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DEVELOPMENT OF A CHROMATOGRAPHIC SEPARATION METHODOLOGY AND IMPURITY PROFILING OF 5-(5-TRIFLUOROMETHYL-ISOXAZOL-3-YL)FURAN-2-SULFONAMIDE, A DRUG CANDIDATE FOR THE GLAUCOMA TREATMENT

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Abstract. *Glaucoma is the leading cause of irreversible blindness. Early diagnosis and effective treatment can reduce the progression of the disease and prevent irreversible decay of visual functions. Indeed, human carboanhydrase isoform II is a classical target for glaucoma treatment. We proposed a compound molecule 5-(5-trifluoromethyl-isoxazol-3-yl)furan-2-sulfonamide under the project name B016, which showed high activity as a human carboanhydrase II inhibitor by in vitro tests. Our team is currently conducting development and preclinical studies of a medicinal product in the eye drops form based on this compound. One of the stages of pharmaceutical research is the development of quality control methods for both the pharmaceutical substance and the finished dosage form. We have developed a chromatographic separation procedure, which can be used for quality control of active pharmaceutical substance by quantification indicators. We also determined the impurity profile, i.e. the structure of the impurities found in the reaction product of compound B016.*

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Introduction

Glaucoma is the leading cause of irreversible blindness. The number of patients expected to rise from 76.0 million in 2020 to 111.8 million in 2040. Early diagnosis and effective treatment can reduce the disease progression and prevent irreversible decay of ophthalmic functions [1].

The human carboanhydrase II isoform (HCA II) is known to be a classic target for the treatment of glaucoma [2-5].

As an extension of our studies on new sulfonamides [6-8], we proposed a drug candidate based on the molecule 5-(5-trifluoromethyl-isoxazol-3-yl)furan-2-sulfonamide under the project name B016, which showed high activity by *in vitro* tests as a KACh II inhibitor [9-11]. Our team is currently conducting development and preclinical drug studies for the treatment of glaucoma in the form of eye drops.

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One of the stages of pharmaceutical research is the development of quality control methods for both the pharmaceutical substance and the finished dosage form. Chromatography is the main method used in quality control of pharmaceuticals. Therefore, the development of a chromatographic technique which allows the separation of the target product and all potentially possible impurities is an urgent task. An equally important task is to establish the structure of impurities detected during the analysis of substance samples. This makes it possible to assess the potential toxic effects of impurities, produce and validate standard samples of impurities, which can then be used in routine quality control of both the pharmaceutical substance and the finished dosage form.

Main body

Fig. 1 shows the chemical process of substance B016 synthesis.

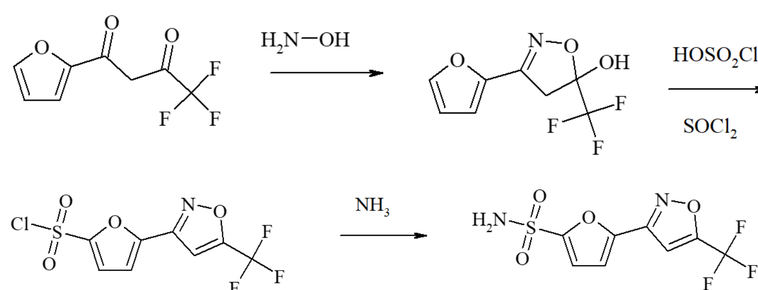
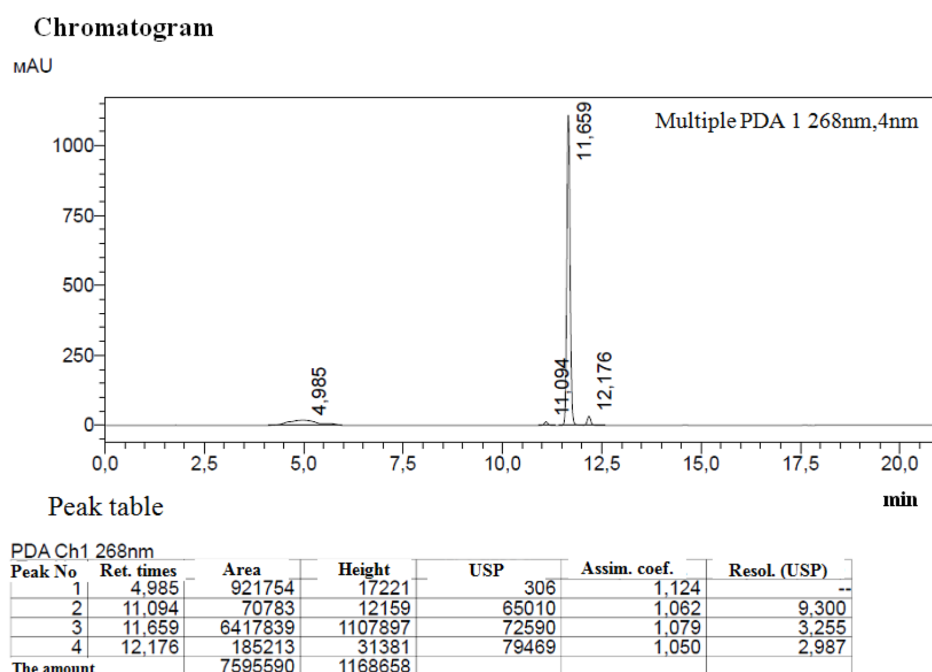


Fig. 1. Synthesis route B016

To develop the chromatographic separation technique, we used the sample obtained in the last stage without further purification.

We have proposed a methodology for the chromatographic separation of the substances contained in this sample. The main parameters are given in the experimental part. A typical chromatogram is shown on Fig. 2. This technique allows us to provide acceptable parameters of components separation (resolution between critical peaks 3.25 and 2.99, respectively).



Peak No	Ret. times	Area	Height	USP	Assim. coef.	Resol. (USP)
1	4,985	921754	17221	306	1,124	--
2	11,094	70783	12159	65010	1,062	9,300
3	11,659	6417839	1107897	72590	1,079	3,255
4	12,176	185213	31381	79469	1,050	2,987
The amount		7595590	1168658			

Fig. 2. Typical chromatogram of B016 reaction products

The chromatogram shows occurring of three additional peaks with retention times of 4.99 min, 11.09 min and 12.18 min, respectively in addition to the main substance peak. We have marked these impurities as impurity A, impurity B, and impurity C, respectively.

We used the LC/MS/MS method to establish the impurity structure.

Fig. 3 shows the mass spectrum of impurity A at quadrupole Q1 and the decay mass spectrum of the parent molecular ion at quadrupole Q3.

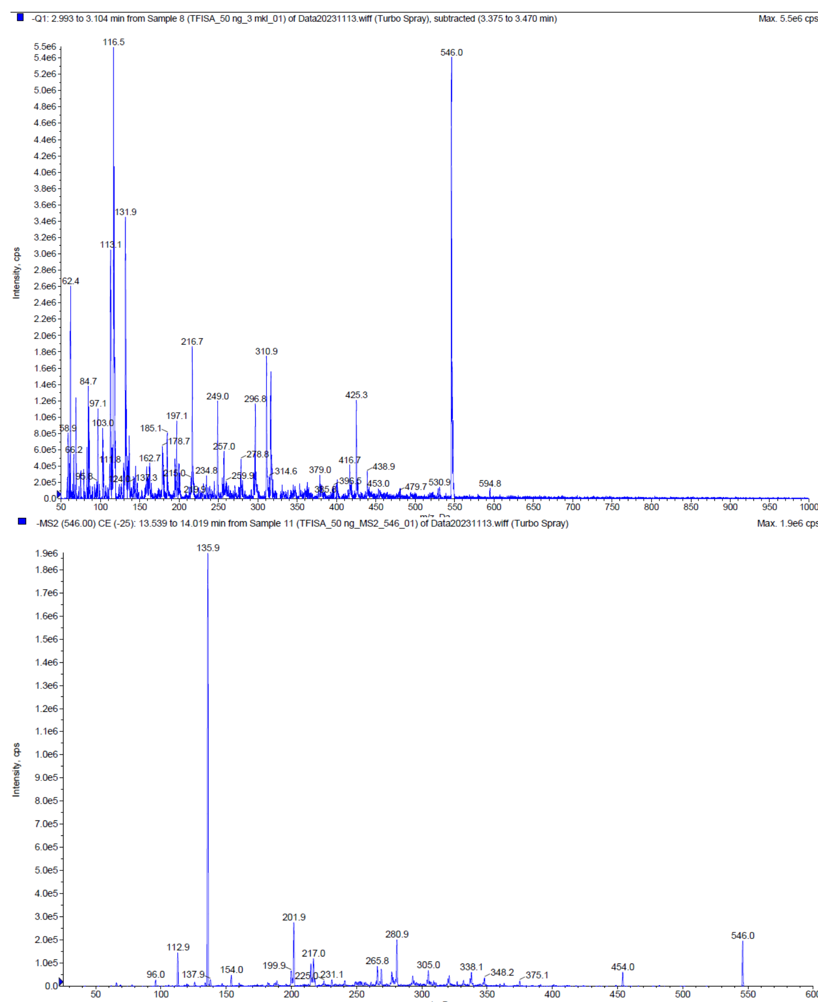


Fig. 3. Mass spectra of impurity A

The main molecular ion M/Z = 546 corresponds to the dimer structure (see Fig. 3). Fig. 4 also shows the structures of the molecular ions formed by the decomposition of the parent ion.

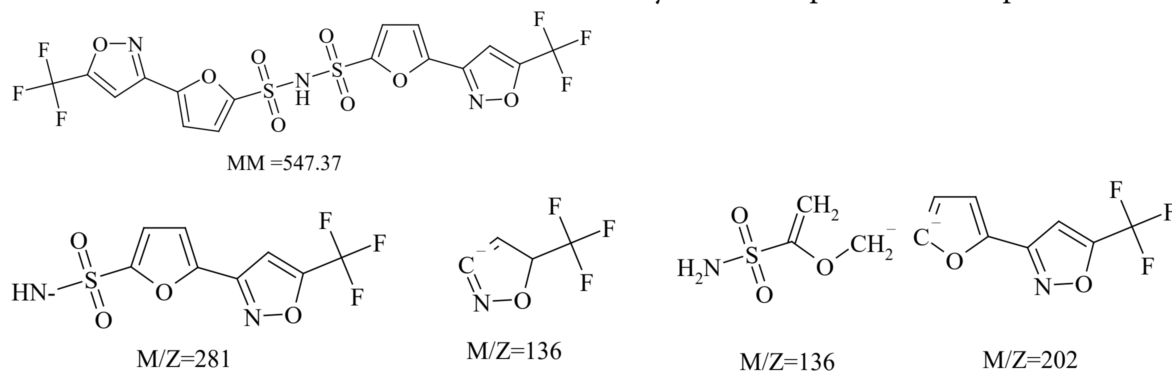


Fig. 4. Impurity A structure and fragment structures corresponding to the mass spectrum peaks on Q3

Hence, the impurity A is a dimer.

Impurities B and C by their physical and chemical properties are close to the basic substance. It is logical to assume the formation of these isomers derivatives during sulfochlorination. And exactly these compounds (impurity B, B016, and impurity C) are critical pairs at chromatographic separation of the mixture.

Fig. 5 shows the mass spectrum of impurity B at quadrupole Q1 and the decay mass spectrum of the parent molecular ion at quadrupole Q3.

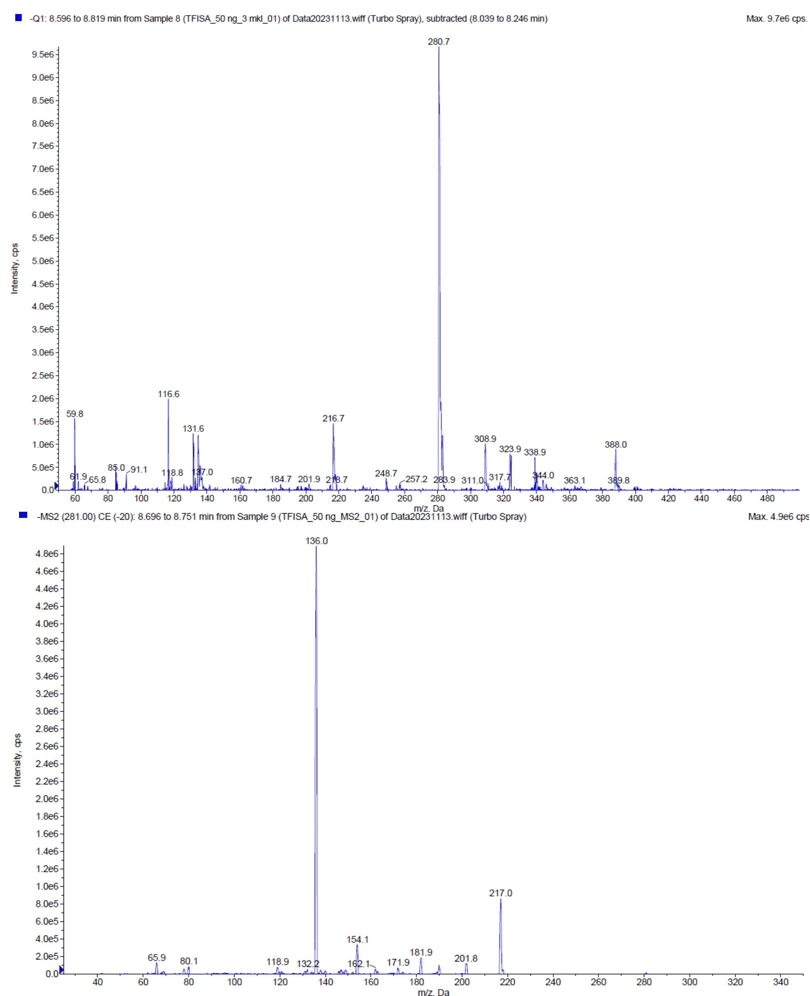


Fig. 5. Mass spectra of impurity B

The main molecular ion M/Z = 281 corresponds to the structure of 2-(5-trifluoromethyl-isoxazol-3-yl]furan-3-sulfonamide (Fig. 6). Fig. 6 shows the structures of the molecular ions formed by the decomposition of the parent ion.

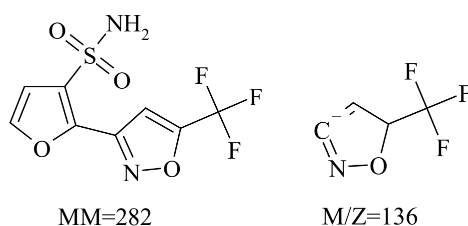


Fig. 6. Impurity B structure and fragment structures corresponding to the mass spectrum peaks on Q3

Hence, the impurity B is 2-(5-trifluoromethyl-isoxazol-3-yl)furan-3-sulfonamide.

Fig. 7 shows the mass spectrum of impurity C at quadrupole Q1 and the decay mass spectrum of the parent molecular ion at quadrupole Q3.

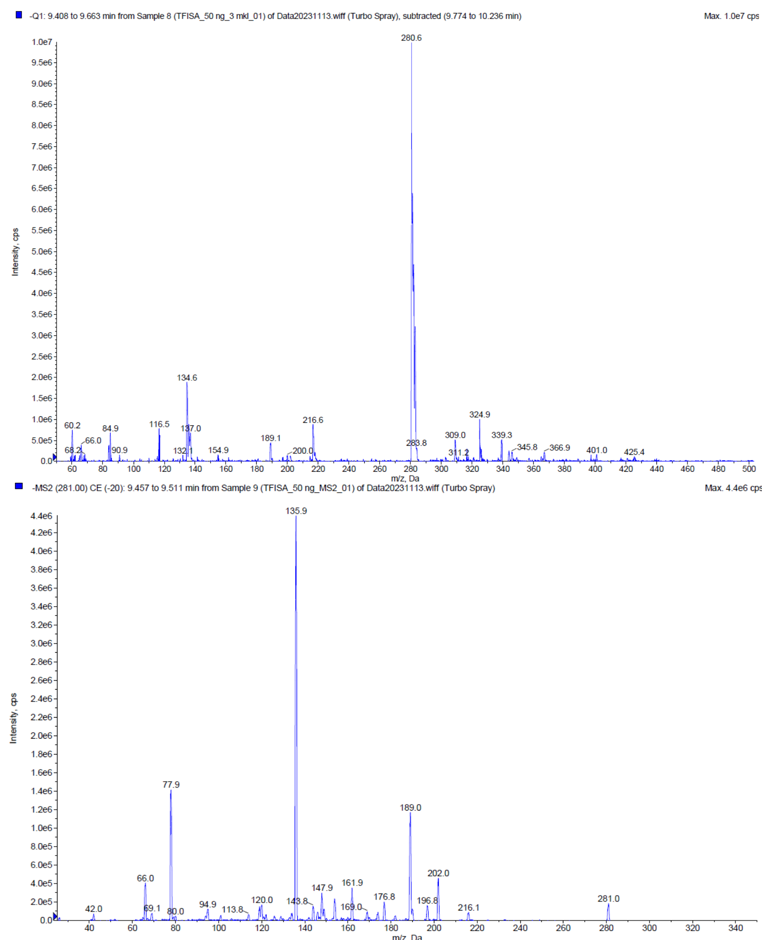


Fig. 7. Mass spectra of impurity C

The main molecular ion M/Z = 281 corresponds to the structure of 5-(5-trifluoromethyl-isoxazol-3-yl)furan-3-sulfonamide (Fig. 8). Fig. 8 shows the structures of the molecular ions formed by the decomposition of the parent ion.

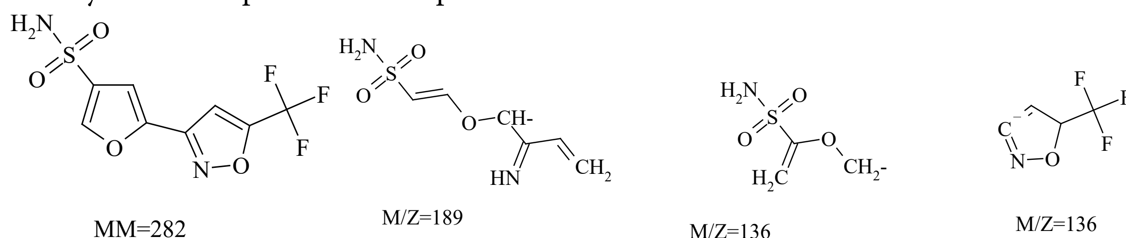


Fig. 8. Impurity C structure and fragment structures corresponding to the mass spectrum peaks on Q3

Hence, the impurity C is 5-(5-trifluoromethyl-isoxazol-3-yl)furan-3-sulfonamide.

Experimental part

Reagents and solvents (Aldrich, Acros) are commercially available and were used without primary purification.

Chromatograms were obtained on a Shimadzu LC-20 Prominence chromatograph.

The model mixture for chromatography and samples of LS B016 were obtained according to the method [5].

Analysis procedure for drug B016. Solvent: 140 ml of acetonitrile and 60 ml of water are put into a conical flask with a lapped stopper; the solution is stirred, filtered, and degassed (Table 1).

Tested solution. We put about 0.01 g (exact suspension) of the substance into a 5 ml volumetric flask, dissolved it in 3 ml of solvent, brought the volume of the solution to the mark with the same solvent, and stirred.

We added 1 ml of the obtained solution to a 50 ml volumetric flask, brought the volume of the solution to the mark with the same solvent, and mixed. The obtained solution was filtered through a membrane filter with a pore size not exceeding 0.45 µm.

Chromatography conditions (Table 2):

Column Kinetex C18 5µm 250x4.6mm or similar;
Suspended phase A deionised water;
Suspended phase B acetonitrile;
Flow rate 1.5 ml/min;
Column temperature 25 ^{0}C;
Spectrophotometric detector, wavelength 268 nm;
Sample volume 10 µl

Table 1. Gradient elution programme

Time, min	Suspended phase A, %	Suspended phase B, %
0	70	30
3	70	30
20	45	55
21	70	30

We identified the reaction products using an HPLC-MS/MS system. The system includes tandem mass spectrometric detector AB Sciex QTRAP5500 (AB Sciex LLC, USA) and chromatograph Agilent 1260 Infinity (Agilent Technologies LLC, USA), consisting of pump G1312B, autosampler G1329B with thermostat G1330B, column thermostat G1316A (instrument control - software "Analyst 1.6.2" (AB Sciex LLC, USA). We used "MultiQuant 3.0.5" software (AB Sciex LLC, USA) for chromatograms processing, metabolite prediction and creation of MRM-methods for metabolite identification - "LightSight" 2.3 software (AB Sciex LLC, USA)).

Table 2. Parameters of mass spectrometric B016 and its metabolites detection

Parameters	Value
Ionisation method	Electrospray ionisation (ESI)
Electric spray voltage	+5500 V
Gas screen	55 psi (Nitrogen)
CAD-gas (collision-activated dissociation)	High (Nitrogen)
Ion source temperature	700 ^{0}C
Gas 1 (Heating gas)	55 psi (Air)
Gas 2 (Gas nebuliser)	55 psi (Air)

Conclusions and recommendations

We have developed a chromatographic separation technique which allows the quality control of an active pharmaceutical substance in terms of quantification and related impurities. This technique provides acceptable separation parameters for the critical components (resolution between the B and B016 impurity peaks, and the B016 and C impurity peaks 3.25 and 2.99, respectively).

We have established the impurity profile, i.e. the structure of the impurities found in the reaction product of compound B016 preparation using LC/MS/MS method.

The study was performed within the framework of the state assignment of the Ministry of Education of the Russian Federation No. 073-00077-21-02 for scientific research on the topic "Development of an innovative drug for the treatment of open-angle glaucoma by selective inhibition of carboanhydrase II" (registry entry No. 730000F.99.1. BV10AAA00006).

References

1. **Schellack, N., Schellack, G. & Bezuidenhout, S.** (2015) Glaucoma: A brief review, *SA Pharmaceutical Journal,* (82), pp. 18-22. DOI: 10520/EJC174862.
2. **Kurysheva, N.I.** (2020) Carboanhydrase inhibitors in glaucoma treatment. Review. Part 1, *Oftal`mologiya,* 17(3), pp. 542-549. DOI: 10.18008/1816-5095-2020-3S-542-549 (in Russian).
3. **Kurysheva, N.I.** (2020) Carboanhydrase inhibitors in glaucoma treatment. Review. Part 2, *Oftal`mologiya,* 17(4), pp. 676-682. DOI: 10.18008/1816-5095-2020-4-676-682 (in Russian).
4. **Scozzafava, A. & Mastrolorenzo, C.T.** (2006) Supuran. Carbonic anhydrase inhibitors and activators and their use in therapy, *Expert Opin. Ther.*, (16), pp. 1627-1664. DOI: 10.1517/13543776.16.12.1627.
5. **Krylov, E.N. & Virzum L.V.** (2021) Quantum chemical analysis of the interaction of alkylarylsulfonamides with α-carboanhydrase HCA II, *Butlerovskie soobshheniya,* 66(5), pp. 11-23. DOI: 10.37952/ROI-jbc-01/21-66-5-11 (in Russian).
6. **Komshina, L.A., Martazova, V.V., Proskurina, I.K., Korsakov, M.K. & Kotov, A.D.** (2020) Synthesis of 3-arylisoxazoles and their sulfamide derivatives, *Butlerovskie soobshheniya,* 63(9), pp.10-18. DOI: 10.37952/ROI-jbc-01/20-63-9-10 (in Russian).
7. **Komshina, L.A., Vasilieva, E.A., Proskurina, I.K., Kotov, A.D. & Korsakov, M.K.** (2021) Regioselectivity of sulfonyl chlorination of 3-aryl-5-N-acylaminoisoxazoles, *Butlerovskie soobshheniya,* 67(8), pp. 123-128. DOI: 10.37952/ROI: jbc-01/21-67-8-123 (in Russian).
8. **Komshina, L.A., Vasilieva, E.A., Proskurina, I.K., Blumina, M.V., Kotov, A.D. & Korsakov, M.K.** 2022. Regioselectivity of 1-Aryl-6-pyrazol-1-yl-pyridazines sulfonyl chlorination, *Butlerovskie soobshheniya,* 69(2), pp. 15-21. DOI: 10.37952/ROI: jbc-01/22-69-2-15 (in Russian).
9. **Krasavin, M., Korsakov, M., Dorogov, M., Tuccinardi, T., Dedeoglu, N. & Supuran, C.T.** (2015) Probing the «bipolar» nature of the carbonic anhydrase active site: Aromatic sulfonamides containing 1,3-oxazol-5-yl moiety as picomolar inhibitors of cytosolic CA I and CA II isoforms, *Eur. J. Med. Chem.*, (101), pp. 334-347. DOI: 10.1016/j.ejmech.2015.06.022.
10. **Krasavin, M., Korsakov, M., Zvonaryova, Z., Semyonychev, E., Tuccinardi, T., Kalinin, S. & Supuran, C.T.** (2017) Human carbonic anhydrase inhibitory profile of mono- and bis-sulfonamides synthesized via a direct sulfochlorination of 3- and 4-(hetero)arylisoxazol-5-amine scaffolds, *Bioorg. Med. Chem.,* 25(6), pp. 1914-1925. DOI: 10.1016/j.bmc.2017.02.018.
11. **Dorogov, M.V. & Krasavin, M.Y.** (2017) *Aromatic derivatives of sulfonamides inhibitors of carboanhydrase II (CA II), their preparation and application methods.* 2607630 RU (in Russian).

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