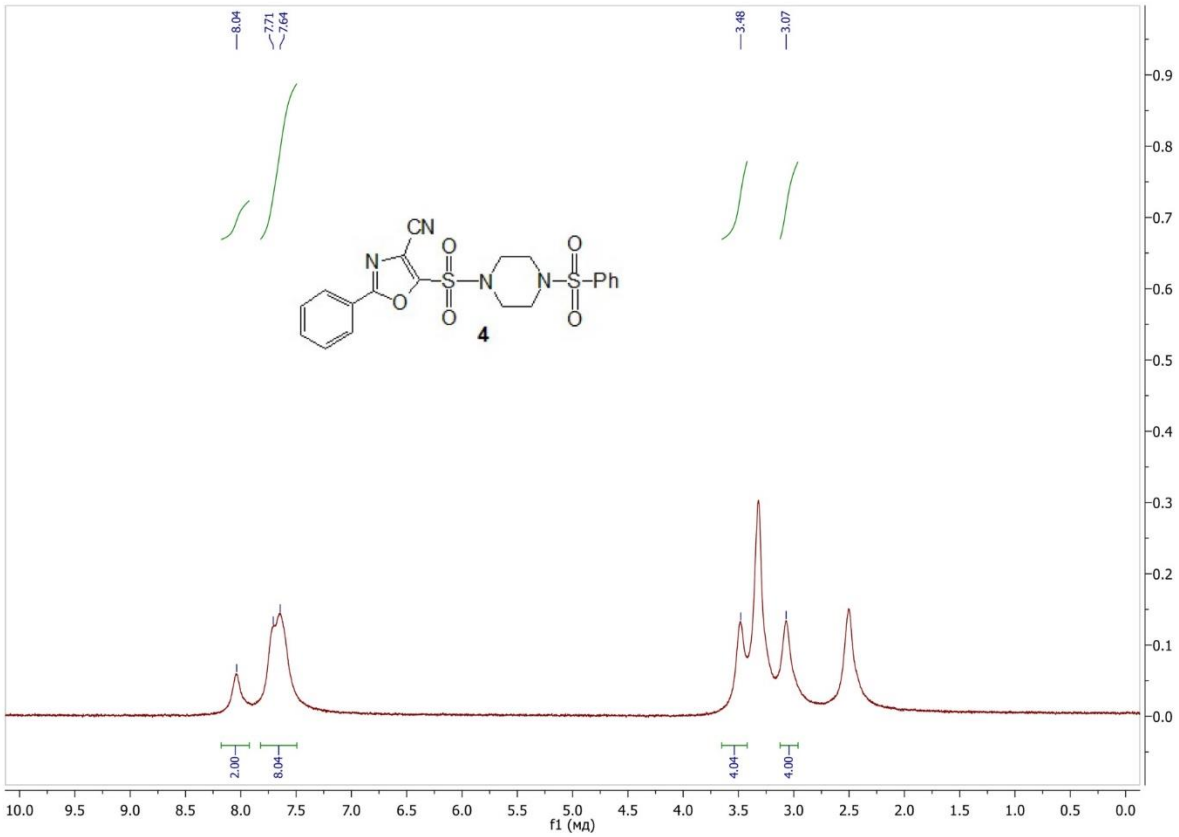

Fig. S14. ^{1}H NMR spectrum of 2-phenyl-5-((4-(phenylsulfonyl)piperazin-1-yl)sulfonyl)-1,3-oxazole-4-carbonitrile **4**.

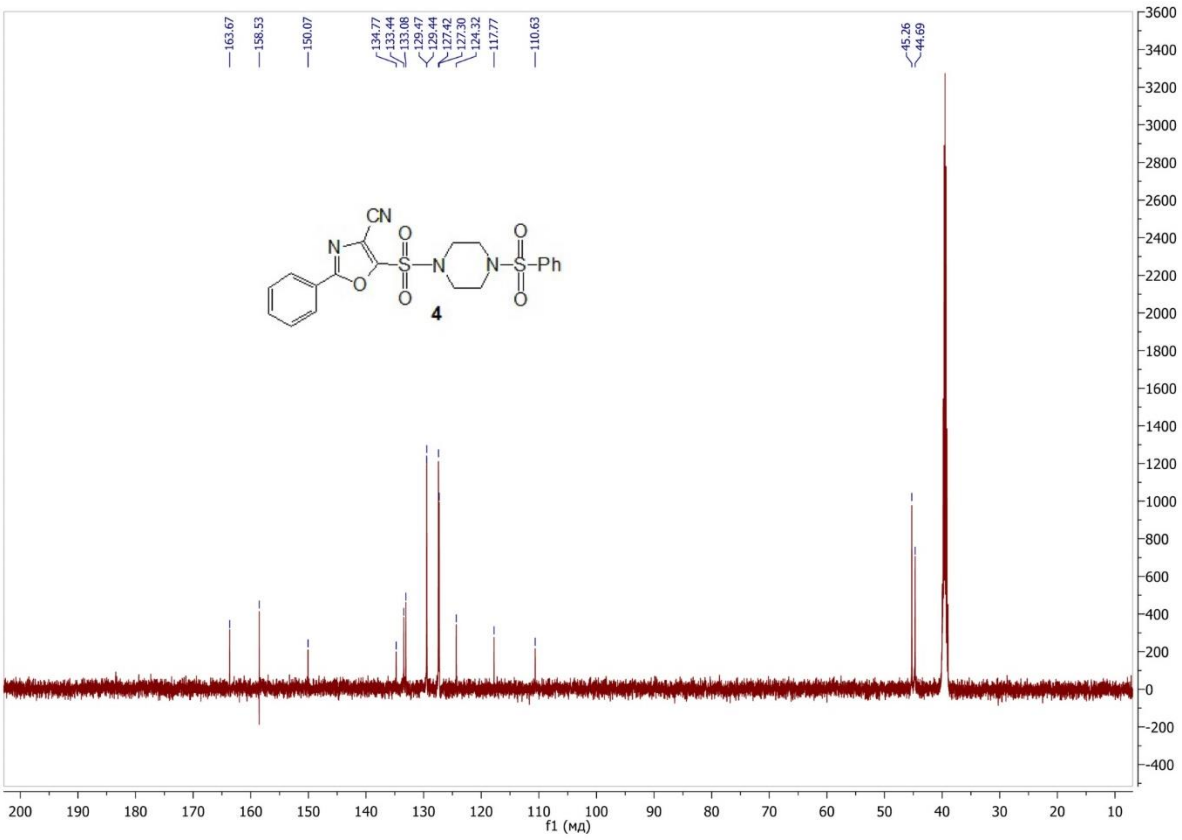

Fig. S15. ^{13}C NMR spectrum of 2-phenyl-5-((4-(phenylsulfonyl)piperazin-1-yl)sulfonyl)-1,3-oxazole-4-carbonitrile **4**.

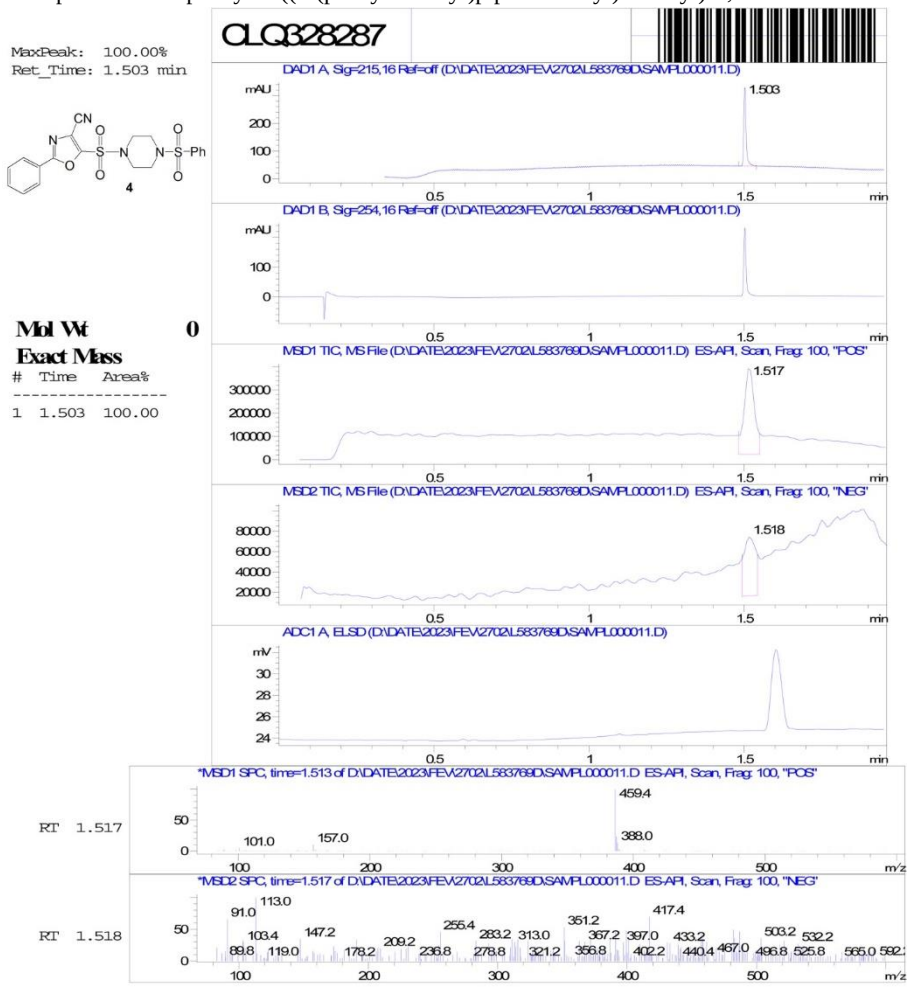

Fig. S16. LSMS spectrum of 2-phenyl-5-((4-(phenylsulfonyl)piperazin-1-yl)sulfonyl)-1,3-oxazole-4-carbonitrile **4**.

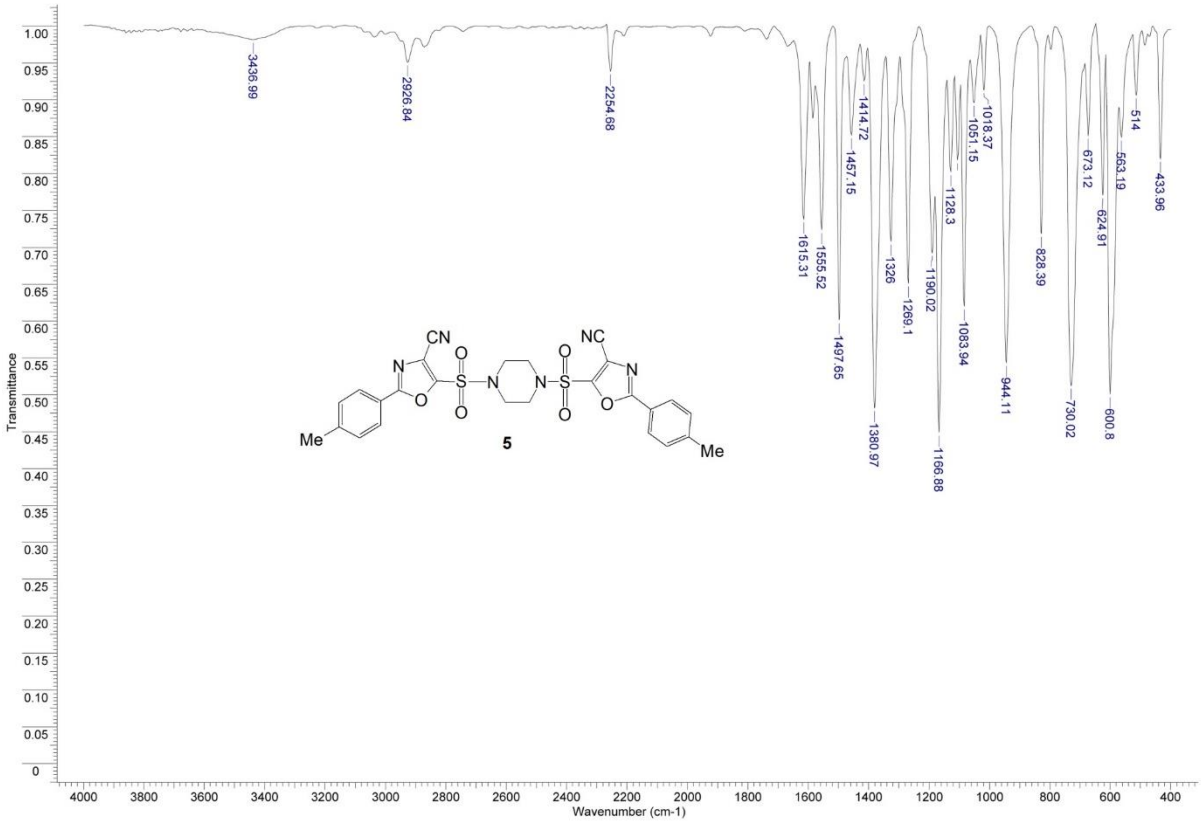

Fig. S17. IR spectrum of 5,5'-(piperazine-1,4-disulfonyl)bis(2-(*p*-tolyl)-1,3-oxazole-4-carbonitrile) **5**.

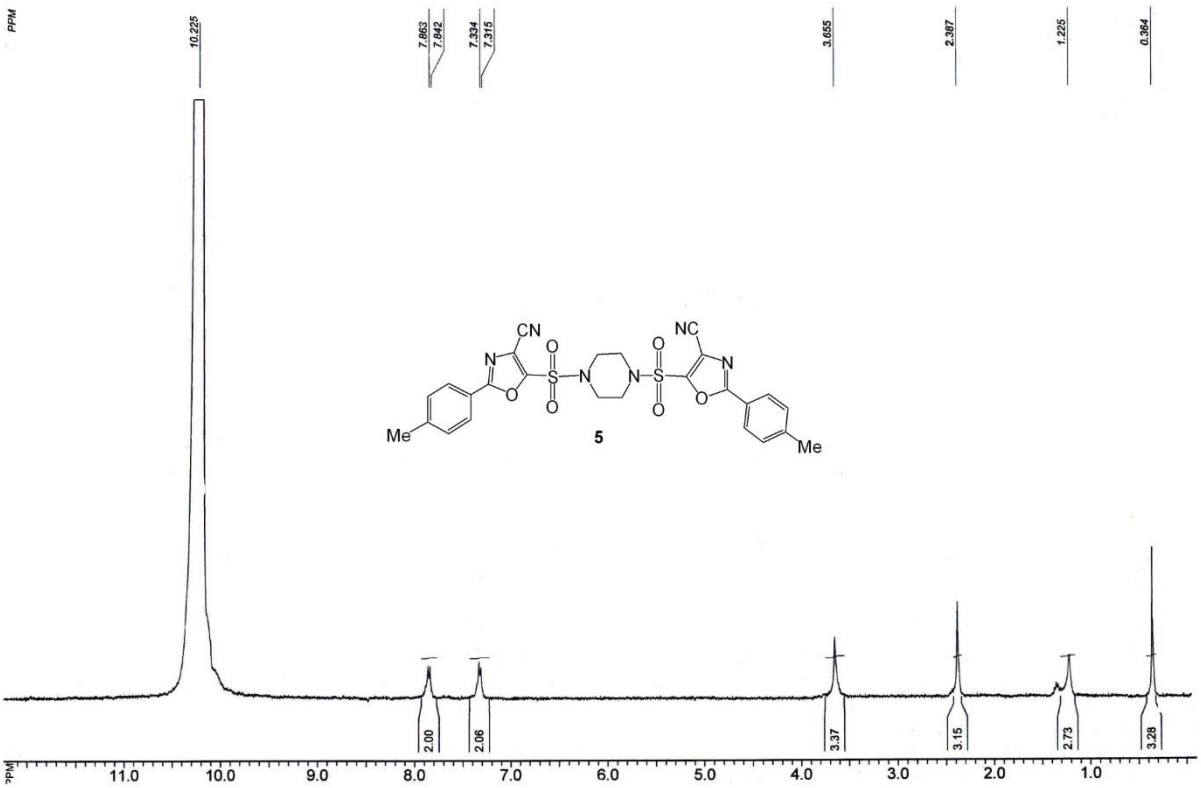

Fig. S18. [1]H NMR spectrum of 5,5'-(piperazine-1,4-disulfonyl)bis(2-(*p*-tolyl)-1,3-oxazole-4-carbonitrile) **5**.

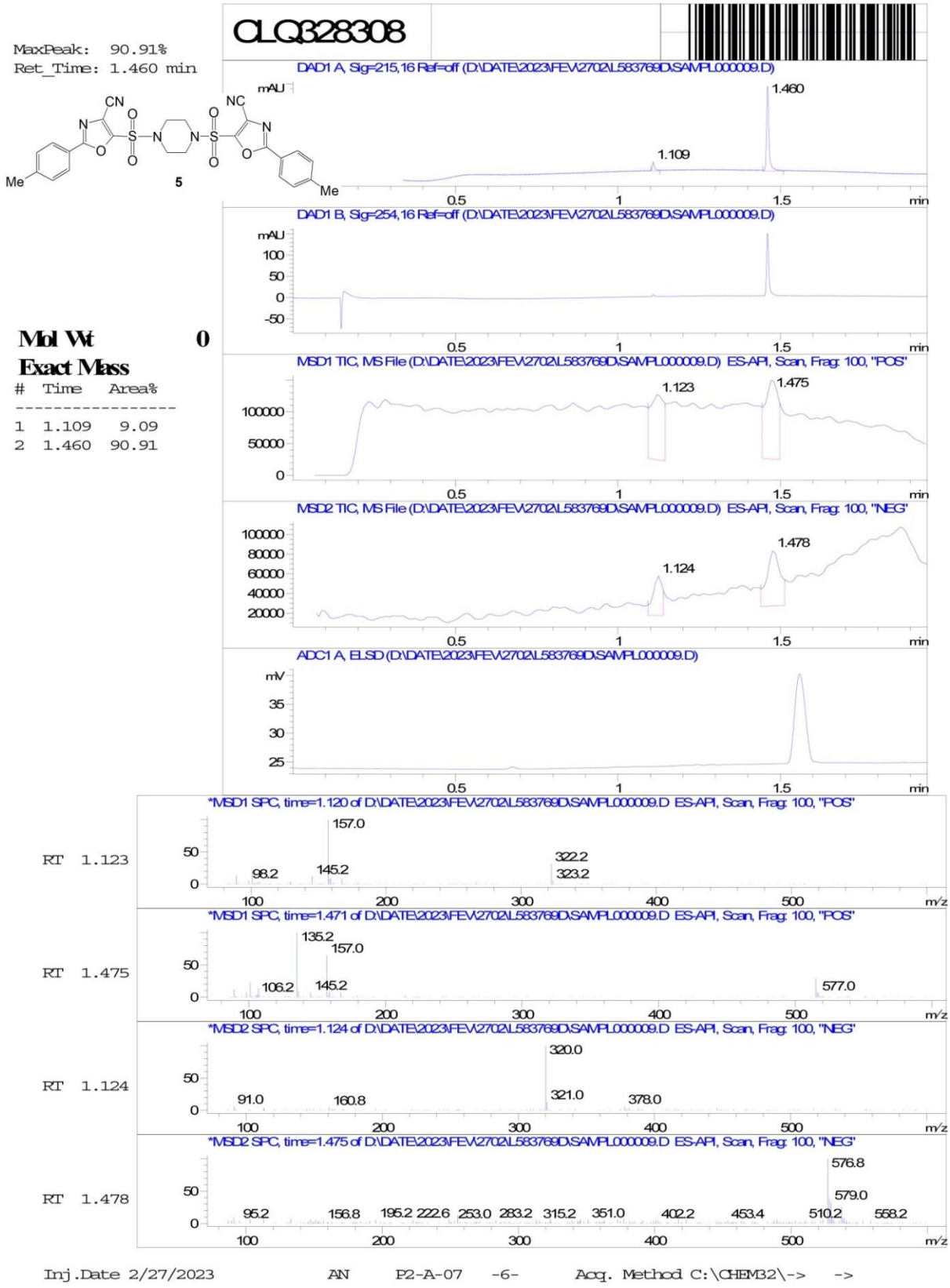

Fig. S19. LSMS spectrum of 5,5'-(piperazine-1,4-disulfonyl)bis(2-(*p*-tolyl)-1,3-oxazole-4-carbonitrile) **5**.

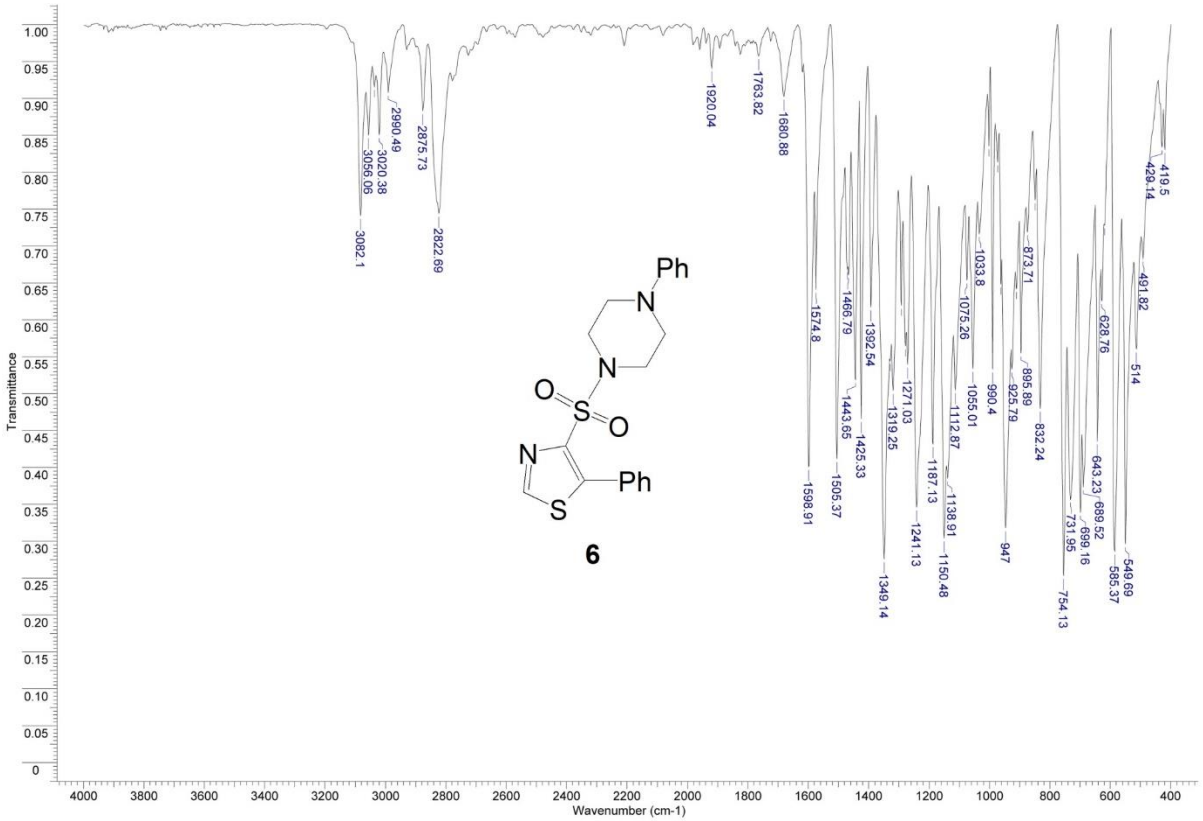

Fig. S20. IR spectrum of 5-phenyl-4-((4-phenylpiperazin-1-yl)sulfonyl)-1,3-thiazole **6**.

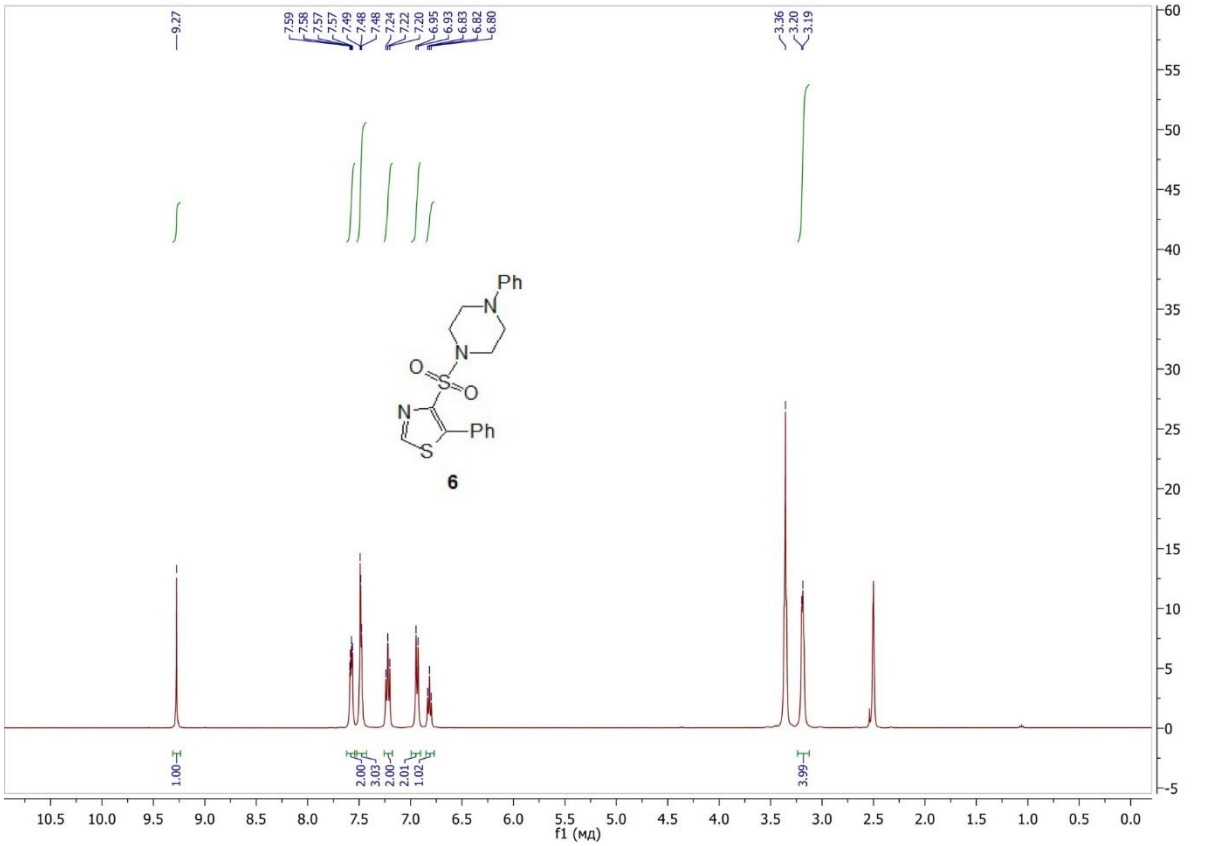

Fig. S21. ^{1}H NMR spectrum of 5-phenyl-4-((4-phenylpiperazin-1-yl)sulfonyl)-1,3-thiazole **6.**

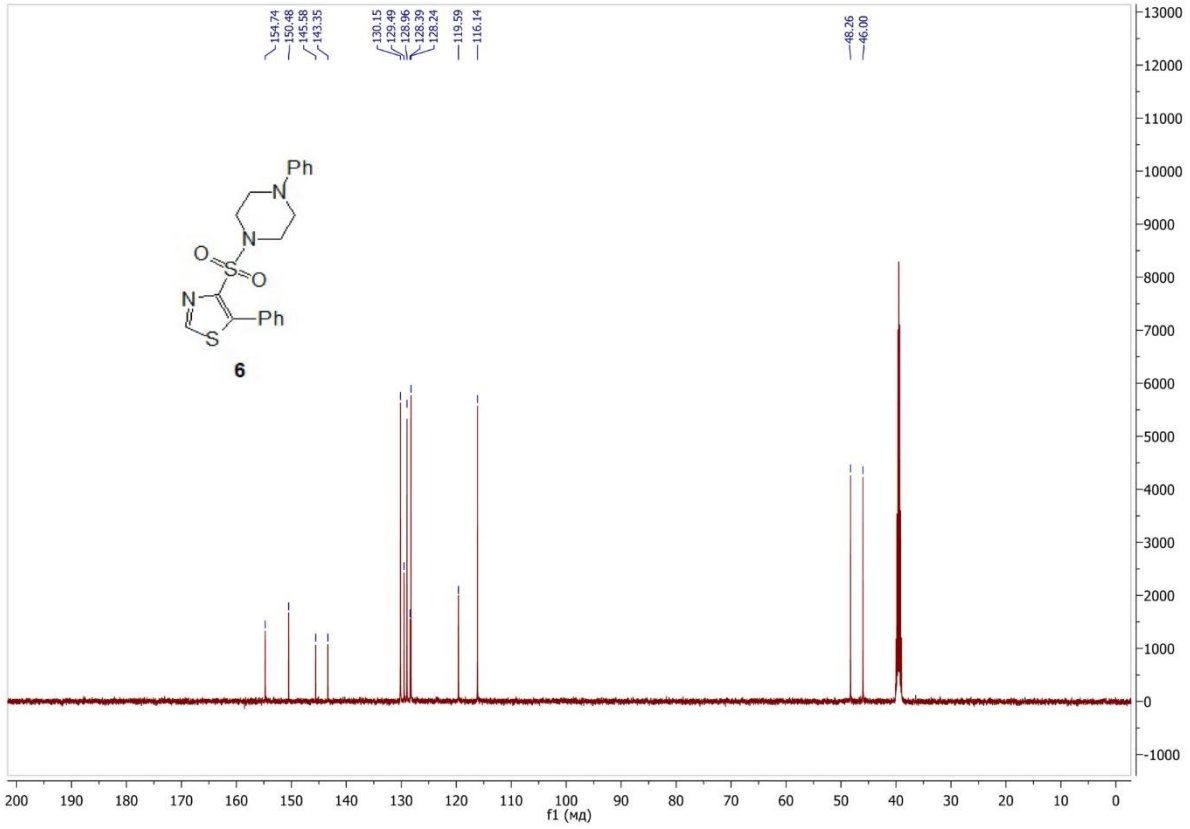

Fig. S22. ^{13}C NMR spectrum of 5-phenyl-4-((4-phenylpiperazin-1-yl)sulfonyl)-1,3-thiazole **6**.

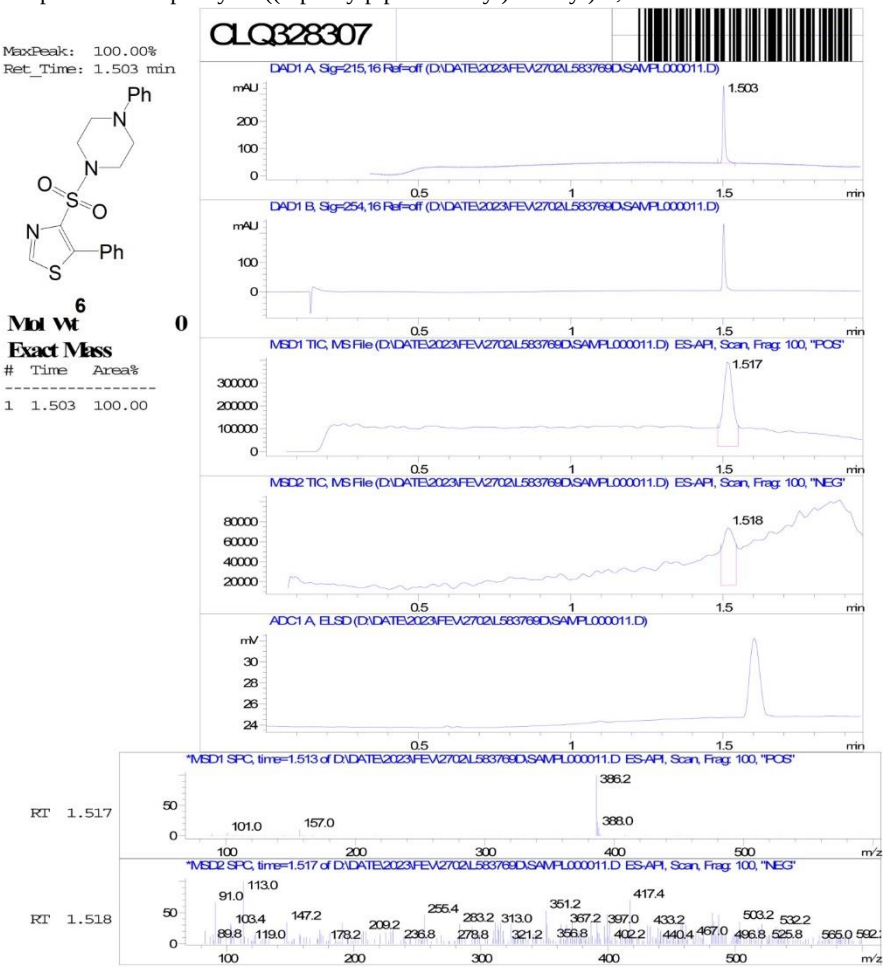

Fig. S23. LSMS spectrum of 5-phenyl-4-((4-phenylpiperazin-1-yl)sulfonyl)-1,3-thiazole **6**.

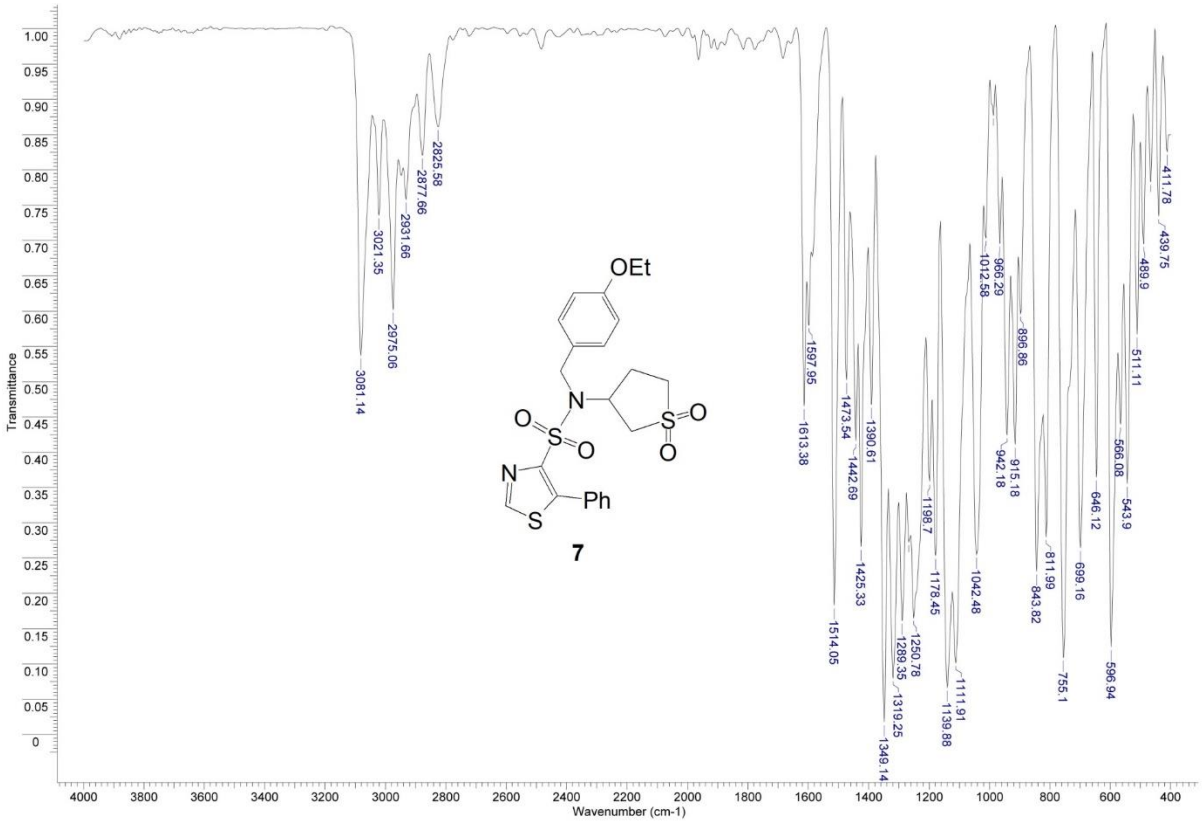

Fig. S24. IR spectrum of *N*-(1,1-dioxidotetrahydrothiophen-3-yl)-*N*-(4-methoxybenzyl)-5-phenyl-1,3-thiazole-4-sulfonamide **7.**

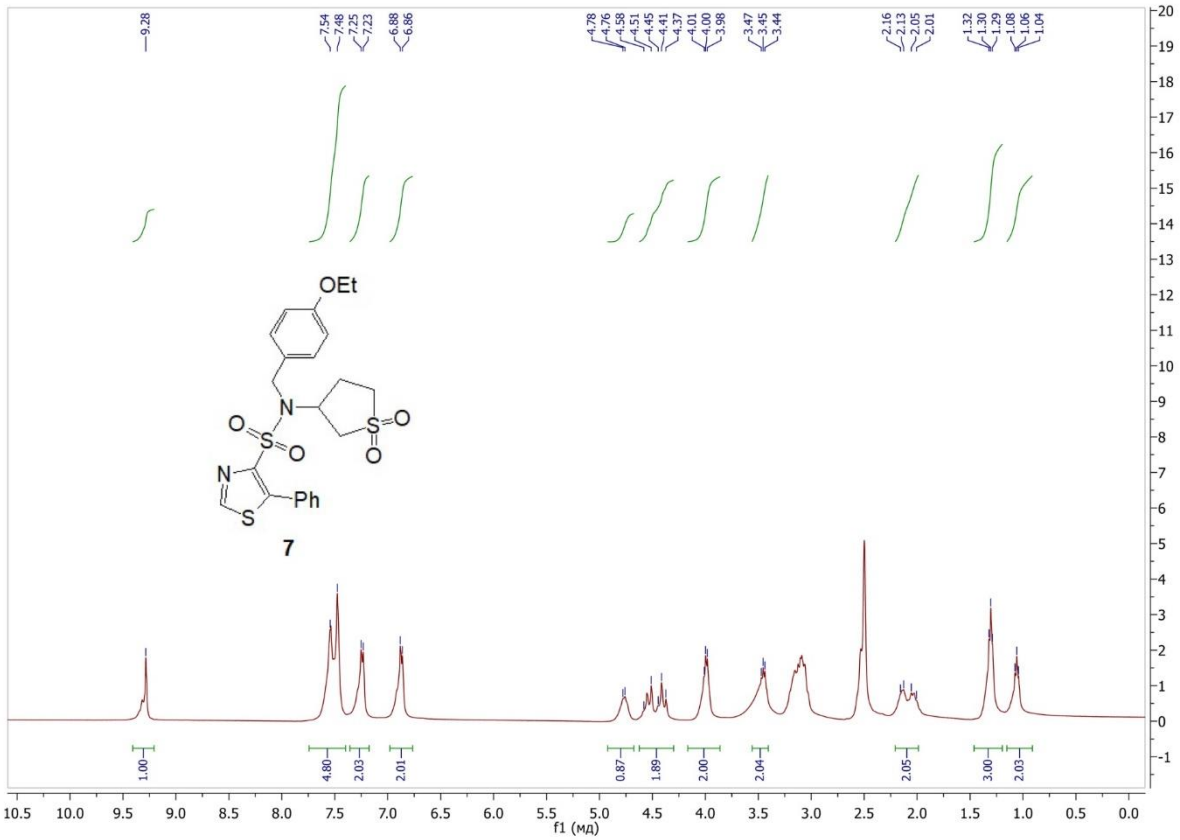

Fig. S25. [1]H NMR spectrum of *N*-(1,1-dioxidotetrahydrothiophen-3-yl)-*N*-(4-methoxybenzyl)-5-phenyl-1,3-thiazole-4-sulfonamide **7**.

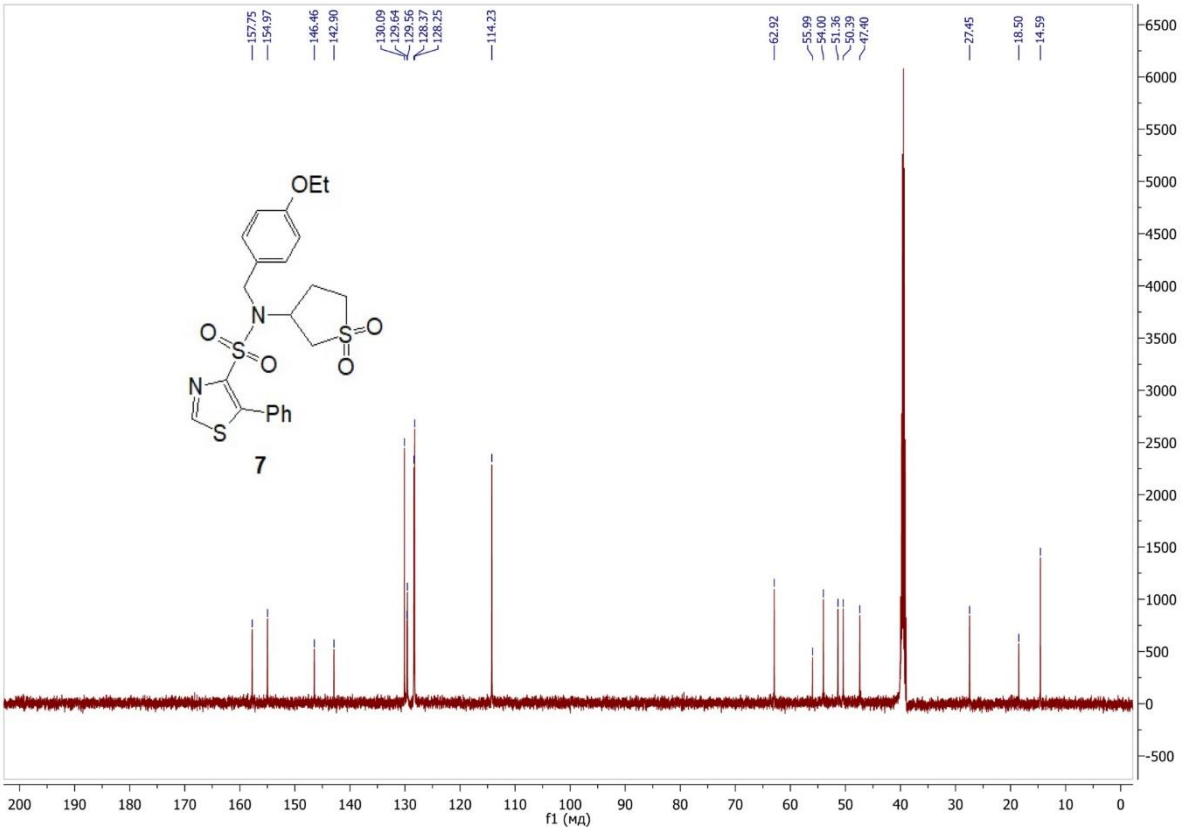

Fig. S26. ^{13}C NMR spectrum of *N*-(1,1-dioxidotetrahydrothiophen-3-yl)-*N*-(4-methoxybenzyl)-5-phenyl-1,3-thiazole-4-sulfonamide **7**.

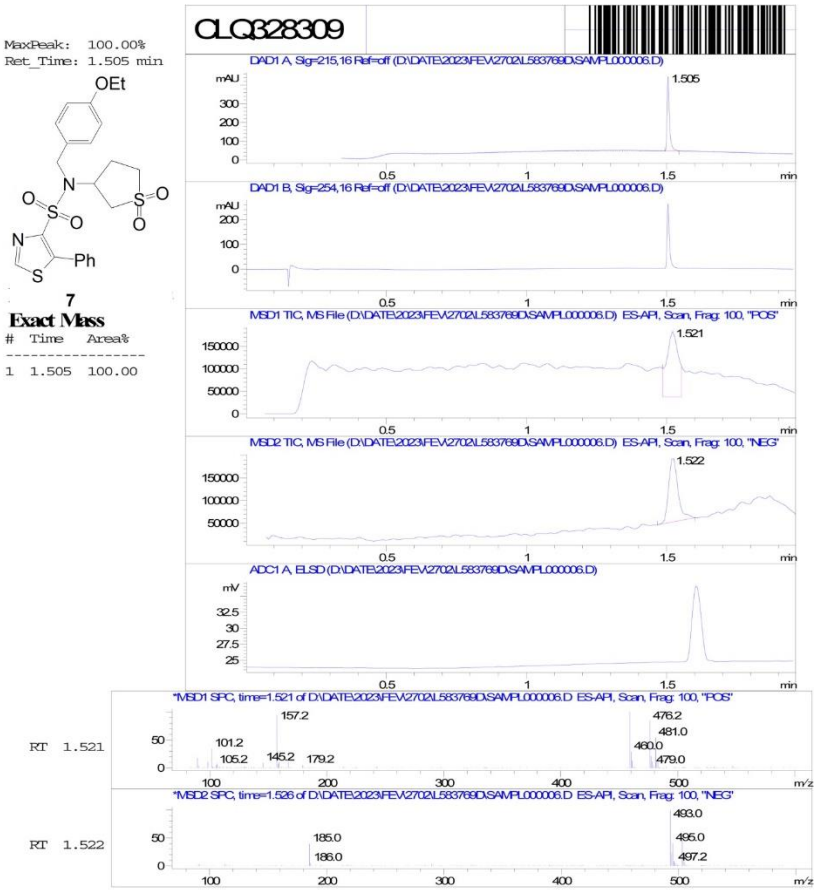

Fig. S27. LSMS spectrum of *N*-(1,1-dioxidotetrahydrothiophen-3-yl)-*N*-(4-methoxybenzyl)-5-phenyl-1,3-thiazole-4-sulfonamide **7**.