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# Monitoring of volatile organic compounds in ambient air of Taldykorgan, Kazakhstan

**[1]Serik L., [2]Ibragimova O.P., [3]Ussenova G.K., [2]Baimatova N.Kh.***

[1]School of Sciences and Humanities, Nazarbayev University, Nur-Sultan, Kazakhstan
[2]Al-Farabi Kazakh National University, Center of Physicochemical methods of research and analysis, Almaty, Kazakhstan
[3]Nazarbayev Intellectual School of Physics and Mathematics, Almaty, Kazakhstan
*E-mail: *baimatova@cfhma.kz*

The pollution of ambient air is one of the main sources of risk to human health in the world. There is a direct relationship between the level of air pollution and risk of the development of cancer, cardiovascular, respiratory and other diseases. Benzene, toluene, ethylbenzene and *o*-xylene (BTEX) are one of the most toxic volatile organic compounds. The aim of this study was to quantify BTEX in air of Taldykorgan, Kazakhstan using solid-phase microextraction followed by gas chromatography with mass-spectrometric detection. In different sampling seasons, average concentrations of four BTEX analytes varied from 7.5 to 27 μg/m$^3$, from 15 to 250 μg/m$^3$, from 2.4 to 12.8 μg/m$^3$ and from 2.6 to 21 μg/m$^3$, respectively. The highest concentrations of TEX were detected in autumn, while the highest concentrations of benzene were observed in winter. Toluene-to-benzene ratios in almost all measurements were above 1 indicating that the traffic emissions are the main source of air pollution with BTEX.

**Keywords:** SPME; GC-MS; BTEX; air pollution; air analysis; Taldykorgan.

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# Қазақстан, Талдықорған қаласының ауасындағы ұшқыш органикалық қосылыстардың мониторингі

**[1]Серик Л., [2]Ибрагимова О.П., [3]Усенова Г.К., [2]Байматова Н.Х.***

[1]Назарбаев Университетінің жаратылыстану, әлеуметтік және гуманитарлық ғылымдар мектебі, Нұр-Сұлтан, Казахстан
[2]Әл-Фараби атындағы Қазақ ұлттық университеті, Физика-химиялық зерттеу әдістері орталығы, Алматы, Қазақстан
[3]Алматы қаласындағы физика-математика бағытындағы Назарбаев Зияткерлік мектебі, Алматы, Қазақстан
*E-mail: *baimatova@cfhma.kz*

Бүкіл әлемде атмосфералық ауаның ластануы адамдардың денсаулығы үшін негізгі қауіп көзі болып саналады.  Ауаның ластану деңгейі мен онкологиялық, жүрек-қан тамырларының, респираторлық және басқа да аурулардың пайда болу қаупі арасында тікелей тәуелділік туғызады. Ең улы ұшқыш органикалық қосылыстардың бірі-бензол, толуол, этилбензол және *о*-ксилол (БТЭК). Бұл жұмыстың мақсаты масс-спектрометриялық детекторы бар газды хроматография және қатты фазалы микроэкстракция әдісімен Талдықорған қаласының ауасындағы БТЭК анықтау және идентификациялау болып табылады. Сынама іріктеудің әртүрлі маусымдарында БТЭК орташа концентрациялары тиісінше 7,5-ден 27-ге дейін, 15-тен 250-ге дейін, 2,4-тан 12,8-ге дейін және 2,6-тан 21 мкг/м$^3$-қа дейін өзгеріп отырды.  ТЭК ең жоғары концентрациясы күзгі кезеңде табылды, ал бензолдың ең жоғары концентрациясы қыста байқалды. Толуолдың бензолға қатынасы барлық өлшемдерде 1-ден жоғары болды, бұл БТЭК ауаны ластаудың негізгі көзі автокөлік шығарындылары болып табылатынын көрсетеді.

**Түйін сөздер:** ҚФМЭ; ГХ-МС; БТЭК; ауаның ластануы; ауа талдауы; Талдықорған.

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# Мониторинг летучих органических соединений в воздухе города Талдыкорган, Казахстан

**[1]Серик Л., [2]Ибрагимова О.П., [3]Усенова Г.К., [2]Байматова Н.Х.***

[1]Школа естественных, гуманитарных и социальных наук, Назарбаев Университет, Нур-Султан, Казахстан
[2]Казахский национальный университет им. аль-Фараби, Центр физико-химических методов исследования и анализа, Алматы, Казахстан
[3]Назарбаев Интеллектуальная Школа физико-математического направления, Алматы, Казахстан
*E-mail: *baimatova@cfhma.kz*

Во всем мире загрязнение атмосферного воздуха считается одним из основных источников риска для здоровья людей. Существует прямая зависимость между уровнем загрязнения воздуха и риском возникновения онкологических, сердечно-сосудистых, респираторных и других заболеваний. Одними из самых токсичных летучих органических соединений (ЛОС) являются бензол, толуол, этилбензол и *о*-ксилол (БТЭК). Целью данной работы было установить концентрации БТЭК в воздухе города Талдыкорган, Казахстан методом твердофазной микроэкстракции в сочетании с газовой хромато-масс-спектрометрией. В разные сезоны пробоотбора средние концентрации БТЭК варьировались от 7,5 до 27, от 15 до 250, от 2,4 до 12,8 и от 2,6 до 21 мкг/м$^3$, соответственно. Максимальные концентрации ТЭК были обнаружены в осенний период, в то время как самые высокие концентрации бензола наблюдались зимой. Отношение толуола к бензолу почти во всех измерениях было выше 1, что указывает на то, что основным источником загрязнения воздуха БТЭК является выбросы автотранспортных средств.

**Ключевые слова:** ТФМЭ; ГХ-МС; БТЭК; загрязнение воздуха; анализ воздуха; Талдыкорган.

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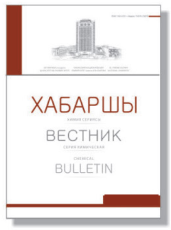


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# Monitoring of volatile organic compounds in ambient air of Taldykorgan, Kazakhstan

**[1]Serik L., [2]Ibragimova O.P., [3]Ussenova G.K., [2]Baimatova N.Kh.***

[1]School of Sciences and Humanities, Nazarbayev University, Nur-Sultan, Kazakhstan
[2]Al-Farabi Kazakh National University, Center of Physicochemical methods of research and analysis, Almaty, Kazakhstan
[3]Nazarbayev Intellectual School of Physics and Mathematics, Almaty, Kazakhstan
*E-mail: *baimatova@cfhma.kz*

## 1. Introduction

Fast and uncontrolled population growth, increase of energy consumption and private transportation lead to a serious problem of air pollution in most cities around the world [1]. Air pollution leads to ecosystem failure and creates huge economic and social harm to the society. WHO reported that in 2016, about 4.2 million of premature deaths were caused by ambient air pollution [1]. 91% of deaths were in low- or middle-income countries [1]. Ambient and indoor air pollution causes the highest health risks around the world.

One of the most important stages in the activities aimed at decreasing the ambient air pollution is its monitoring [2]. It allows predicting the trends in change of concentrations of contaminants, estimating an efficiency of anti-pollution activities, identification of new dangerous contaminants and key, most dangerous and illegal pollution sources.

One of the most dangerous group of air pollutants is volatile organic compounds (VOCs) which are released into the atmosphere due to biogenic and anthropogenic activity, as well as in the result of the photolysis of gases in the air [2]. The main sources of VOCs in the air are exhaust gases of vehicles, power plants, industry, construction as well as the emissions from household activities: cigarette smoke, paints, aerosols and cleaning products [3]. Special attention is paid to benzene, toluene, ethylbenzene and xylenes (BTEX) due to their high toxicity. Many countries regulate and mandate monitoring BTEX concentrations in ambient air [4].

The problem of air pollution exists not only in large cities, but also in small ones such as Taldykorgan, Kazakhstan. Taldykorgan is the center of Almaty region and a neighboring city with Almaty. For several decades, it was considered one of the most environmentally friendly cities in Kazakhstan, as there have never been large industrial plants. Nowadays, the air quality in Taldykorgan has deteriorated. This can be caused by the intensive expansion of the city, the construction of new residential areas and, accordingly, the increase in the number of vehicles and amount of the heating systems in the cold seasons. Taldykorgan does not have access to the gas pipeline. As a result, coal is the main fuel for obtaining electricity and heat generation in the city.

No data on the concentrations of the most common and dangerous pollutants, including BTEX, in air of Taldykorgan are available. For BTEX , it can be caused by a complexity of standard analytical methods [5]. Currently, three main approaches are most widely used for determination of BTEX in air [6-11]:

1. Air sampling in containers or canisters with different volume [7-10]. Containers for sampling are made from materials such as Teflon, glass or stainless steel. To concentrate the analytes, the sampled air is passed through sorbent tubes followed by desorption in a thermal desorption unit (TDU) connected to the inlet of gas chromatograph.

2. Passing of air samples through a suitable VOCs-retaining sorbent, followed by transferring the analytes to the inlet of gas chromatograph using thermal desorption unit [10];

3. Continuous analysis of VOCs concentrations using mobile monitoring stations and portable devices [11,12].

The disadvantages of the first two approaches are:

– the need for cleaning of containers and sorption tubes with high purity helium;

– the need for additional thermal desorption unit for desorption of analytes;

– thermal desorption of analytes from sorption tubes and its transfer to a gas chromatograph is a slow process, which causes wide and poorly separated peaks observed in chromatograms.

For achieving proper accuracy, the third approach requires costly equipment, which is unavailable in Kazakhstan.

All these factors result in the absence of information from the official sources about air pollution with BTEX in Kazakhstan although the required equipment is available in responsible laboratories.

Solid-phase microextraction is one of the most perspective methods for sampling and quantification of VOCs in air developed by Arthur and Pawliszyn from Waterloo University (Canada) in 1989 [13-15]. SPME is based on a sorption of analytes onto a polymeric coating followed by a desorption in a GC inlet. SPME is very efficient and popular for screening of VOCs in air. Available commercial fibers allow detecting all VOCs or a narrow group of analytes depending on their polarity and volatility. Using SPME in combination with gas chromatography mass-spectrometry (GC-MS), Carlsen et al. identified more than 100 VOCs in the air of Almaty [16]. Baimatova et al. [17] developed a very simple and accurate method for quantification of BTEX in ambient air and applied it in Almaty, Kazakhstan.

The objective of this research was to determine the levels of BTEX in ambient air of Taldykorgan in different seasons during 2018-2019 by SPME-GC-MS using the method developed by Baimatova et al. [17].

**2. Experiment**

*2.1 Air sampling sites*

Sampling was conducted at three sites located in different districts of Taldykorgan: Karatal, Center and the 2$^{nd}$ Microdistrict. Sampling sites (A1, A2, A3) were chosen in different parts of the city for determination of mean BTEX concentrations. Sampling sites were located close to the main roadways of the city, but at a distance of more than 15 m from road – Almaty highway, Zhansygurov street and Kabanbay Batyr street (Table 1). Meteorological parameters such as temperature, wind speed and humidity (Table 2) were taken from publicly available database Gismeteo.

**Table 1** – Description of sampling sites

| Sampling site | Crossroad (coordinates) | Objects within a radius of 200 m |
|---|---|---|
| A1 | Rakishev – Kablisa-Zhyrau (45°00’31.6”N, 78°20’49.1”E) | Almaty highway, new residential microdistricts, university, gas station |
| A2 | Shevchenko – Kabanbay batyr (45°01’13.3”N, 78°22’32.6”E) | Low-rise buildings, shopping complexes Karagash, Shagan, Eurasia, bazaar |
| A3 | Zhansygurov – Naberezhnaya (45°00’36.7”N, 78°24’10.3”E) | Residential buildings, shopping and entertainment complex City Plus, Nazarbaev Intellectual School, school No. 9, Karatal river, riverside |

**Table 2** – Weather conditions on sampling days

| Sampling date | Air temperature, °C | Weather conditions | Wind velocity, m/s | Pressure, mmHg | Humidity, % |
|---|---|---|---|---|---|
| 10/3/2018 | 13 | rainy | 1 | 713 | 77 |
| 12/4/2018 | 15 | sunny | 6 | 715 | 53 |
| 14/4/2018 | 24 | cloudy | 3 | 705 | 60 |
| 12/07/2018 | 33 | sunny | 5,8 | 714 | 25 |
| 14/07/2018 | 32 | rainy | 5 | 702 | 38 |
| 16/07/2018 | 34 | cloudy | 0 | 701 | 64 |
| 16/10/2018 | 0 | snow | 0 | 716 | 94 |
| 18/10/2018 | 5 | sunny | 2 | 713 | 39 |
| 20/10/2018 | 12 | cloudy | 3 | 719 | 62 |
| 14/01/2019 | -13 | sunny | 2 | 710 | 72 |
| 16/01/2019 | -3 | cloudy | 3 | 715 | 77 |
| 18/01/2019 | -10 | sunny | 0 | 709 | 91 |

Sampling was conducted four times a year between 5 PM and 6 PM on April 10, 12 and 14; July 12, 14 and 16; October 16, 18 and 20, 2018 and January 14, 16 and 18, 2019. Nine air samples were collected per one sampling day and 27 samples per season. In Almaty sampling was conducted at six different districts between 8 and 9 AM and 8 and 9 PM on April 3, 5 and 7 (Table 3). 36 air samples were collected per day and 108 samples in total.

**Table 3** – Sampling sites in Almaty

| Sampling site | Crossroad (coordinates) | Height, m |
|---|---|---|
| S1 | Radostovets st. – al-Farabi av. (N43°12.007’, E76°53.774’) | 978 |
| S2 | Mendikulov st. – al-Farabi av. (N43°13.654’, E76°57.252’) | 944 |
| S3 | Nauryzbay Batyr st. – Raiymbek av. (N43°16.099’, E76°56.062’) | 764 |
| S4 | Papanin st. – Suyunbay av. (N43°19.095’, E76°57.781’) | 700 |
| S5 | Raiymbek av. – Akhrimenko st. (N43°14.950’, E76° 50.844’) | 770 |
| S6 | Shevchenko st. – Gagarin av. (N43°14.612’, E76°53.586’) | 803 |

*2.2 Air sampling using SPME*

Air sampling was conducted as described by Baimatova et al. [17]. Ambient air samples were collected into 20-mL crimp-top vials (Agilent, USA) in triplicates by opening vial to air and shaking of ~60 sec to increase air exchange and sealed with aluminum caps with polytetrafluoroethylene/silicone septa (Agilent, USA). Vials were transported to the laboratory in 1-L glass jars. Prior to sampling all vials and caps were washed by

**Table 4** – Results of calibration using SPME and GC-MS

| Analyte | Retention time, min | Calibration range, µg/m³ | $R^2$ | | | |
|---|---|---|---|---|---|---|
| | | | Spring | Summer | Autumn | Winter |
| Benzene | 7.7 | 20-200 | 0.9603 | 0.9906 | 0.9780 | 0.9967 |
| Toluene | 9.3 | 20-200 | 0.9947 | 0.9931 | 0.9988 | 0.9297 |
| Ethylbenzene | 10.6 | 2-20 | 0.9905 | 0.9976 | 0.9956 | 0.9729 |
| *o*-Xylene | 11.7 | 2-20 | 0.9922 | 0.9927 | 0.9711 | 0.9992 |

distilled water and conditioned at 160°C during 4 h. Vials with sampled air were placed on Combi-PAL tray (CTC Analytics AG, Switzerland) for further GC-MS analysis. The analytes were extracted from vials by exposed 85 µm Carboxen/polydimethylsiloxane (Car/PDMS) SPME fiber at room temperature (T=22°C) for 7 min.

*2.3 Air sample analyses with GC-MS*

Analytes were desorbed from a fiber in the split/splitless inlet of 7890A/5975C GC-MS system (Agilent, Santa Clara, USA). Inlet was equipped with a 0.75 mm ID SPME liner (Supelco, USA) operating in splitless mode at 250°C. Separation of BTEX was conducted in a 60 m x 0.25 mm DB-WAXetr (Agilent, USA) column with 0.50 µm film thickness at a constant (1 mL/min) helium (>99.995%, Orenburg-Tehgas, Orenburg, Russia) flow. Temperatures of MS interface, quadrupole and ion source were 250, 150 and 230°C, respectively. Oven temperature was programmed from 40°C (held for 1 min) to 160°C (held for 2 min) with a heating rate of 10°C/min. Total GC run time was 15 min. MS detector was running in selected ion monitoring (SIM) mode for better sensitivity at *m/z* 78, 91, 106 and 106 amu for BTEX, respectively.

*2.4 Calibration and quantification of BTEX*

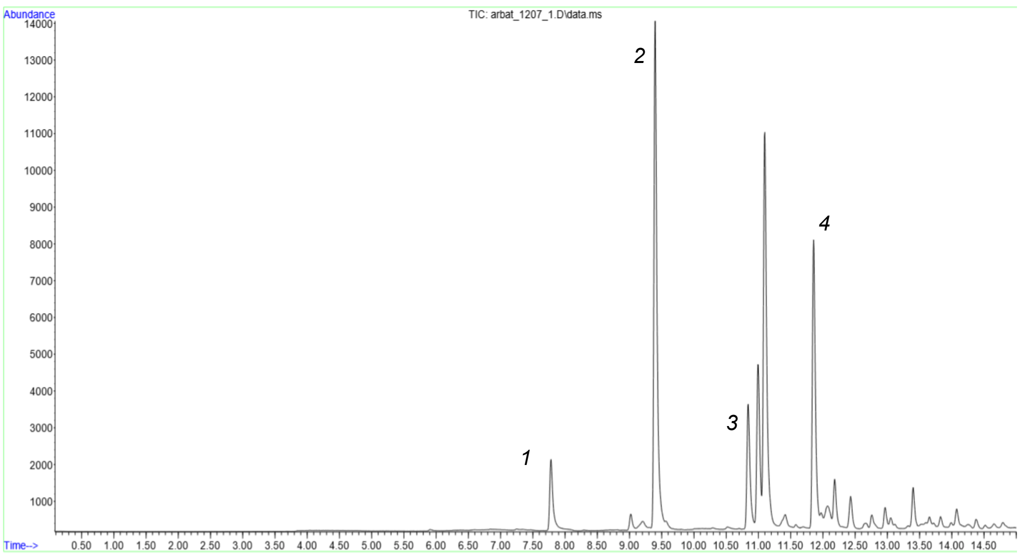
Benzene (99.8%) and toluene (99.8%) were obtained from "EKOS-1" LLP (Moscow, Russia). Ethylbenzene (99.0%) and *o*-xylene (99.0%) were purchased from Sigma-Aldrich (St. Louis, MO, USA). All solutions were prepared in methanol (≥99.9%) purchased from Sigma-Aldrich (St. Louis, MO, USA).

Calibration was conducted using standard addition method. Standard solutions (1.00 µL) were injected into 20-mL vials. Concentrations of benzene, toluene and ethylbenzene, *o*-xylene were different due to their different background concentrations in ambient air. Addition concentrations of BT were 20, 50, 100 and 200 µg/m³; and 2, 5, 10 and 20 µg/m³ for EX. Obtained calibration plots were linear ($R^2$>0.99). Calibration results are presented in Table 4. Mean relative standard deviations (RSD) ranged from 1 to 5%.

## 3. Results and discussion

*3.1 General characterization of data*

Chromatograms obtained for air samples provided a proper separation (Figure 1). Signal-to-noise ratios were




1 – benzene, 2 – toluene, 3 – ethylbenzene, 4 – *o*-xylene

**Figure 1** – Chromatogram of air sample at sampling point A2 at 5 PM on July 12, 2018

higher than 15:1 for all analytes. Mean concentrations of BTEX were calculated for all 27 air samples in Taldykorgan in each sampling period (Tables 5 and 6). RSDs of concentrations at three sampling sites at one sampling day did not exceed 25% in the most cases. The greatest RSDs between sampling sites were observed in winter (55% for toluene at third sampling day), which can be explained by random factors such as a car passing close to sampling site or a smoking person nearby. RSDs of most replicates were in the range ±10%. During entire sampling period, only 14 outliers were found (<11% from total samples), most probably, caused by damage of vials and subsequent leakage of analytes. Thus, the previously developed method [17] is simple, accurate, reproducible and can be applied for air monitoring in different cities.

*3.2 Difference between districts*

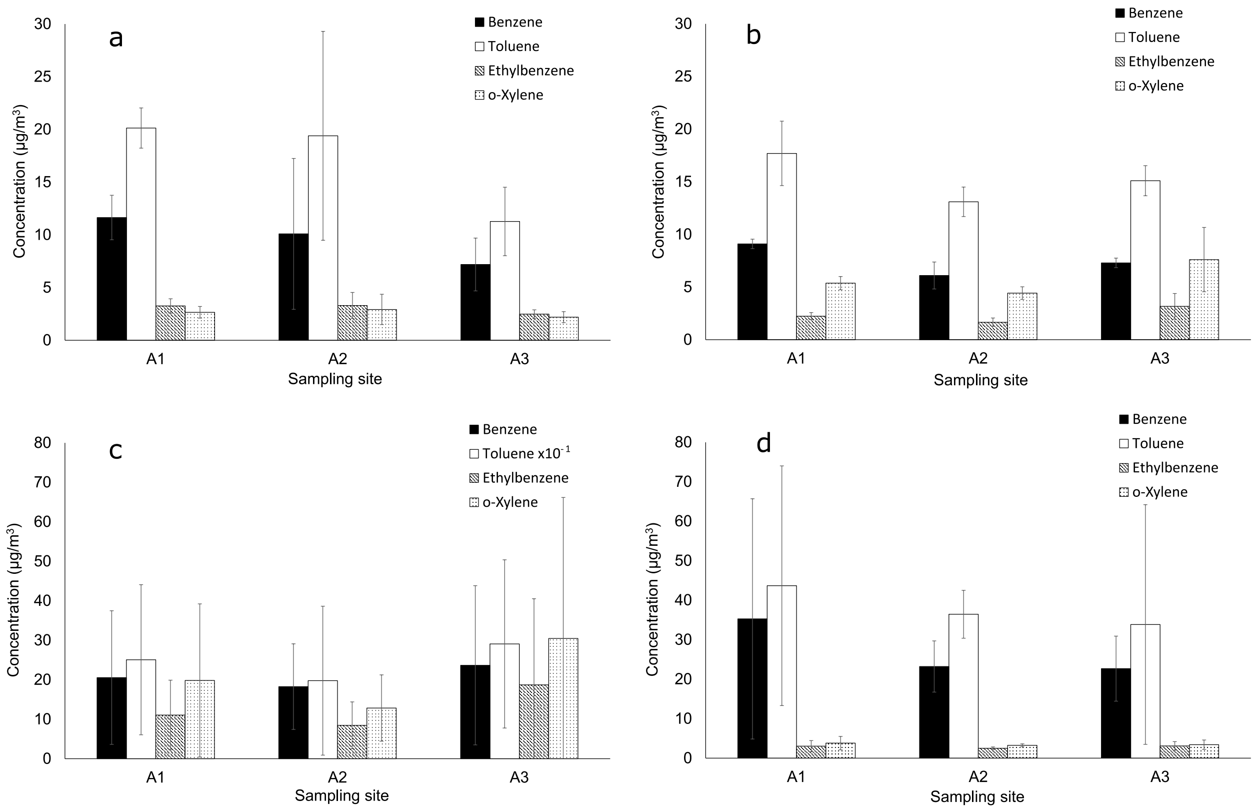
Concentrations of BTEX were different at three districts of Taldykorgan (Karatal, Center and the 2nd microdistrict) (Figure 2). The lowest concentrations of BTEX were detected in Karatal district while the highest – close to Almaty highway in 2nd Microdistrict. Possible reason is that Karatal district is

**Table 5** – Mean concentrations of BTEX in air of Taldykorgan in spring and summer, 2018

| | | Concentration ± SD (μg/m³) | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Sampling season | | Spring 2018 | | | Mean | Summer 2018 | | | Mean |
| Sampling date | | 10/04 | 12/04 | 14/04 | | 12/07 | 14/07 | 16/07 | |
| Benzene | Mean | 7±3 | 13±5 | 10±4 | | 6.1±1.3 | 7.3±0.5 | 9.1±0.5 | |
| | Max | 11 | 18 | 14 | 10±4 | 7.2 | 21.3 | 11.5 | 7.5±0.7 |
| | Min | 4 | 9 | 7 | | 5.4 | 3.5 | 7.6 | |
| Toluene | Mean | 14±6 | 20±10 | 17±4 | | 13.1±1.4 | 15.1±1.4 | 17.7±3.1 | |
| | Max | 20 | 31 | 22 | 17±7 | 16.3 | 32.1 | 22.3 | 15.3±1.9 |
| | Min | 9 | 10 | 15 | | 11.4 | 13.2 | 14.1 | |
| Ethylbenzene | Mean | 3.0±0.9 | 3.5±1.1 | 2.5±0.4 | | 1.9±0.4 | 2.4±1.1 | 2.7±0.8 | |
| | Max | 4.0 | 4.7 | 2.8 | 3.0±0.8 | 2.3 | 4.1 | 3.6 | 2.4±0.9 |
| | Min | 2.4 | 2.9 | 2.1 | | 1.5 | 1.4 | 2.1 | |
| *o*-Xylene | Mean | 1.8±0.3 | 3.3±0.8 | 2.7±0.7 | | 4.8±1.0 | 7±3 | 5.7±1.5 | |
| | Max | 2.0 | 4.3 | 3.1 | 2.6±0.6 | 5.8 | 11 | 7.5 | 6±2 |
| | Min | 1.4 | 2.8 | 1.9 | | 3.8 | 4 | 4.7 | |

**Table 6** – Mean concentrations of BTEX in air of Taldykorgan in autumn 2018 and winter 2018-2019

| | | Concentration ± SD (μg/m³) | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Sampling season | | Autumn 2018 | | | Mean | Winter 2018-2019 | | | Mean |
| Sampling date | | 16/10 | 18/10 | 20/10 | | 14/01 | 16/01 | 18/01 | |
| Benzene | Mean | 39±8 | 15.2±0.4 | 8.4±1.1 | | 45±25 | 20±2 | 17.4±1.4 | |
| | Max | 47 | 15.7 | 9.4 | 21±3 | 70 | 21 | 18.2 | 27±9 |
| | Min | 30 | 14.8 | 7.2 | | 31 | 17 | 15.9 | |
| Toluene | Mean | 530±40 | 160±30 | 65±9 | | 50±20 | 39±13 | 24±13 | |
| | Max | 810 | 230 | 74 | 250±30 | 80 | 53 | 38 | 38±16 |
| | Min | 370 | 120 | 57 | | 35 | 30 | 13 | |
| Ethylbenzene | Mean | 27±4 | 7.5±1.0 | 4.1±0.6 | | 3.9±0.9 | 2.6±0.4 | 2.1±0.3 | |
| | Max | 44 | 8.5 | 4.8 | 13±6 | 4.6 | 2.8 | 2.5 | 2.9±0.5 |
| | Min | 15 | 6.6 | 3.7 | | 2.9 | 2.2 | 1.8 | |
| *o*-Xylene | Mean | 45±20 | 11.6±1.2 | 6.3±0.7 | | 4.5±1.2 | 3.3±0.4 | 2.7±0.8 | |
| | Max | 70 | 12.7 | 7.1 | 21±9 | 5.7 | 3.5 | 3.6 | 3.5±0.8 |
| | Min | 20 | 10.3 | 5.8 | | 3.4 | 2.8 | 2.1 | |




a – spring, b – summer, c – autumn, d – winter

**Figure 2** – Mean concentrations of BTEX at different districts of Taldykorgan

located in the eastern part of the city, almost on its suburb close to the Karatal River that provides air circulation (Table 1). In summer season, on the contrary, the lowest concentrations of BTEX were found in the Center, and the highest – in Karatal district. However, the most popular entertainment places are located in Karatal district, which results in an increased number of people and cars in the summer season.

In the 2nd microdistrict, there is a "ring" of 5 roads, one of which is the Almaty highway with traffic jams and weak air circulation. Results of monitoring in autumn and winter seasons are similar to the ones in the summer. The minimum concentrations of BTEX were determined on Tuesday (April 10) and on Thursday (July 12), most probably, due to rain. Toluene/benzene (T/B) ratios were lower in Karatal district and higher in 2nd microdistrict, but in both cases, T/B ratios were higher than 1. During all sampling period, T/B ratios were lower than 1 in 4 of 36 samples. Such ratios show that the main sources of BTEX originated from transport related sources [18].

*3.3 Seasonal variations*

Sampling of air and analyses were conducted during four seasons (Figure 3). Despite the three-month difference, the seasonal mean concentrations in spring and summer were similar: 9.6 and 7.5 µg/m$^3$ for benzene, 16.9 and 15.3 µg/m$^3$ for toluene, 3.0 and 2.4 µg/m$^3$ for ethylbenzene, 2.6 and 5.8 µg/m$^3$ for *o*-xylene, respectively.

A substantial difference was observed only in the concentration of *o*-xylene that can be caused by an increase in the number of cars in the summer season. Both seasons were characterized by abundant flowering of trees, flowers and other plants, which promotes photosynthesis purifying the air at the same time. A substantial concentration raise of all compounds was in autumn: 21, 250, 12.8 and 21 µg/m$^3$, for BTEX, respectively. These changes could be caused by the beginning of the heating season in October, and also the burning of leaves in open areas. Another factor is the temperature decrease that results in slowing down air circulation. A particular increase in concentration was observed for toluene. Even in winter, the average concentration of toluene (38 µg/m$^3$) was about six times lower than in autumn. The source of such high concentrations of toluene in autumn is impossible to explain using the available data. To answer this question, additional research is needed.

The maximum concentration of benzene (27 µg/m$^3$) was detected in winter season. Mean concentrations of ethylbenzene (2.9 µg/m$^3$) and *o*-xylene (3.5 µg/m$^3$) were in the same range as in spring and summer. In most samples, concentrations of ethylbenzene and *o*-xylene were ten times lower than those of benzene and toluene (Tables 5 and 6), most probably, due to their lower stability in air [19], content in gasoline [20], exhaust gases of cars [21] and other emissions.

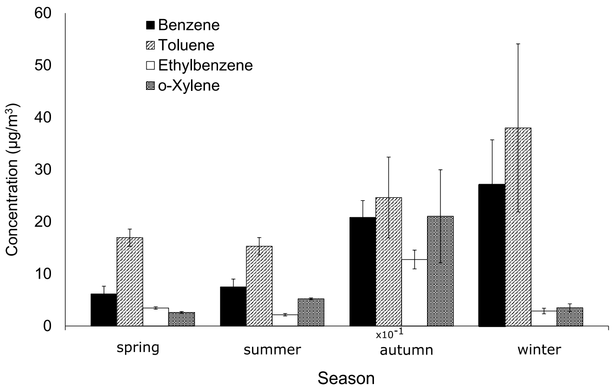



**Figure 3** – Concentrations of BTEX in different seasons

Mean T/B ratio were in the range from 1.2 and 1.8 in all seasons except autumn, when T/B was 10.6.

*3.4 Comparison with other cities*

In the middle of spring, concentrations of BTEX in air of Taldykorgan were compared with BTEX concentrations in Almaty, Kazakhstan (Figure 4). Mean concentrations of benzene and toluene in air collected in spring in Taldykorgan (10 and 17 μg/m³) were about two times lower than at the same season in Almaty (19 and 32 μg/m³). Concentrations of ethylbenzene and *o*-xylene in two cities ranged from 2 to 4 μg/m³. Mean concentrations of BTEX in air of Almaty were close to those in cities around the world with high levels of air pollution such as New Delhi, Cairo, Rome, Ho Chi Minh city, San-Paulo and Manila [17], while in Taldykorgan, BTEX concentrations were substantially lower, which indicates a lower level of air pollution with BTEX.

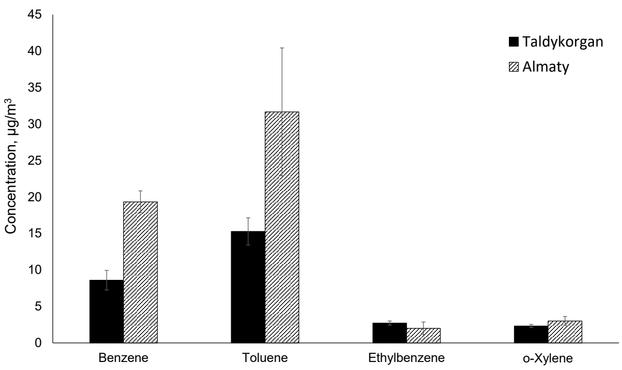



**Figure 4** – Concentrations of BTEX in spring 2018 in air of Taldykorgan and Almaty

**4. Conclusions**

Thus, the monitoring of BTEX in ambient air of Taldykorgan, Kazakhstan was conducted for the first time. Highest concentrations of TEX were observed in autumn, except benzene, maximum concentrations of which were in winter. In Taldykorgan, T/B ratio were higher than 1 in most samples indicating the greatest contribution of transport-related sources of BTEX. Concentrations of benzene and toluene in spring in air of Taldykorgan were about two times lower than those in Almaty at the same period. The concentrations of ethylbenzene and *o*-xylene were similar in both cities. The obtained results prove that the method developed by Baimatova et al. [17] is efficient and can be applied for air monitoring in many other cities. The obtained results can be used for developing air pollution monitoring network in Taldykorgan. For better decision making, Taldykorgan can be compared to other cities using the partial order ranking methodology proposed by Carlsen et al. [22].

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# Determination of chemical composition of the *Ligularia narynensis* root by gas chromatography-mass spectrometry

**[1,2]Nurlybekova A.K., [3]Ye Y., [1]Abilov Zh.A., [1,2,3]Jenis J.***

[1]Faculty of Chemistry and Chemical Technology, al-Farabi Kazakh National University, Almaty, Kazakhstan
[2]Research Center for Medicinal Plants, al-Farabi Kazakh National University, Almaty, Kazakhstan
[3]Shanghai Institute of Materia Medica, Chinese Academy of Sciences, Shanghai, China
*E-mail: *janarjenis@mail.ru*

*Ligularia* is a medicinally important herb of the family Compositae. *Ligularia narynensis* is a perennial herb growing in the mountains, rich in sesquiterpenes, triterpenes, lignans, alkaloids, and steroids. In this work chemical constituents of the root part of medicinal plant *L. narynensis* from Kazakhstan have been determined for the first time. The constituents of the root part of *L. narynensis* were extracted with hexane and analyzed by gas chromatography – mass spectrometry (GC-MS). Thirty compounds were detected, and their concentrations were determined by the method of normalization of peak areas. Among them, the major components are (9Z,12E)-octadeca-9,12-dienoic acid (16.7%), ethyl (9Z,12Z)-octadeca-9,12-dienoate (11.1%), n-hexadecanoic acid (11.0%), (3a,5a,5b,8,8,11a-hexamethyl-1-prop-1-en-2-yl-1,2,3,4,5,6,7,7a,9,10,11,11b,12,13,13a,13b-hexadecahydrocyclopenta[a]chrysen-9-yl) acetate (9.1%), [(3R)-4,4,6a,6b,8a,11,11,14b-octamethyl-1,2,3,4a,5,6,7,8,9,10,12,12a,14,14a-tetradecahydropicen-3-yl] acetate (5.1%). Presence of these bioactive constituents may indicate that the plant extract possesses anti-inflammatory, antimicrobial and anticancer activities, which can serve as a basis for the development of new phytopreparations.

**Keywords:** *Ligularia narynensis*; hexane extract; liposoluble constituents; GC-MS.

# Газ хроматография – масс-спектрометрия әдісімен *Ligularia narynensis* тамырларының химиялық құрамын анықтау

**[1,2]Нурлыбекова А.К., [3]Е Я., [1]Абилов Ж.А., [1,2,3]Жеңіс Ж.***

[1]Химия және химиялық технология факультеті, Әл-Фараби атындағы Қазақ ұлттық университеті, Алматы, Қазақстан
[2]Дәрілік өсімдіктерді ғылыми зерттеу орталығы, Әл-Фараби атындағы Қазақ ұлттық университеті, Алматы, Қазақстан
[3]Шанхай дәрілік препараттар институты, Қытай ғылым академиясы, Шанхай, Қытай
*E-mail: *janarjenis@mail.ru*

*Ligularia* – терапиялық маңызды шөпті өсімдік. *Ligularia narynensis* сесквитерпен, тритерпен, лигнан, алкалоид және стероидтарға бай тауда өсетін көпжылдық өсімдік. Бұл жұмыста Қазақстанда өсетін *L. narynensis* дәрілік өсімдігі тамырларынан химиялық компоненттерінің талдауы бірінші рет жүргізілді. *L. narynensis* өсімдігі тамыр бөлігінен майда ергіш заттар гексанмен экстрагирленген және газды хроматография – масс-спектрометрияның (ГХ-МС) әдісімен талданды. Отыз қосылыс сарапталды және олардың концентрациялары пик аудандарын қалыпқа келтіру әдісімен анықталды, олардың ішінде негізгі (9Z,12E)-октадека-9,12-диен қышқылы (16,7%), этил (9Z,12Z)-октадека-9,12-диеноат (11,1%), n-гексадекан қышқылы (11,0%), (3a,5a,5b,8,8,11a-гексаметил-1-проп-1-ен-2-ил-1,2,3,4,5,6,7,7a,9,10,11,11b,12,13,13a,13b-гексадекагидроциклопента[a]хризен-9-ил) ацетат (9,1%), [(3R)-4,4,6a,6b,8a,11,11,14b-октаметил-1,2,3,4a,5,6,7,8,9,10,12,12a,14,14a-тетрадекагидропицен-3-ил] ацетат (5,1%) болып табылады. Осы биологиялық белсенді компоненттердің болуы өсімдік сығындысы қабынуға қарсы, микробқа қарсы және ісікке қарсы белсенділікке ие екенін көрсетуі мүмкін, бұл жаңа фитопрепараттарды әзірлеуге негіз бола алады.

**Түйін сөздер:** *Ligularia narynensis*; гександі экстракт; майда ергіш заттар; ГХ-МС.

# Определение химического состава корней *Ligularia narynensis* методом газовой хроматографии – масс-спектрометрии

**[1,2]Нурлыбекова А.К., [3]Е Я., [1]Абилов Ж.А., [1,2,3]Жеңіс Ж.***

[1]Факультет химии и химической технологии, Казахский национальный университет имени аль-Фараби, Алматы, Казахстан
[2]Научно-исследовательский центр лекарственных растений, Казахский национальный университет имени аль-Фараби, Алматы, Казахстан
[3]Шанхайский институт лекарственных препаратов, Китайская академия наук, Шанхай, Китай
*E-mail: *janarjenis@mail.ru*

*Ligularia* является терапевтически важным травянистым растением из семейства Compositae. *Ligularia narynensis* – многолетнее растение, произрастающее в горах, богатое сесквитерпенами, тритерпенами, лигнанами, алкалоидами и стероидами. В данной работе впервые был исследован химический состав корней лекарственного растения Казахстана *L. narynensis*. Жирорастворимые компоненты из корневой части *L. narynensis* были экстрагированы гексаном и проанализированы методом газовой хроматографии – масс-спектрометрии (ГХ-МС). Обнаружено тридцать соединений и их концентрации определены методом нормализации площадей пиков, среди которых основными составляющими являются (9Z,12E)-октадека-9,12-диеновая кислота (16,7%), этил (9Z,12Z)-октадека-9,12-диеноат (11,1%), n-гексадекановая кислота (11,0%), (3a,5a,5b,8,8,11a-гексаметил-1-проп-1-ен-2-ил-1,2,3,4,5,6,7,7a,9,10,11,11b,12,13,13a,13b-гексадекагидроциклопента[a]хризен-9-ил) ацетат (9,1%), [(3R)-4,4,6a,6b,8a,11,11,14b-октаметил-1,2,3,4a,5,6,7,8,9,10,12,12a,14,14a-тетрадекагидропицен-3-ил] ацетат (5,1%). Наличие этих биологически активных компонентов, может свидетельствовать о том, что растительный экстракт обладает противовоспалительной, противомикробной и противоопухолевой активностью, что может послужить основой для разработки новых фитопрепаратов.

**Ключевые слова:** *Ligularia narynensis*; гексановый экстракт; жирорастворимые компоненты; ГХ-МС.

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# Determination of chemical composition of the *Ligularia narynensis* root by gas chromatography-mass spectrometry

**[1,2]Nurlybekova A.K., [3]Ye Y., [1]Abilov Zh.A., [1,2,3]Jenis J.***

[1]Faculty of Chemistry and Chemical Technology, al-Farabi Kazakh National University, Almaty, Kazakhstan
[2]Research Center for Medicinal Plants, al-Farabi Kazakh National University, Almaty, Kazakhstan
[3]Shanghai Institute of Materia Medica, Chinese Academy of Sciences, Shanghai, China
*E-mail: *janarjenis@mail.ru*

## 1. Introduction

*Ligularia* is a medicinally important herb of the family Compositae containing about 180 Eurasian species, 17 species growing in mountains of Kazakhstan [1]. Some species in this genus have been used for a long time as folk remedies for their antibacterial, anticancer, and antitumor activities [2-5]. More than 27 *Ligularia* species have been used as traditional Kazakh and Chinese medicinal herbs for the treatment of fever, pain, inflammation, intoxication, cough phlegm, removing blood stasis, emetic, diuresis, cholagogue [6,7]. Previous studies confirmed the presence of sesquiterpenes, triterpenes, sinapyl alcohol derivatives, lignans, alkaloids, and steroids in *Ligularia* [8]. Eremophilane sesquiterpenes are considered as the major secondary metabolites and taxonomic markers of *Ligularia* genus. More than 500 eremophilane sesquiterpenes have been reported from this genus [9,10].

*Ligularia narynensis* is a *perennial* herb growing in Almaty region of Kazakhstan and in Xinjiang province of China. Gao et al. [2,7,11,12] determined the structures of oplopane-type sesquiterpenes, a new 8-O-4′-type neolignan-, oplopane- and guaiane-type sesquiterpenoids, monoterpenoids from the roots of *L. narynensis*.

We have previously reported the chemical investigation results on total bioactive components from root part of *L. narynensis* such as organic acids, flavonoids, moisture content, total ash, and extractives content. Together with eleven macro-, microelements from the ash of plant were determined by using method of multi-element atomic emission spectral analysis. And same time, twenty amino and eight fatty acids were quantified in this plant [13]. In addition, fifty nine liposoluble constituents in chloroform extract from the root part of *L. narynensis* have been identified by gas chromatography-mass spectrometry (GC-MS) method [14].

In our continuously study of the plant, thirty liposoluble constituents in hexane extract from medicinal plant *L. narynensis* have been determined by GC-MS method which grown in Almaty region of Kazakhstan for the first time.

## 2. Experiment

### *2.1 Plant material*

The root part of plant *L. narynensis* was collected in September 2017 from the Zailiysky Alatau Mountains of Almaty region and identified by Dr. Alibek Ydyrys. Specimens (1217-BN-17) were deposited in the Herbarium of Laboratory Plant Biomorphology, Faculty of Biology and Biotechnology, al-Farabi Kazakh National University, Almaty, Kazakhstan.

### *2.2 Extraction*

The dried and powdered *L. narynensis* (100 g) was extracted three consecutive portions of 95% ethanol. Volume of each portion was 800 mL. Extraction time of each portion was 7 days. Filtered extracts were combined and concentrated under reduced pressure with a vacuum rotary evaporator R-300s (Buchi, Switzerland). A residue was dissolved in 150 mL of water and extracted with 150 mL of hexane (99%, China). Then the dry extract (133 mg) was stored at 4°C. For GC-MS analysis, 1 mg of dry extract was dissolved in 1 mL of hexane.

### *2.3 GC-MS conditions*

Analyses were conducted on Agilent 7890A/5975C gas chromatograph coupled to mass spectrometer equipped with a 7683B auto injector (Agilent Technologies, USA). Separation was carried out with a HP-5MS fused silica capillary column (0.25 mm x 30 m, 0.25 μm film, J&W Scientific, USA). The injection port temperature was 310°C. The injection volume was 1 µL, split ratio 5:1. Helium (99.99 %, China) was used as the carrier gas at a rate of 1.0 mL/min. The column temperature was held at 50°C for 10 min, increased by 10°C/min to 300°C, and then held for 40 min. Mass spectra were obtained by electron impact (EI) ionization at 70 eV in scan mode (*m/z* 30-1000 amu). Solvent delay was 3 min. The detector, ion source and transfer line temperature were set to 150, 230 and 250°C, respectively.

*2.4 Identification and quantitation*

The compounds were identified using NIST14 library. Mass fraction of each detected compound was estimated using normalization of peak areas. The sample was analyzed three times. All data are expressed as the mean ± standard deviation of three replicate measurements.

**3. Results and discussion**

The liposoluble constituents present in hexane extract from the root part of *L. narynensis* were analyzed by GC-MS for the first time (Figure 1). Thirty compounds were detected on a chromatogram with a NIST MS library match >70% (Table 1). The prevailing constituents are: (9Z,12E)-octadeca-9,12-dienoic acid (16.7%), ethyl (9Z,12Z)-octadeca-9,12-dienoate (11.1%), n-hexadecanoic acid (11.0%), (3*a*,5*a*,5*b*,8,8,11*a*-hexamethyl-1-prop-1-en-2-yl-1,2,3,4,5,6,7,7*a*,9,10,11,11*b*,12,13,13*a*,13*b*-hexadecahydrocyclopenta[a]chrysen-9-yl) acetate (9.1%), [(3*R*)-4,4,6*a*,6*b*,8*a*,11,11,14*b*-octamethyl-1,2,3,4*a*,5,6,7,8,9,10,12,12*a*,14,14*a*-tetradecahydropicen-3-yl] acetate (5.1%). Table 1 report the composition of the liposoluble constituents of *L. narynensis.*

The earlier reports on the essential oil from *L. virgaurea* has been reported to possess 4-methyl-1-(1-methylethyl) 3-cyclohexen-1-ol (14.4%), 2-methyl-heptane (9.8%), 3-methyl-heptane (8.3%), heptane (7.9%), 4-methyl-1-(methylethyl)-bicyclo [3,1,0] hex-2-ene (7.8%), 3-methyl-hexane (6.4%), 2-methyl-hexane (5.5%) and limonene (4.7%) [15]. *L. stenocephala* growing in Korea was reported to possess *α*-pinene (41.1%), limonene (17.7%), 2,7-bis(spirocyclopropane) bicycle [2.2.1] heptan-5-one (13.2%), *o*-anisaldehyde (5.9%) and phellandrene (5.2%) as the major constituents of its oil [16]. The oil from *L. persica* (from Iran) contained (Z)-*β*-ocimene (12.5%), *cis*-*m*-mentha-2,8-diene (8.8%), *α*-eudesmol (8.7%), valencene (5.9%) and 14-hydroxy-*δ*-cadinene (5.7%) as the major constituents [17].

On correlating the liposoluble constituents' composition of these species, it appears that *L. virgaurea, L. stenocephala* and *L. persica* are chemotaxonomically not related to *L. narynensis.* These results indicated that the differences in the volatile profiles of the species are primarily qualitative. Taken together, these data suggest that *L. narynensis* may play very important role in the development of new phytopreparations.

The main liposoluble constituent of *L. narynensis* (9Z,12E)-octadeca-9,12-dienoic acid (16.7%) have been reported to have antimicrobial activity [18]. And second major liposoluble constituent ethyl (9Z,12Z)-octadeca-9,12-dienoate (11.1%) has a hypocholesterolemic, nematicide, antiarthritic, hepatoprotective, antiandrogenic, hypocholesterolemic, 5-alpha reductaseinhibitor, antihistaminic, anticoronary, insectifuge, antieczemic, antiacne activities [19]. n-Hexadecanoic acid (11.0%) might function as an anti-inflammatory agent [20]. Furthermore, this acid has an inhibitory activity. These findings further confirm the medicinal value of plant and its anticancer cytotoxic potential [21,22].

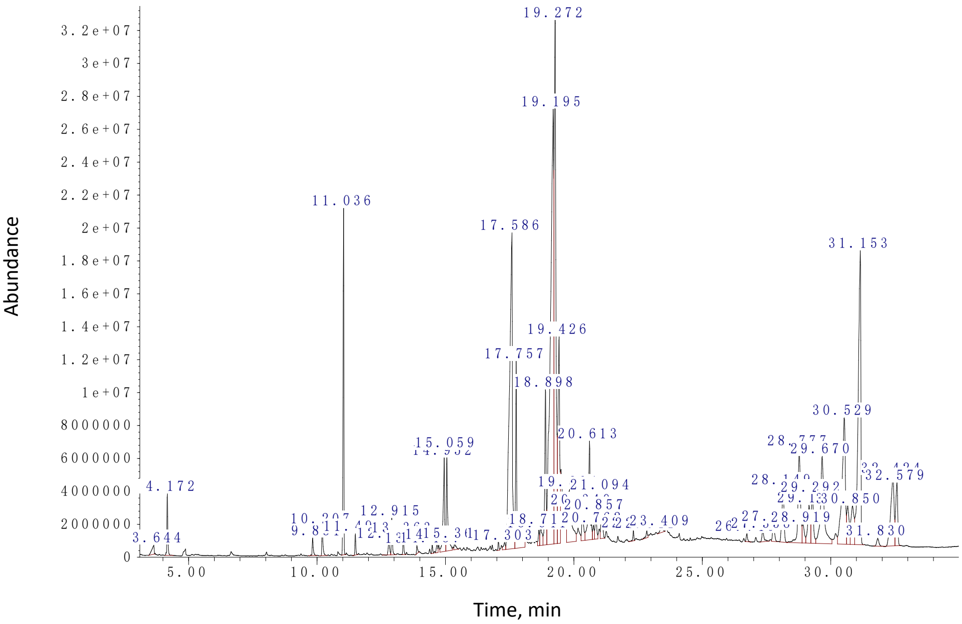


**Figure 1** – Total ion (*m/z* 30-1000) chromatogram of hexane extract from the root part of *L. narynensis*

**Table 1** – The liposoluble constituents from the root part of *L. narynensis*

| Peak No. | Constituent | Cas No. | Retention time, min | Molecular formula | Molecular weight, amu | Content[a], % | NIST Match, % |
|---|---|---|---|---|---|---|---|
| 1 | 2-Methoxy-4-vinylphenol | 7786-61-0 | 9.831 | $C_9H_{10}O_2$ | 150 | 0.23±0.03 | 94 |
| 2 | 2,6-Dimethoxyphenol | 91-10-1 | 10.207 | $C_8H_{10}O_3$ | 154 | 0.39±0.01 | 98 |
| 3 | Phenoxybenzene | 101-84-8 | 11.036 | $C_{12}H_{10}O$ | 170 | 3.4±0.1 | 87 |
| 4 | Dimethylbenzene-1,2-dicarboxylate | 131-11-3 | 11.494 | $C_{10}H_{10}O_4$ | 194 | 0.22±0.03 | 97 |
| 5 | 1-Chloro-4-phenoxybenzene | 7005-71-3 | 13.363 | $C_{12}H_9ClO$ | 204 | 0.22±0.02 | 97 |
| 6 | 4-Hydroxy-3,5-dimethoxybenzaldehyde | 134-96-3 | 13.892 | $C_9H_{10}O_4$ | 182 | 0.09±0.01 | 96 |
| 7 | 2,6-Dimethoxy-4-[(E)-prop-1-enyl]phenol | 20675-95-0 | 14.487 | $C_{11}H_{14}O_3$ | 194 | 0.10±0.01 | 98 |
| 8 | 1-Tetradecanoic acid | 544-63-8 | 15.362 | $C_{14}H_{28}O_2$ | 228 | 0.15±0.01 | 93 |
| 9 | Methyl hexadecanoate | 112-39-0 | 17.067 | $C_{17}H_{34}O_2$ | 270 | 0.10±0.01 | 98 |
| 10 | n-Hexadecanoic acid | 57-10-3 | 17.586 | $C_{16}H_{32}O_2$ | 256 | 11.0±0.8 | 99 |
| 11 | Ethyl hexadecanoate | 628-97-7 | 17.757 | $C_{18}H_{36}O_2$ | 284 | 3.1±0.5 | 99 |
| 12 | Methyl (10E,12Z)-octadeca-10,12-dienoate | 21870-97-3 | 18.641 | $C_{19}H_{34}O_2$ | 294 | 0.23±0.03 | 99 |
| 13 | Methyl (9Z,11E)-octadeca-9,11-dienoate | 79790-32-2 | 18.713 | $C_{19}H_{34}O_2$ | 294 | 0.46±0.04 | 87 |
| 14 | (9Z,12E)-Octadeca-9,12-dienoic acid | 506-21-8 | 19.195 | $C_{18}H_{32}O_2$ | 280 | 16.7±1.0 | 99 |
| 15 | Ethyl (9Z,12Z)-octadeca-9,12-dienoate | 6114-21-2 | 19.272 | $C_{20}H_{36}O_2$ | 308 | 11.1±0.9 | 99 |
| 16 | Octadecanoic acid | 57-11-4 | 19.426 | $C_{18}H_{36}O_2$ | 284 | 4.4±0.9 | 95 |
| 17 | 1,4-Dimethyl-7-(1-methylethyl)-azulen-2-ol | 18937-66-1 | 19.834 | $C_{15}H_{18}O$ | 214 | 2.5±0.3 | 74 |
| 18 | 2-Butyl-5-hexyloctahydro-1H-indene | 55044-33-2 | 20.762 | $C_{19}H_{36}$ | 264 | 0.23±0.03 | 94 |
| 19 | Bis(2-ethylhexyl) benzene-1,2-dicarboxylate | 117-81-7 | 22.321 | $C_{24}H_{38}O_4$ | 390 | 0.12±0.01 | 96 |
| 20 | Ethyl docosanoate | 5908-87-2 | 22.841 | $C_{24}H_{48}O_2$ | 368 | 0.12±0.02 | 95 |
| 21 | (9Z,12Z)-1,3-Dihydroxypropan-2-yl octadeca-9,12-dienoate | 3443-82-1 | 23.409 | $C_{21}H_{38}O_4$ | 354 | 0.15±0.02 | 97 |
| 22 | (2*S*)-2,5,7,8-Tetramethyl-2-[(4*S*,8*S*)-4,8,12-trimethyltridecyl]-3,4-dihydrochromen-6-ol | 1406-18-4 | 26.740 | $C_{29}H_{50}O_2$ | 430 | 0.13±0.01 | 97 |
| 23 | (3*S*,8*S*,9*S*,10*R*,13*R*,14*S*,17*R*)-17-[(2*R*,5*R*)-5,6-Dimethylheptan-2-yl]-10,13-dimethyl-2,3,4,7,8,9,11,12,14,15,16,17-dodecahydro-1*H*-cyclopenta[a]phenanthren-3-ol | 474-62-4 | 27.774 | $C_{28}H_{48}O$ | 400 | 0.41±0.04 | 99 |
| 24 | (3*b*,24*S*)-Stigmast-5-en-3-ol | 83-47-6 | 28.777 | $C_{29}H_{50}O$ | 414 | 2.8±0.3 | 99 |
| 25 | (3*S*,4*aR*,6*aR*,6*bS*,8*aR*,12*aR*,14*aR*,14*bR*)-4,4,6*a*,6*b*,8*a*,11,11,14*b*-Octamethyl-1,2,3,4*a*,5,6,7,8,9,10,12,12*a*,14,14*a*-tetradecahydropicen-3-ol | 559-70-6 | 29.160 | $C_{30}H_{50}O$ | 426 | 1.14±0.08 | 99 |
| 26 | (6aR,6bS,8aR,12aS,14aR,14bR)-4,4,6a,6b,8a,11,11,14b-Octamethyl-2,4a,5,6,7,8,9,10,12,12a,14,14a-dodecahydro-1H-picen-3-one | 638-97-1 | 29.292 | $C_{30}H_{48}O$ | 424 | 1.27±0.07 | 94 |
| 27 | (3*S*,4*aR*,6*aR*,6*bS*,8*aR*,11*R*,12*S*,12*aR*,14*aR*,14*bR*)-4,4,6*a*,6*b*,8*a*,11,12,14*b*-Octamethyl-2,3,4*a*,5,6,7,8,9,10,11,12,12*a*,14,14*a*-tetradecahydro-1*H*-picen-3-ol | 638-95-9 | 29.670 | $C_{30}H_{50}O$ | 426 | 3.9±0.5 | 93 |
| 28 | [(3R)-4,4,6a,6b,8a,11,11,14b-Octamethyl-1,2,3,4a,5,6,7,8,9,10,12,12a,14,14a-tetradecahydropicen-3-yl] acetate | 1616-93-9 | 30.529 | $C_{32}H_{52}O_2$ | 468 | 5.1±0.5 | 97 |
| 29 | (3S,8aS)-5,8a-Dimethyl-3-prop-1-en-2-yl-2,3,4,4a,7,8-hexahydro-1H-naphthalene | 84238-29-9 | 30.665 | $C_{15}H_{24}$ | 204 | 1.11±0.09 | 90 |
| 30 | (3a,5a,5b,8,8,11a-Hexamethyl-1-prop-1-en-2-yl-1,2,3,4,5,6,7,7a,9,10,11,11b,12,13,13a,13b-hexadecahydrocyclopenta[a]chrysen-9-yl) acetate | 1617-68-1 | 31.153 | $C_{32}H_{52}O_2$ | 468 | 9.1±0.6 | 95 |

[a] Data are expressed as means ± standard deviation of three replicate measurements

**4. Conclusion**

In this work, the investigation of the liposoluble constituents from the roots of *L. narynensis* of Kazakhstan have been made for the first time. As the results of this study, thirty liposoluble compounds were quantified from medicinal plant in which the major constituents are (9Z,12E)-octadeca-9,12-dienoic acid (16.7%), ethyl (9Z,12Z)-octadeca-9,12-dienoate (11.1%), n-hexadecanoic acid (11.0%), (3*a*,5*a*,5*b*,8,8,11*a*-hexamethyl-1-prop-1-en-2-yl-1,2,3,4,5,6,7,7*a*,9,10,11,11*b*,12,13,13*a*,13*b*-hexadecahydrocyclopenta[a]chrysen-9-yl) acetate (9.14%), [(3*R*)-4,4,6*a*,6*b*,8*a*,11,11,14*b*-octamethyl-1,2,3,4*a*,5,6 7,8,9,10,12,12*a*,14,14*a*-tetradecahydropicen-3-yl] acetate (5.10%). Presence of these bioactive constituents may indicate that the plant extract possesses anti-inflammatory, antimicrobial and anticancer activities. The results can be used in future investigations of *L. narynensis*, to improve the knowledge about this plant, and to provide a venue to develop and debate new ideas. Further phytochemical study of the root part of *L. narynensis* opens prospects for the creation of new plant-based preparations. The practice of using medicinal plants in recent years is expanding due to their low cost, complex therapeutic effect on the body, low toxicity and the possibility of long-term use without side effects. The development of this direction through introduction of medicinal plants into medical practice and expansion of the assortment of phytopreparations is quite promising.

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# Adsorption modification of the zeolite surface with chitosan

[1]Tattibayeva Zh.A.*, [1]Tursynbetov M.T., [1]Tazhibayeva S.M., [2]Kujawski W., [1]Musabekov K.B.

[1]Al-Farabi Kazakh National University, Almaty, Kazakhstan
[2]Nicolaus Copernicus University in Torun, Faculty of Chemistry, Torun, Poland
*E-mail: *jani-1989@mail.ru*

In order to modify the surface, thermal acid activation of the zeolite of the Chankanai deposit was conducted. It was found that the treatment of the mineral with acid at high temperature leads to a decrease in the content of Ca, Al and Sr in its composition. Adsorption of chitosan on the surface of thermoacid-activated zeolite was also studied. Processing of the adsorption isotherms according to Langmuir and Freundlich models showed that the maximum adsorption of chitosan on the zeolite surface is 30.1 mg/g and the Freundlich constant 1/n is 0.75. On the IR-spectra of chitosan-modified zeolite, a certain shift to the higher frequencies of the peak was found at the oscillation frequency of 1638 $cm^{-1}$, which can be explained by the contribution of amino groups adsorbed on the surface of the mineral. The shift to the left of the peak at 581 $cm^{-1}$, typical for aluminosilicate groups, is also an evidence of their interactions with chitosan. When studying the effect of chitosan concentration on the wetting of the modified zeolite powder, it was found that at concentration of $2 \cdot 10^{-3}$ base mol/L, an increase in the wetting angle from 10° to 47° occurs due to surface overcharging. According to the data of adsorption, IR spectroscopy and wetting of the surface, the main mechanism for binding chitosan to the zeolite surface was due to the electrostatic interaction of polymer amino groups with silicate and aluminosilicate groups of the mineral, stabilized by hydrogen bonds between the OH-groups of the polymer and ≡Si-O-groups of the solid phase.

**Keywords:** zeolite; chitosan; modification; adsorption; thermal acid activation.

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# Цеолит бетін хитозанмен адсорбциялық өңдеу

[1]Тәттібаева Ж.А.*, [1]Тұрсынбетов М.Т., [1]Тәжібаева С.М., [2]Куявский В., [1]Мұсабеков Қ.Б.

[1]Әл-Фараби атындағы Қазақ ұлттық университеті, Алматы, Қазақстан
[2]Николай Коперник университеті, Торунь, Польша
*E-mail: *jani-1989@mail.ru*

Бетті өңдеу мақсатында Шаңқанай кен орнының цеолитін термоқышқылдық активациялау жүргізілді. Жоғары температурада минералды қышқылмен өңдеу оның құрамындағы Ca, Al және Sr үлесінің төмендеуіне апаратындығы анықталды. Термоқышқылды белсендірілген цеолит бетінде хитозанның адсорбциясы зерттелді. Адсорбция мәліметтерін Ленгмюр және Фрейндлих модельдері бойынша өңдеу цеолит бетіндегі хитозанның максималды адсорбциясының мәні 30,1 мг/г, ал 1/n константасының 0,75-ке жететіндігін көрсетті. Хитозанмен өңделген цеолиттің ИҚ-спектрінде 1638 $см^{-1}$ тербеліс жиілігіндегі шыңның жоғары жиілігі анықталды, бұл жайт минерал бетінде адсорбцияланған амин топтарының үлесімен негізделді. Алюмосиликатты топтарға тән 581 $см^{-1}$ аймағындағы шыңның да сол жаққа ығысуы олардың хитозанмен өзара әрекеттесуінің дәлелі болып табылады. Хитозан концентрациясының цеолит ұнтағына су тамшыларының жұғуына әсерін зерттеу барысында полимердің $2 \cdot 10^{-3}$ негіз-моль/л концентрациясында жұғу бұрышының 10°-тан 47°-қа дейін артуы байқалды және бұл өзгерістер беттің теріс зарядының оң зарядқа ауысуымен негізделді. Адсорбция, ИҚ-спектроскопия, сканерлеуші электрондық микроскопия және жұғу мәліметтері бойынша хитозан макромолекулаларының цеолит бетімен байланысуының негізгі механизмі полимердің амин топтарының минералдың силикаттық және алюмосиликаттық топтарымен электростатикалық әрекеттесуі болып табылады, бұл әрекеттесу полимердің ОН-топтары мен қатты фазаның ≡Si-O- топтары арасындағы Н-байланыстармен тұрақтандырылған.

**Түйін сөздер:** цеолит; хитозан; өңдеу; адсорбция; термоқышқылдық активация.

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# Адсорбционная модификация поверхности цеолита хитозаном

[1]Таттибаева Ж.А.*, [1]Турсынбетов М.Т., [1]Тажибаева С.М., [2]Куявский В., [1]Мусабеков К.Б.

[1]Казахский национальный университет имени аль-Фараби, Алматы, Казахстан
[2]Университет Николая Коперника, Торунь, Польша
*E-mail: jani-1989@mail.ru

С целью модификации поверхности проведена термокислотная активация цеолита Чанканайского месторождения. Установлено, что обработка минерала кислотой при повышенной температуре приводит к снижению содержания Ca, Sr и Al в его составе. Изучена адсорбция хитозана на поверхности термокислотно-активированного цеолита. Обработка данных адсорбции по Ленгмюру и Фрейндлиху показала, что значение максимальной адсорбции хитозана на поверхности цеолита составляет 30,1 мг/г, а константы 1/n - 0,75. На ИК-спектрах модифицированного хитозаном цеолита обнаружено некоторое смещение влево пика при частоте колебаний 1638 $см^{-1}$, что объяснено вкладом аминогрупп, адсорбированных на поверхности минерала. Смещение влево пика при 581 $см^{-1}$, характерного для алюмосиликатных групп, также является свидетельством их взаимодействия с хитозаном. При изучении влияния концентрации хитозана на смачивание порошка цеолита установлено, что при концентрации $2 \cdot 10^{-3}$ осново-моль/л происходит увеличение угла смачивания от 10° до 47°, обусловленное перезарядкой поверхности. На основании данных адсорбции, ИК-спектроскопии и смачивания поверхности сделано заключение, что основным механизмом связывания хитозана с поверхностью цеолита является электростатическое взаимодействие аминогрупп полимера с силикатными и алюмосиликатными группами минерала, стабилизированное Н-связями между ОН-группами полимера и ≡Si-O- группами твердой фазы.

**Ключевые слова:** цеолит; хитозан; модификация; адсорбция; термо-кислотная активация.

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# Adsorption modification of the zeolite surface with chitosan

[1]Tattibayeva Zh.A.*, [1]Tursynbetov M.T., [1]Tazhibayeva S.M., [2]Kujawski W., [1]Musabekov K.B.

[1]Al-Farabi Kazakh National University, Almaty, Kazakhstan
[2]Nicolaus Copernicus University in Torun, Faculty of Chemistry, Torun, Poland
*E-mail: *jani-1989@mail.ru*

## 1. Introduction

Clay minerals are the most widely used natural raw materials, ranging from building materials to enterosorbents [1-5]. Among them, zeolites, so-called "molecular sieves", are particularly distinguished, due to their ability to adsorb molecules smaller than 10 Å. Along with high adsorption capacity, they also have ions-exchange properties [6-8]. The use of zeolites for purification of gases was also reported [9]. The high porosity and the ability to control particle size by dispersion determine the prospects for using this mineral in the development of composite materials: in construction chemistry by combining with other minerals, in biotechnology as carriers of enzymes, microorganism cells to create selective biosorbents and biocatalysts. However, as natural minerals, zeolites have a negative charge on their surface. Most microbial cells are also negatively charged. For combination with other minerals and microbial cells, the modification of the zeolite surface with positively charged polymers and surfactants is needed. The well-known cationic polymers and surfactants such as polydimethyldiallylammonium chloride, polyvinylpyridinium chloride, cetylpyridinium bromide and chloride can have toxic effects on enzymes and microbial cells. Among cationic polymers, chitosan obtained from chitin is most harmless to living organisms and environment [10,11]. However, there is no information in the literature about the use of chitosan to regulate the surface properties of clay minerals. In this regard, the aim of this study was to modify the surface of the zeolite using chitosan.

## 2. Experiment

Zeolites of Chankhanai deposit (Almaty region) were used. Zeolite samples were crushed to particle size of 10-30 µm, then washed twice with distilled water and dried at 200°C. However, zeolite particles with long-term exposure in the aqueous medium increased the optical density of suspensions due to leaching of fine particles of impurities, which complicated their analysis. In this respect, the method of treatment with mineral acid and high temperature was used, which is widely used for purification of clays from impurities [12, 13]. 50 g of zeolite were mixed with 250 mL of 15% (w/w) $H_2SO_4$, boiled for 4 h at 100°C on a water bath. Then the mineral was washed with distilled water, pH of which was brought to 7 by 0.1 M NaOH. The purified zeolite was dried at 200°C for 2 h.

Zeolite composition was determined using X-ray fluorescence spectrometer Focus 2M (Russia) using Fe-radiation in the range from 2 V to 37 V with a measurement accuracy 3%. The intensity of the diffraction maxima was estimated by an analytical method in a tetragonal singoniya. Experiments were carried out at 25±0.2°C.

Chitosan purchased from Sigma Aldrich (USA) was used for the modification of zeolite. The concentration of chitosan was varied in the range of $(0.1\text{-}1.0)\cdot10^{-2}$ base mol/L. For this purpose, 100 mL of a solution containing 0.16 g of chitosan was prepared. Then 1 mL of the solution was mixed with 9 mL of distilled water. Other solutions were prepared in a similar way. For modification of the zeolite surface, 1 g of mineral sample was put in 20 mL chitosan solution with a concentration of $(0.1\text{-}1.0)\cdot10^{-2}$ base mol/L for 2 h. Adsorption of chitosan on the zeolite surface was calculated by the formula: $A=(C_1-C_2)\cdot V/m$, where $C_1$ and $C_2$ – initial and equilibrium concentrations of chitosan, base mol/L; $V$ – solution volume, L; $m$ – zeolite mass, g.

Determination of chitosan concentration was performed using UV–7504 (Shanghai, China) spectrophotometer with a measurement accuracy ±2%. Analysis was based on the dependence of the optical density of polymer solutions on the concentration. Experiment was carried out in cuvettes with an absorbing layer thickness of 1 cm. The dependence of the optical density of the chitosan solution with concentration of $1{\cdot}10^{-2}$ base mol/L on a wavelength in the range of 200-800 nm in 10 nm increments was obtained. The maximum optical density corresponded to a wavelength of 210 nm. Then, at this wavelength, a concentration dependence of the optical density of chitosan was obtained. Range of polymer concentration from $10^{-3}$ base mol/L to $10^{-2}$ base mol/L was used. For analysis, the linear range of the curve was used.

FTIR-spectroscopy studies were performed using the Fourier-transform infrared spectrometer Avatar 370-CsI (Thermo Nicolet, USA) in tablets with KBr. For this, the samples of thermal-acid-activated zeolite, chitosan powder, and zeolite treated with $1{\cdot}10^{-2}$ base mol/L concentration solution of chitosan were used. Each of them was individually pressed with KBr at the ratio 2:250 (mg/mg). Studies were conducted in the frequency range from 400 to 4000 cm$^{-1}$.

KBr with quality "pure for analysis" (ChemPlus, Russia) was used during FTIR analysis for the preparation of tablets. Dried sample of KBr was used.

The wetting angle was determined by applying water droplets to the powder of zeolite samples modified with chitosan solution with concentration from $0.1{\cdot}10^{-2}$ to $1.0{\cdot}10^{-2}$ base mol/L, and drawing the tangents to the images of droplets.

### 3. Results and discussion

There are two large zeolite deposits in Kazakhstan: Tayzhuzgen (East Kazakhstan Region) and Chankanay (Almaty Region). The zeolites of the Chankanay deposit are smaller than the Tayzhuzgen ones. In addition, the latter contain quartz in their composition, which compicates their grinding. Thermal-acid treatment improves the quality of clay sorbents, however, it significantly changes their composition. It was found that thermal-acid treatment of the Chankanay deposit zeolite results in the decrease of the amount of Ca, Al, Ti, Mn and Sr in the composition of the mineral, and the decrease in the content of Ca, Al and Sr is particularly observable (Figure 1, Table 1). The Ca content in the initial sample decreased from 8.9 wt% to 1.8 wt% after treatment; the Sr content decreased from 2.1 wt% to 0.5 wt%. In the case of Al, a decrease of content from 24.3 to 16.6 wt% was observed. At the same time, there is a significant increase in the amount of silicon and iron. This change in the composition of zeolite indicates that Ca, Mn, and Sr compounds in the composition of the mineral are presented as impurities, which can be easily removed by thermal-acid treatment. The decrease of Al content can be explained by specific decomposition of mineral structure. An increase in the amount of Si and Fe can be associated with an increase in their specific contribution to the mass of zeolite upon dissolution of other elements. It also follows from these data that Ca, Al, Ti, Mn and Sr ions play the role of exchange cations, and atoms of Si and Fe, being the main components of the crystal lattice of the mineral, will play a decisive role in the adsorption of other substances on the zeolite surface.

**Table 1** – Effect of thermal-acid activation on the composition of the zeolite

| Base elements | Mass content, % | |
|---|---|---|
| | Natural zeolite | Thermal-acid activated zeolite |
| K | 4.7 | 5.0 |
| Ca | 8.9 | 1.8 |
| Si | 18.4 | 29.1 |
| Fe | 40.0 | 45.8 |
| Al | 24.3 | 16.6 |
| Ti | 1.1 | 0.9 |
| Mn | 0.5 | 0.3 |
| Sr | 2.1 | 0.5 |

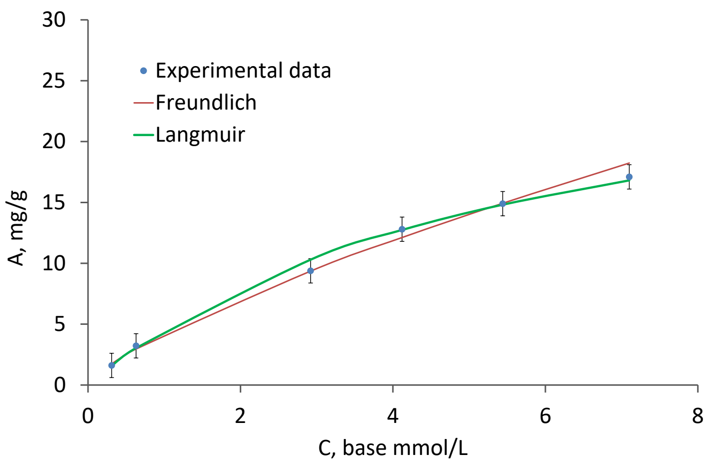


**Figure 1** – Adsorption isotherm of chitosan on the surface of thermal-acid activated zeolite, T=25°C

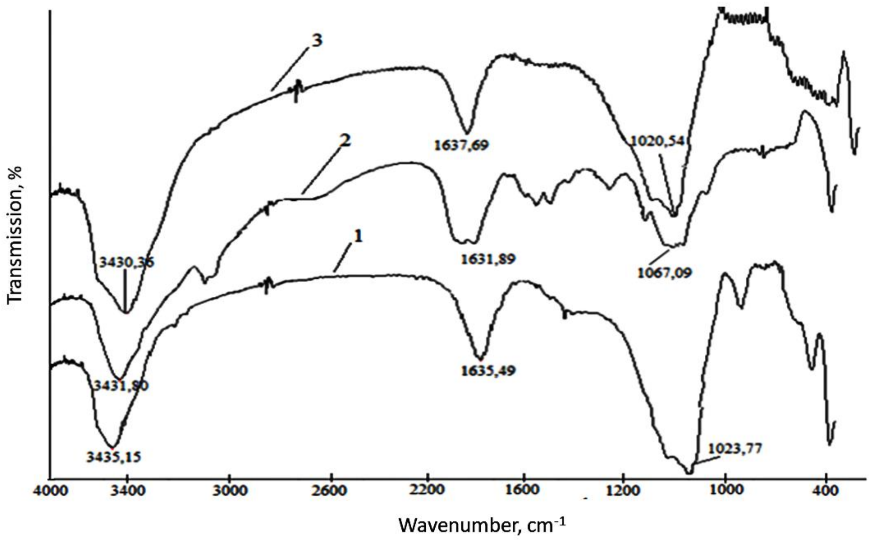


**Figure 2** – FTIR spectrum of thermal-acid activated zeolite (1), chitosan (2) and zeolite-chitosan system (3)

Studies on the surface modification of zeolite particles after thermal-acid treatment were aimed at determining the optimal concentration of the modifying agent (Figure 1). The isotherm of chitosan adsorption on zeolite has the form of a rising curve. The data of adsorption were described using Langmuir and Freundlich models [14]. To do this, Langmuir's equation was transformed into the straight-line equation:

$$\frac{C}{A} = \frac{1}{A_{max} \cdot K} + \frac{C}{A_{max}} \quad (1)$$

The intercept was equal to $\frac{1}{A_{max} \cdot K}$ , and the slope was equal to $1/A_{max}$. The value of maximum adsorption of chitosan on the surface of zeolite according to Langmuir model was 30.1 mg/g, which is close to the values of adsorption of low-molecular surfactants on the surface of clays [15]. To calculate the Freundlich constants, the adsorption data $A=K_F \cdot C^{1/n}$ were represented graphically as a function of $lgA=f(lg\,C)$. The intercept was equal to $lgK_F$, and the slope was equal to $1/n$. The value of constant $K_F$ indicates the adsorption capacity of adsorbent, and $1/n$ indicates the affinity of the adsorbed substance to the adsorbent, i.e. the intensity of interaction of an adsorbate-adsorbent. If the value of $1/n$ is in the range of 0.6-0.8, the adsorbent is considered favorable for adsorption [16,17]. In the case of chitosan adsorption on zeolite, $1/n$ was equal to 0.75 meaning that it is within this interval.

The adsorption isotherms constructed using Langmuir and Freundlich constants coincide with the isotherms obtained from experimental data. The values of the determination coefficient $R^2$ were 0.98 for the Langmuir model and 0.99 for the Freundlich model (Table 2). It follows that both models satisfactorily describe the process of adsorption of chitosan on the zeolite surface.

FTIR-spectroscopic studies were conducted to obtain information on the mechanism of zeolite-chitosan interaction. The data of FTIR-spectroscopic measurements of zeolite before and after chitosan modification are shown in Figure 2. In the case of the initial zeolite, the greatest adsorption bands were recorded at the frequencies of vibration of 3435 $cm^{-1}$, 2488 $cm^{-1}$, 1635 $cm^{-1}$, 1024 $cm^{-1}$, 856 $cm^{-1}$ and 581 $cm^{-1}$. Fluctuations in the range from 1600 to 3600 $cm^{-1}$ can be caused by deformation vibrations of OH-groups of water molecules and SiOH-groups of mineral. The peak at 1024 $cm^{-1}$ can be provided with deformation vibrations of Si-O-Si groups, and the peak at 581 $cm^{-1}$ is due to the presence of Si-O-Si and Al-O-Si groups in the zeolite [18,21].

In the FTIR-spectrum of chitosan, the peak at 3432 $cm^{-1}$ can be due to OH-groups of water molecules. In addition, it can be attributed to the $NH_2$-groups of the polymer. The adsorption band at 2881 $cm^{-1}$can be attributed to the vibrations of C-H bonds of the hydrocarbon chains of chitosan, and band at 1632 $cm^{-1}$ is caused by $NH_2$- groups. The wide adsorption band at the 1067 $cm^{-1}$ frequency can be explained by the skeletal vibrations of the C-O groups [19,20].

**Table 2** – The comparison of models of chitosan adsorption on the surface of zeolite

| System | Temperature, °C | Langmuir model | | | Freundlich model | | |
|---|---|---|---|---|---|---|---|
| | | $K_L$, L/mg | $A_{max}$, mg/g | $R^2$ | 1/n | $K_F$, mg/g | $R^2$ |
| Zeolite-chitosan | 25 | 0.18 | 30.1 | 0.98 | 0.75 | 4.18 | 0.99 |

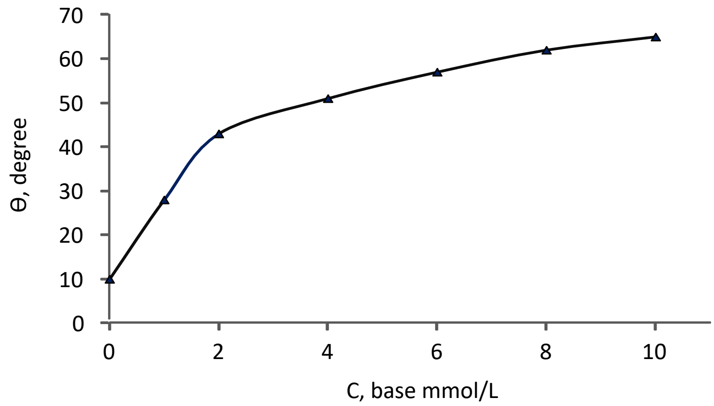


**Figure 3** – Effect of chitosan concentration on the wetting of zeolite particles surface

In the FTIR-spectrum of the modified zeolite, all characteristic bands of the initial zeolite were observed (Figure 3). Some shifts to the higher frequencies of the peak at 1638 $cm^{-1}$ can be explained by the contribution of amino groups adsorbed on the surface of the mineral. The shift to the left of the peak at 581 $cm^{-1}$, typical for aluminosilicate groups, is also evidence of their interaction with chitosan. Vibrations at 856 $cm^{-1}$ and 921 $cm^{-1}$ can be assigned to Si-O-Si bonds [21, 22].

Based on the FTIR-spectroscopy data it can be assumed that the adsorption of chitosan on the zeolite surface takes place due to the electrostatic interaction of amino groups of the polymer with the silicate and aluminosilicate groups of the zeolite, stabilized by hydrogen bonds between the OH, $NH_2$-groups of chitosan and the oxygen atoms of the silicate groups.

Studies of the wettability of the surface of mineral particles were also conducted to confirm the fact of modification of the zeolite surface with chitosan solution (Figure 3). Water droplets instantly spread on the surface of the initial mineral with a wetting angle of 10°. After modification, there is a sharp increase in the wetting angle due to the adsorption of macromolecules of the cationic polymer on the zeolite surface. At concentration of $2·10^{-3}$ base-mol/L, the contact angle increased to 47°. When changing the polymer concentration from $4·10^{-3}$ to $1·10^{-2}$ base-mol/L, the contact angle values were within 60° indicating the high hydrophilicity of the zeolite surface [23]. The hydrophilicity of the mineral surface covered with the polymer can be caused by the presence of OH-groups along the macromolecules of chitosan. In addition, some of the polymer amino groups can remain free, without participating in electrostatic interaction with the zeolite surface. Such phenomenon takes place in the case of excessive amount of polymer in the system in relation to the solid surface. In fact, electron micrographs of zeolite after modification show a tendency to increase the particle size (Figure 4), which can result from the flocculating action of the adsorbed polymer. Thus, the polymer adsorption leads to a recharge of the surface or change of the charge from negative to positive.

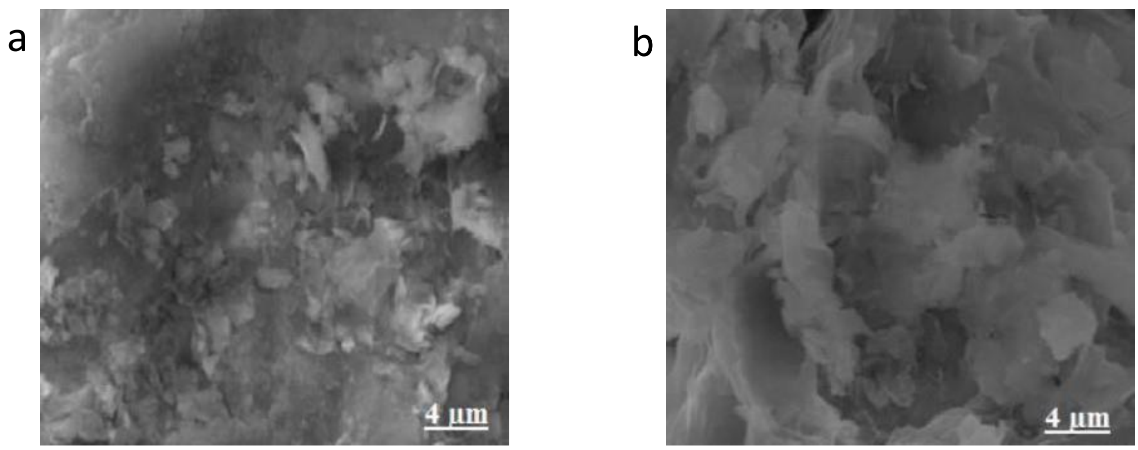


**Figure 4** – Electron microscopic images of the thermal-acid activated zeolite before (a) and after modification (b) by chitosan (x 30000)

**4. Conclusions**

Thus, using the adsorption of chitosan on the surface of thermal acid-activated zeolite, the modification of its surface was conducted. It was shown that the surface modification leads to a change of the negative charge of the mineral to positive. The main forces responsible for the adsorption of chitosan are electrostatic interactions and hydrogen bonds.

In order to increase the adsorption capacity of the zeolite, thermal-acid activation has been proposed. Modification of thermal-acidactivated zeolite surface with a negative charge by adsorption of cationic polymer chitosan conducted, and the maximum adsorption of chitosan on the zeolite surface was found to be 30.1 mg/g. Modification was proved using FTIR analysis. It was found that the modification of the surface leads to change of negative charge of the mineral to a positive. Shift of the frequencies from 1635 $cm^{-1}$ to 1638 $cm^{-1}$ is the evidence of presence of positive charged amino groups of the zeolite surface. Changing of the peak at 581 $cm^{-1}$, characterizing aluminosilicate groups, is also evidence of their interaction with chitosan.

The main forces responsible for the adsorption of chitosan on the zeolite surface are electrostatic interactions and hydrogen bonds. Studies of the wettability of the surface of mineral particles showed that the contact angle was increasing with the increase of concentration of the polymer and the maintenance of the high hydrophilicity of the surface of zeolite.

These results can be used for sorbents preparation in sewage treatment, for the development of effective biocatalysts and biosorbents in biotechnology, for targeted changing of built materials hydrophilicity in construction.

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# Synthesis, antimicrobial evaluation and *in silico* studies of novel 3,4-disubstituted pyrrolidinesulfonamides

[1]Santosh Kumar B.*, [2]Madhu G., [3]Ravindranath L.K.

[1]Department of Chemistry, Gayatri Vidya Parishad College of Engineering, Vishakapatnam, India
[2]Department of Chemistry, Dr. APJ Abdulkalam IIIT-Ongole, Rajiv Gandhi University of Knowledge Technologies-AP, Ongole, India
[3]Department of Chemistry, Sri Krishnadevaraya University, Anantapur, India
*E-mail: *besantosh1985@gmail.com*

3,4-Disubstituted pyrrolidinesulfonamides were synthesized and screened for their antimicrobial activity. Title compounds were established as potent antibacterial and antifungal agents. Noteworthy antimicrobial activity was found for the title compounds against the tested microorganisms. They exhibit comparable results with standard drugs. Besides the *in vitro* antimicrobial activity, the synthesized compounds were evaluated for their *in silico* inhibitory activity on active site of β-glucosidase enzyme. *In silico* studies were done by GOLD docking method against β-glucosidase 3VKK (PDB Id). In silico studies were conducted to evaluate the ability of synthesized compounds to inhibit the β-glucosidase enzyme. The results revealed that 3,4-disubstitutedpyrrolidinesulfonamides are the potent β-glucosidase inhibitors by binding at the active site. A sensible inhibition against β-glucosidases was observed for the compound with 13,4-oxadizole ring has higher β-glucosidase inhibition activity than the other compounds. The free energy of binding and inhibition constant ($K_i$) of the docked compounds were evaluated and presented.

**Keywords:** pyrrolidinesulfonamides; synthesis; *in silico* studies; β-glucosidase; antimicrobial activity.

# Жаңа 3,4-алмастырылған пирролидинсульфонамидтердің синтезі, микробтарға қарсы қабілетін бағалау және *in silico* зерттеулері

[1]Сантош Кумар Б.*, [2]Маду Б., [3]Равиндранат Л.К.

[1]Department of Chemistry, Gayatri Vidya Parishad College of Engineering, Вишакхапатнам, Индия
[2]Department of Chemistry, Dr. APJ Abdulkalam IIIT-Ongole, Rajiv Gandhi University of Knowledge Technologies-AP, Онголе, Индия
[3]Department of Chemistry, Sri Krishnadevaraya University, Анантапур, Индия
*E-mail: *besantosh1985@gmail.com*

Жұмыста 3,4-алмастырылған пирролидинсульфонамидтері синтезделді және олардың микробтарға қарсы белсенділігі тексерілді. Бұл қосылыстар бактерияларға қарсы және антифунгицидті күшті агенттер екендігі анықталды. Қосылыстардың алынған микроорганизмдерге қарсы жоғары белсенділігі анықталды. Олар стандартты дәрілермен салыстырылатын нәтижелерді көрсетеді. *In vitro* антимикробтық белсенділігімен қатар, олардың β-глюкозидаза ферментінің белсенді орнына *in silico* ингибиторлық белсенділігі бағаланды. In silico зерттеулерді GOLD қондыру әдісі арқылы β-глюкозидазаға 3VKK (PDB Id) қарсы жүргізілді. *In silico* зерттеулер синтезделген қосылыстардың β-глюкозидаза ферментін ингибирлеу қабілетін бағалау үшін жүргізілді. Нәтижелер 3,4-алмастырылған пирролидинсульфонамидтер ферменттің активті орындарында байланысатын β-глюкозидазаның күшті ингибиторлары екенін көрсетті. 13,4-оксадизол сақинасы бар қосылыс үшін β-глюкозидазалардың айтарлықтай ингибирлеуі байқалды, ол басқа қосылыстарға қарағанда β-глюкозидазаға қатысты ингибирлеу жоғары белсенділігін көрсетеді. Жұмыста қосылыстардың бос байланыстырушы энергиясы мен ингибирлеу тұрақтылықтары ($K_i$) бағаланды.

**Түйін сөздер:** пирролидинсульфонамидтері; синтез; *in silico* зерттеулері; β-глюкозидаза; антимикробтық белсенділік.

# Синтез, антимикробная оценка и *in silico* исследования новых 3,4-дизамещенных пирролидинсульфонамидов

[1]Сантош Кумар Б.*, [2]Маду Б., [3]Равиндранат Л.К.

[1]Department of Chemistry, Gayatri Vidya Parishad College of Engineering, Вишакхапатнам, Индия
[2]Department of Chemistry, Dr. APJ Abdulkalam IIIT-Ongole, Rajiv Gandhi University of Knowledge Technologies-AP, Онголе, Индия
[3]Department of Chemistry, Sri Krishnadevaraya University, Анантапур, Индия
*E-mail: *besantosh1985@gmail.com*

В работе синтезированы 3,4-дизамещенные пирролидинсульфонамиды с последующей проверкой их антимикробной активности. Установлено, что данные соединения являются сильными антибактериальными и противогрибковыми агентами. Обнаружена высокая антимикробная активность данных соединений против выбранных микроорганизмов. Они показывают сопоставимые результаты со стандартными препаратами. Помимо антимикробной активности *in vitro*, оценивали их ингибирующую активность *in silico* на активном участке фермента β-глюкозидазы. Исследования *in silico* проводили методом стыковки GOLD против β-глюкозидазы 3VKK (PDB Id). Исследования in silico проводили для оценки способности синтезированных соединений ингибировать фермент β-глюкозидазу. Результаты показали, что 3,4-дизамещенные пирролидинсульфонамиды являются мощными ингибиторами β-глюкозидазы, связываясь в активном центре. Заметное ингибирование β-глюкозидаз наблюдалось для соединения с 13,4-оксадизольным кольцом, которое обладает более высокой активностью ингибирования β-глюкозидазы, чем другие соединения. В работе также оценены свободные энергии связывания и константы ингибирования ($K_i$) присоединенных соединений.

**Ключевые слова:** пирролидинсульфонамиды; синтез; исследования *in silico*; β-глюкозидаза; антимикробная активность.

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# Synthesis, antimicrobial evaluation and *in silico* studies of novel 3,4-disubstituted pyrrolidinesulfonamides

**[1]Santosh Kumar B.*, [2]Madhu G., [3]Ravindranath L.K.**

[1]Department of Chemistry, Gayatri Vidya Parishad College of Engineering, Vishakapatnam, India
[2]Department of Chemistry, Dr. APJ Abdulkalam IIIT-Ongole, Rajiv Gandhi University of Knowledge Technologies-AP, Ongole, India
[3]Department of Chemistry, Sri Krishnadevaraya University, Anantapur, India
*E-mail: *besantosh1985@gmail.com*

## 1. Introduction

In the field of medicinal chemistry, chiral 3,4-disubstituted pyrrolidines derived from tartaric acid are widely used. Most of these pyrrolidine rings are found in biological compounds as their frameworks. These frameworks were successfully mutated into receptor molecules, amino-sugar derivatives as glycosidase inhibitors as well as sugar simulate in nucleoside analog. Besides kinases, pyrrolidines are also suitable substitutes for inhibitor design to recognize the specificity pockets of the corresponding enzymes in proteases [1,2]. Proline based pyrrolidines are used as drug candidates in the treatment of hepatitis C [3]. The solid-phase construction of a guanidine based bis-cyclic pyrrolidine exhibited marked bactericidal activity against known human pathogens, it may represent a newfangled category of antimicrobial therapeutics [4]. Pyrrolidineoxadiazole and pyrrolidine thiadiazole derivatives are useful in the treatment and prevention of oxytocin mediated disease states like preterm labor, premature birth and dysmenorrheal because of their markable oxytocin receptor antagonist activity [5], pyrrolidine and piperidine as antidiabetic agents [6].

Sulfonamides are promising antibacterial/antibiotic agents for over several years. In addition to their commercialized utilization as antibacterial/antibiotic agents, several sulfonamides are reported to inhibit enzymes such as carbonic anhydrase [7], cysteine protease [8], HIV protease [9] and cyclo-oxygenase [10]. Besides these potential applications, various other therapeutic applications, in cancer chemotherapy [11], diuretics [12], hypoglycemia [13] and the anti-impotence agent [14] and in metabolic syndrome treatment [15] are also reported for sulfonamides.

Glucosidases catalyze the cleavage of glycosidic bonds in oligosaccharides or glycoconjugates. The arrangement of hydroxyl groups in a sugar molecule influences the enzymatic action of several glucosidases. Accordingly, α- and β-glucosidases are able to catalyze the cleavage of glycosidic bonds bearing terminal glucose linked at the site of cleavage, respectively, through α- or β-linkages at the anomeric center [16]. The activity of glucosidases is fundamental to several biochemical operations like degradations of diet polysaccharides to furnish monosaccharide units, lysosomal glycoconjugate catabolism and glycoprotein processing and biosynthesis of oligosaccharide units in glycoproteins or glycolipids [17]. These multidimensional biochemical activities of glucosidases cater to the needs for developing new and potential therapeutic inhibitors to be used in diabetes [18], obesity [17], glycosphingolipid lysosomal storage disease [19], HIV infections [20] and tumors in general [21].

Considering the vitality of pyrrolidine and sulfonamides in view, a new series of N,N'-(pyrrolidine-3,4-diyl)sulfonamide derivatives containing 1,3,4-oxadiazole/azetidinone/thiazolidinone were synthesized and examined for their antimicrobial activity and inhibitory activity against human β-glucosidase enzyme.

## 2. Experiment

### *2.1 Materials and Methods*

All chemicals and reagents were procured from Merck India Ltd. X-6 digital display binocular microscope (uncorrected) was used to determine the melting points. Nicolet nexus 470 FT-IR spectrometer (USA) using deploying KBr crystal or KBr plate was used to record the IR spectra of the synthesized compounds. $^{1}$H NMR (400 MHz) and $^{13}$C NMR (75 MHz) spectra were recorded on a Bruker Avance (Switzerland) spectrometer. The elemental analysis was carried on Vario Micro Cube Elementar (Germany) instrument. The reaction progress was monitored by TLC with a mixture of cyclohexane and ethylacetate (9:1) as an elutent. A 300 mesh silica gel was used to perform flash column chromatography. The yields were calculated by the last step reaction.

The standard bacterial and fungal strains were procured from National Centre for Cell Science (Pune, India). The antimicrobial activity was expressed in terms of minimum inhibitory concentration (MIC). The MIC was found by the agar cup plate method for antibacterial activity and disc diffusion method for antifungal activity. Streptomycin and clotrimazole were used as standards (20 µg/mL) for antibacterial studies and antifungal studies respectively.

*2.2 Docking method*

A genetic algorithm (GA) based software namely GOLD (Genetic Optimization of Ligand Docking, Cambridge Crystallographic Data Centre, Cambridge, UK) was used to carry out the docking studies. GOLD version 3.0.1 program was used to perform the molecular docking method for studying the binding affinities of synthesized molecules into the active site of the β-glucosidase protein. The location and measurement of the protein pockets and cavities were done automatically by a program named CASTP server (Cambridge Crystallographic Data Centre, Cambridge, UK), which is used for active site identification [22].

*2.3 General Procedures*

*2.3.1 Synthesis of ethyl 2-((3S,4S)-3,4-bis(N-cyclopropylthiophene-2-sulfonamido)pyrrolidin-1-yl)acetate **(2)***

To the solution of ethyl 2-((3S,4S)-3,4-bis(thiophene-2-sulfonamido)pyrrolidin-1-yl)acetate **(1)** (1.3 g, 2.71 mmol) in acetonitrile (12 mL), potassium carbonate (1.39 g, 10.03 mmol), cyclopropyl bromide (0.33 g, 2.71 mmol) and few crystals of KI were added and refluxed for 20 h. The residue obtained after the removal of the solvent was poured into water under reduced pressure and extracted with $CH_2Cl_2$ (3x10 mL). The combined organic layer was dried over anhydrous $Na_2SO_4$. A crude solid was obtained on filtration and concentration of the organic layer under reduced pressure, which was then purified by column chromatography using 60-120 mesh silica gel as an adsorbent and dichloromethane and methanol (10:1) mixture as an eluent [23,24]. The spectral and physical characterization data of compound **2** are shown in Table 1.

*2.3.2 Synthesis of N,N′-((3S,4S)-1-(2-hydrazinyl-2-oxoethyl) pyrrolidine-3,4-diyl)bis(N-cyclopropylthiophene-2-sulfonamide) **(3)***

A solution of compound **2** (1.1 g, 1.9 mmol) and hydrazine hydrate in ethanol (85%, 3.8 mmol) was refluxed for 5 h. The crude product obtained on evaporation of the reaction mixture under reduced pressure was purified by recrystallization from the proper absolute alcohol. The spectral and physical characterization data of compound **3** are shown in Table 1.

*2.3.3 Synthesis of N,N′-((3S,4S)-1-((5-phenyl-1,3,4-oxadiazol-2-yl)methyl)pyrrolidine-3,4-diyl)bis(N-cyclopropyl thiophene-2-sulfonamide) **(4a)***

A mixture of benzoic acid (0.122 g, 1.0 mmol) and compound-3 (0.55 g, 1.0 mmol) in phosphoryl chloride (5 mL) was refluxed over a steam bath for 5-6 h. The cooled reaction mixture was poured on to crushed ice (~300 g) under continuous stirring. The separated solid mass was neutralized using sodium bicarbonate solution (10% w/v), collected by filtration, washed with cold water and dried in vacuum. The resulting solid thus obtained was recrystallized from absolute ethanol (95%) to obtain the desired product **4a**.

Compounds **4b-4f** were prepared from compound **3** and the appropriate 4-substituted benzoic acid by using a procedure similar to that described for the synthesis of **4a**.

The IR (KBr) spectrum of compound **4a** showed peaks ($cm^{-1}$) around 3135 (Ar-H), 1645 & 1232 (characteristic peaks for oxadiazole), 1322 & 1182 (asymmetric & symmetric stretching of O=S=O), 1140 & 1125 (C-N exo) respectively. The $^1H$ NMR (400 MHz, $^{\delta}$ppm) spectrum exhibits the signals 8.02-7.62 (m, 5H, Ar-H), 7.59-7.17 (m, 6H, thiophene), 3.64 (s, 2H, N-$CH_2$-), 3.28 (m, 2H, -$SO_2$-N-CH-), 3.07 (m, 2H, $H_a$ protons of pyrrolidine), 2.69 (m, 2H, cyclopropyl C-H attached to N), 2.28 (m, 2H, $H_b$ protons of pyrrolidine), 0.51&0.39(m, 8H, -$CH_2$ of cyclopropane). The $^{13}C$ NMR (75 MHz, $^{\delta}$ppm) spectrum has the peaks at 144.4 & 131.8 (thiophene), 170.4 & 162.9 (oxadiazole), 116.4, 142.7, 128.8 & 131.3 (Ar). The spectral and physical characterization data of compounds **4a-4f** are shown in Table 1.

*2.3.4 General procedure for the synthesis of N,N′-((3S,4S)-1-(2-((E)-2-(4-substitutedbenzylidene hydrazinyl)-2-oxoethyl) pyrrolidine-3,4-diyl)bis(N-cyclopropylthiophene-2-sulfonamide) **(5a-f)***

To an equimolar methanolic solution of compound **3** (0.83 g, 1.52 mmol) and benzaldehyde (0.16 g, 1.52 mmol) mixture, few drops of glacial acetic acid were added. The mixture was then refluxed on a water bath for 5 h, allowed to cool, poured into crushed ice and filtered. 60-120 mesh silica gel and cyclohexane-ethylacetate (9:1) solvent mixture as an eluent were used to purify the crude mass by column chromatography.

Compounds **5b-5f** were prepared from compound-**3** and the appropriate 4-substituted benzaldehyde by using a procedure similar to that described for the synthesis of **5a**.

*2.3.5 Synthesis of 2-((3S,4S)-3,4-bis(N-cyclopropylthiophene-2-sulfonamido)pyrrolidin-1-yl)-N-(3-chloro-2-oxo-4-phenylazetidin-1-yl)acetamide **(6a)***

A solution of **5a** (0.64 g, 1.0 mmol) in dioxane (8 mL) was added to a well stirred mixture of chloroacetylchloride (0.24 g, 2.0 mmol) and triethylamine (0.2 g, 2.0 mmol) in dioxane (10 mL) at 0-5°C. The reaction mixture was then stirred for 8 h, kept at room temperature for 2 days and then washed with cold water. The obtained solid was filtered, washed with water and recrystallized from methanol to yield the desired product **6a**.

Compounds **6b-6f** were prepared from **5a** by using a procedure adopted for the synthesis of **6a**.

The IR (KBr) spectrum of compound **6a** showed peaks ($cm^{-1}$) around 3490 (N-H), 3135 (Ar-H), 1689 (C=O of azitidinone), 1322 & 1182 (asymmetric and symmetric stretching), 1215(C-N of azitidinone), 810 (C-Cl). The $^1H$ NMR (400 MHz, $^{\delta}$ppm) spectrum exhibits the signals 9.35 (s, 1H, -CO-NH), 7.65-7.25 (m, 6H, thiophene), 7.47-7.32(m, 5H, Ar-H), 5.51 (d, 1H, Cl-C-H of

**Table 1** – The spectral and physical characterization data

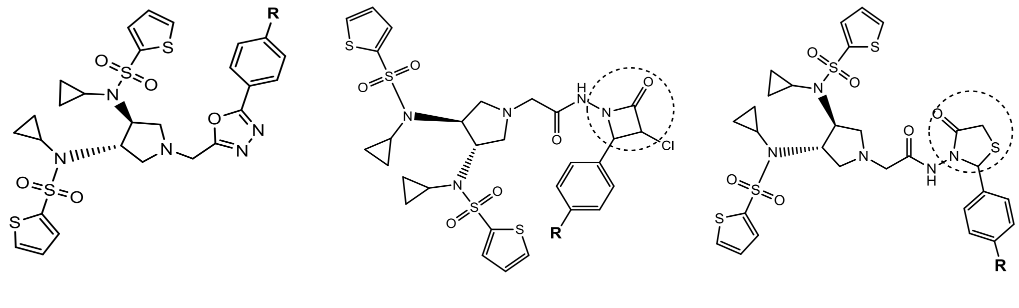
| Com No. | Molecular Formula | M.P., °C | Yield, % | IR (KBr, $cm^{-1}$) | $^{1}$H NMR (DMSO-$d_6$, 400 MHz, $^{δ}$ppm) | $^{13}$C NMR (DMSO-$d_6$, 75MHz, $^{δ}$ppm) | Results of elemental analysis |
|---|---|---|---|---|---|---|---|
| 2 | $C_{22}H_{29}N_3O_6S_4$ | 162-164 | 70 | 3496&3424 (asym.& sym.$NH_2$),3221(N-H), 1698(C=O), 1125 (C-N), 1100 (pyrrolidine C-N), 1322&1182 (sym.&assym. O=S=O),1388 (thiophene C-S) | 7.68-7.18(m,6H), 4.55(m,2H,), 3.4(q, 2H, J=7.2 Hz), 3.33(s,2H), 3.14&2.25(m,4H)*, 2.70(m,2H), 1.3(t, 3H, J=5.2 Hz), 0.70-0.30(m,8H,) | 171.5 (C=O)144.4 & 131.8 (thiophene), 64.3(O-$CH_3$), 15.6($CH_3$), 5.2&25.0 (cyclopropyl) | C, 47.29 (47.22); H, 5.28 (5.24); N, 7.57 (7.55) |
| 3 | $C_{20}H_{27}N_5O_5S_4$ | 174-176 | 65 | 3430 & 3370 (asym.&sym -$NH_2$), 1739 (C=O), 1125 (exo C-N), 1100 (pyrrolidine C-N), 1388 (thiophene C-S), 1322 & 1182 (asym.& sym. O=S=O) | 8.03 (s, H), 7.59-7.15(m,6H), 4.23(m,2H), 3.17(s,2H), 3.12&2.35 (m,4H), 2.74(m,2H), 2.0 (s, 2H), 0.51&0.39(m,8H) | 170.7 (C=O), 144.4 & 131.8 (thiophene), 5.2&25.0 (Cyclopropyl) | C, 44.09 (44.02); H, 4.92 (4.99); N, 12.87 (12.83) |
| 4a | $C_{27}H_{29}N_5O_5S_4$ | 182-184 | 70 | 3135 (Ar-H), 1645 & 1232 (characteristic peaks for oxadiazole), 1322 & 1182 (asymmetric and symmetric stretching O=S=O), 1140 & 1125 (two C-Nexo) | 8.02-7.62(m,5H), 7.62(m,3H), 7.59-7.17 (m, 6H), 3.64 (s,2H), 3.28 (m,2H), 2.69 (m,2H), 3.07&2.28 (m,4H), 0.51&0.39(m,8H) | 144.4 & 131.8 (thiophene), 170.4 & 162.9(oxadiazole), 116.4, 142.7, 128.8 & 131.3(Ar) | C, 51.37 (51.33); H, 4.59 (4.63); N, 11.13 (11.08) |
| 4b | $C_{28}H_{31}N_5O_5S_4$ | 156-158 | 70 | 3135(Ar-H), 1649 & 1233(Characteristic peaks for oxadiazole), 1327 & 1185(asymmetric and symmetric stretching O=S=O), 1142 & 1126(two C-Nexo) | 7.97-7.26 (m,4H), 7.59-7.17 (m, 6H), 3.64 (s,2H), 3.28 (m,2H), 3.07&2.28 (m,4H), 2.69 (m,2H,), 2.34 (s,3H), 0.51&0.39(m,8H) | 144.4 & 131.8 (thiophene), 170.4 & 162.9(oxadiazole), 117.1, 129.1, 131.2 & 139.1(phenyl), 23.1($CH_3$) | C, 52.12 (52.07); H, 4.86(4.84); N, 10.89(10.84) |
| 4c | $C_{28}H_{31}N_5O_6S_4$ | 138-140 | 70 | 3139(Ar-H), 1651 & 1234(Characteristic peaks for oxadiazole), 1329 & 1188(asymmetric and symmetric stretching O=S=O), 1144 & 1128(two C-Nexo) | 8.02-7.03 (m,4H), 7.59-7.17 (m, 6H), 3.81(s,3H), 3.64 (s,2H), 3.28(m,2H), 3.07 &2.28 (m,4H), 2.69 (m,2H), (m,2H), 0.51&0.39(m,8H) | 144.4 & 131.8 (thiophen), 170.4 & 162.9(oxadiazole), 117.7, 129.6, 115.5 & 163.4(phenyl), 57.0(-$OCH_3$) | C, 50.89(50.81); H, 4.79(4.72); N, 10.63(10.58) |
| 4d | $C_{27}H_{28}ClN_5O_5S_4$ | 172-174 | 70 | 3141(Ar-H), 1653 & 1237(Characteristic peaks for oxadiazole) , 1331 & 1189(asymmetric and symmetric stretching O=S=O), 1145 & 1129(two C-Nexo) | 7.58-7.53(m,4H), 7.70-7.22 (m, 6H), 3.63 (s,2H), 4.11 (m,2H), 3.32 & 2.17 (m,4H), 2.69 (m,2H), 2.17 (m,2H,), 0.51&0.39(m,8H) | 144.4 & 131.8 (thiophen), 170.4 & 162.9(oxadiazole), 117.7, 131.7, 129.6 & 131.3(phenyl) | C, 48.71(48.67); H, 4.29(4.24); N, 10.59(10.51) |
| 4e | $C_{27}H_{28}N_6O_7S_4$ | 166-168 | 75 | 3140(Ar-H), 1651 & 1233(Characteristic peaks for oxadiazole), 1329 & 1185(asymmetric and symmetric stretching O=S=O), 1143 & 1126(two C-Nexo) | 7.29-7.09(m,4H), 7.70-7.22(m, 6H), 3.63(s,2H), 4.11 (m,2H), 3.32 & 2.17 (m,4H), 0.51&0.39(m,8H) | 144.4 & 131.8 (thiophene), 170.4 & 162.9(oxadiazole), 116.8, 127.7, 128.4 & 136.7(phenyl) | C, 45.69(45.63); H, 3.893.97); N, 9.81(9.85) |
| 4f | $C_{27}H_{28}N_6O_7S_4$ | 202-204 | 75 | 3148(Ar-H), 1656& 1237(asymmetric and symmetric stretching O=S=O), 1335&1189(asymmetric and symmetric stretching O=S=O) 1147&1129(two C-Nexo) | 7.95-7.4(m,4H), 7.59-7.17 (m, 6H), 3.64 (s,2H), 3.28 (m,2H), 3.07 & 2.28 (m,2H), 2.69 (m,2H), 0.51&0.39(m,8H) | 144.4 & 131.8 (thiophene), 170.4 & 162.9(oxadiazole), 116.4, 127.0, 124.1 & 150.5(phenyl) | C, 47.93(47.91); H, 4.21(4.17); N, 12.35(12.42) |
| 5a | $C_{27}H_{31}N_5O_5S$ | 166-168 | 75 | 3135(Ar-H), 1620(C=N), 1388(thiophene),1322 & 1182(asymmetric and symmetric stretching O=S=O), 1125 & 1100 (two C-Nexo) | 11.07(s,1H), 8.53(s,1H), 7.93-7.58(m,5H), 7.59-7.15 (m, 6H), 3.64 (s,2H), 3.28 (m,2H), 3.23 & 2.58 (m,4H), 2.69 (m,2H), 0.51&0.26 (m,8H) | 171.3(C=O)145.1 & 132.3(thiophen), 144.1 (C=N), 133.1,131.2 & 129 (phenyl) | C, 51.11(51.16); H, 4.96(4.93); N, 11.10(11.05) |
| 5b | $C_{28}H_{33}N_5O_5S_4$ | 134-136 | 65 | 3134(Ar-H), 1615(C=N),1387(thiophe ne),1322&& 1181(asymmetric and symmetric stretching O=S=O), 1127 & 1102(two C-Nexo) | 11.07(s,1H), 8.53(s,1H), 7.91-7.40(m,4H), 7.61-7.17 (m, 6H), 3.64 (s,2H), 3.28 (m,2H), 3.07 & 2.28 (m,4H), 2.69 (m,2H), 2.28 (m,2H), 2.41(s,3H), 0.51&0.26 (m,8H) | 171.3(C=O), 145.1 & 132.3(thiophen), 144.4(C=N), 142, 131, 129 & 126 (phenyl), 21.3($CH_3$) | C, 51.88(51.91); H, 5.10(5.13); N, 10.77(10.71) |

**Table 1** – The spectral and physical characterization data (continued)

| Com No. | Molecular Formula | M.P., °C | Yield, % | IR (KBr, $cm^{-1}$) | $^{1}$H NMR (DMSO-$d_6$, 400 MHz, $^{\delta}$ppm) | $^{13}$C NMR (DMSO-$d_6$, 75MHz, $^{\delta}$ppm) | Results of elemental analysis |
|---|---|---|---|---|---|---|---|
| 5c | $C_{28}H_{33}N_5O_6S_4$ | 148-150 | 70 | 3135(Ar-H), 1617(C=N), 1388(thio-phene),1321&& 1182(asymmetric and symmetric stretching O=S=O), 1129 & 1101(two C-Nexo) | 11.07(s,1H), 8.53(s,1H), 8.02-7.03(m,4H), 7.59-7.17 (m, 6H), 3.81(s,3H), 3.64 (s,2H), 3.28(m,2H), 3.07 & 2.28 (m,2H), 2.69 (m,2H), 2.28 (m,2H), 0.51&0.26 (m,8H) | 171.3(C=O), 145.1 & 132.3(thio-phen), 144.4(C=N), 162.6, 131.0, 126.3 & 114.9(phenyl), 56.0(-$OCH_3$) | C, 50.59(50.66); H, 4.95(5.01); N, 10.51(10.55) |
| 5d | $C_{27}H_{30}ClN_5O_5S_4$ | 154-156 | 75 | 3136(Ar-H), 1625(C=N) , 1387(thio-phene),1322&& 1182(asymmetric and symmetric stretching O=S=O), 1125 & 1100 | 11.07(s,1H), 8.53(s,1H), 7.58-7.53(m,4H), 7.70-7.22 (m, 6H), 3.63 (s,2H), 4.11 (m,2H), 3.32&2.17 (m,2H), 2.69 (m,2H), 2.17 (m,2H), 0.51&0.26 (m,8H) | 171.0(C=O), 145.1 & 132.3(thio-phen), 144.3(C=N), 136.6, 131.5, 130.3 & 129.0(phenyl) | C, 48.49(48.53); H, 4.49(4.52); N, 10.45(10.48) |
| 5e | $C_{27}H_{30}BrN_5O_5S_4$ | 174-176 | 75 | 3135(Ar-H), 1622(C=N), 1388(thio-phene),1322&& 1182(two C-Nexo) | 11.07(s,1H), 8.53(s,1H), 7.29-7.09(m,4H), 7.70-7.22(m, 6H), 3.63(s,2H), 4.11 (m,2H), 3.32&2.17(m,2H), 2.69(m,2H), 0.51&0.26 (m,8H) | 171.0(C=O), 145.1 & 132.3(thio-phen), 144.3(C=N), 137.1,132.9, 131.5 & 128.4(phenyl) | C, 45.52(45.50); H, 4.29(4.24); N, 9.89(9.83) |
| 5f | $C_{27}H_{30}N_6O_7S_4$ | 185-187 | 70 | 3135(Ar-H), 1626(C=N), 1389(thio-phene),1322&& 1182(asymmetric and symmetric stretching O=S=O), 1125 & 1100(two C-Nexo) | 11.07(s,1H), 8.53(s,1H), 8.03-7.62(m,4H), 7.59-7.17 (m, 6H), 3.64 (s,2H), 3.28 (m,2H), 3.07&2.28(m,2H), 2.69 (m,2H), 0.51&0.26 (m,8H) | 171.0(C=O), 145.1 & 132.3(thio-phen), 144.3(C=N), 150.1, 140.2 & 124.5 (phenyl) | C, 47.79(47.77); H, 4.42(4.45); N, 12.34(12.38) |
| 6a | $C_{29}H_{32}ClN_5O_6S_4$ | 142-143 | 70 | 3490(N-H), 3135 (Ar-H), 1689 (C=O of Azitidinone), 1322 & 1182 (asymmetric and symmetric stretching O=S=O),1215(C-N of Azitidinone), 810 (C-Cl) | 9.35(s,1H), 7.65-7.25 (m, 6H),7.47-7.32(m,5H), 5.51(d,1H, J=4.7Hz), 3.97(d,1H, J=9.11Hz), 3.21(s,2H), 3.52 (m,2H), 3.16&2.18 (m,4H), 2.69 (m,2H), 0.51&0.26 (m,8H) | 144.4 & 131.8(thiophen), 142.7, 128.1, 129.2 & 130.3 (phenyl) | C, 49.09(49.04); H, 4.62(4.54); N, 9.79(9.86) |
| 6b | $C_{30}H_{34}ClN_5O_6S_4$ | 158-159 | 65 | 3485(N-H), 3137(Ar-H), 1686(C=O of Azitidinone), 1322 & 1182(asymmetric and symmetric stretching O=S=O),1212(C-N of Azitidinone), 808(C-Cl) | 9.35(s,1H), 7.65-7.25 (m, 6H),7.19-7.09(m,4H), 5.51(d,1H, J=4.7Hz), 3.97(d,1H, J=9.11Hz), 3.21(s,2H), 3.52(m,2H), 3.16&2.18(m,4H), 2.69 (m,2H), 2.18 (m,2H), 2.19(s,3H), 0.51&0.26 (m,8H) | 144.4 & 131.8(carbons of thiophen), 131.1, 130.0, 129.7 & 133.7 (phenyl), 21.1($CH_3$) | C, 49.73(49.79); H, 4.79(4.73); N, 9.63(9.67) |
| 6c | $C_{30}H_{34}ClN_5O_7S_4$ | 171-172 | 70 | 3480(N-H), 3135(Ar-H), 1683(C=O of Azitidinone), 1322 & 1182(asym.&sym. $SO_2$) (asymmetric and symmetric stretching O=S=O),1210(C-N of Azitidinone), 806(C-Cl) | 9.42(s,1H), 7.65-7.28 (m, 6H), 7.22-6.88 (m,4H), 5.51(d,1H, J=4.7Hz), 3.97(d,1H, J=9.11Hz), 3.45(s,2H), 3.28 (m,2H), 3.07&2.28 (m,4H), 2.69 (m,2H), 0.51-0.26 (m,8H) | 144.4 & 131.8(thiophen), 132.6, 128.0, 125.6 & 132.3 (phenyl), 57.0 ($OCH_3$) | C, 48.61(48.67); H, 4.69(4.63); N, 9.51(9.46) |
| 6d | $C_{29}H_{31}C_{l2}N_5O_6S_4$ | 138-139 | 75 | 3495(N-H), 3143(Ar-H), 1692(C=O of Azitidinone), 1322 & 1182(asymmetric and symmetric stretching O=S=O),1218(C-N of Azitidinone), 814(C-Cl) | 9.33(s,1H), 7.66-7.18 (m, 6H),7.49(m,4H), 5.51(d,1H, J=4.7Hz), 3.97(d,1H, J=9.11Hz), 3.45(s,2H), 3.28 (m,2H, -), 3.07&2.28 (m,4H), 2.69 (m,2H), 0.51&0.26 (m,8H) | 144.4 & 131.8(thiophen), 139.4, 128.1, 127.3 & 135.7 (phenyl) | C, 46.71(46.77); H, 4.23(4.20); N, 9.46(9.40) |
| 6e | $C_{29}H_{31}BrClN_5O_6S_4$ | 166-167 | 77 | 3493(N-H), 3142(Ar-H), 1690(C=O of Azitidinone), 1322 & 1182(asymmetric and symmetric stretching O=S=O),1216(C-N of Azitidinone), 812(C-Cl) | 9.30(s,1H), 7.6-7.18 (m, 6H), 7.78-7.18 (m,4H), 5.51(d,1H, J=4.7Hz), 3.97(d,1H, J=9.11Hz), 3.45(s,2H), 3.28 (m,2H), 3.07&2.28 (m,4H), 2.69 (m,2H), 0.51&0.26 (m,8H) | 144.4 & 131.8(thiophen), 135.7, 127.3, 128.1 & 139.4 (phenyl) | C, 44.19(44.13); H, 4.05(3.96); N, 8.91(8.87) |
| 6f | $C_{29}H_{31}ClN_6O_8S_4$ | 187-188 | 80 | 3493(N-H), 3142(Ar-H), 1694(C=O of Azitidinone), 1322 & 1182(asymmetric and symmetric stretching O=S=O),1223(C-N of Azitidinone), 817(C-Cl) | 9.34(s,1H), 7.59-7.15 (m, 6H), 8.18-7.55(m,4H), 5.51(d,1H, J=4.7Hz), 3.97(d,1H, J=9.11Hz), 3.45(s,2H), 3.28 (m,2H), 3.07&2.28 (m,2H, 2.69 (m,2H), 0.51&0.26 (m,8H) | 144.4 & 131.8(thiophen), 147.3, 126.5, 130.0 & 139.5 (phenyl) | C, 46.23(46.12); H, 4.16(4.14); N, 11.23(11.13) |

**Table 1** – The spectral and physical characterization data (continued)

| Com No. | Molecular Formula | M.P., °C | Yield, % | IR (KBr, $cm^{-1}$) | $^{1}H$ NMR (DMSO-$d_6$, 400 MHz, $^{\delta}$ppm) | $^{13}C$ NMR (DMSO-$d_6$, 75MHz, $^{\delta}$ppm) | Results of elemental analysis |
|---|---|---|---|---|---|---|---|
| 7a | $C_{29}H_{33}N_5O_6S_5$ | 162-164 | 73 | 3482(N-H), 3135 (Ar-H), 1712 (C=O of thiazolidine), 1322 & 1182 (asymmetric and symmetric stretching O=S=O),1215(C-N of thiazolidine) | 8.38(s,1H), 7.65-7.15(m, 6H),7.36-7.23(m,5H), 6.14(s,1H), 4.25(m,2H), 3.79(s,2H),3.31(s,2H), 3.10&2.88 (m,4H), 3.24(m,2H), 0.51& 0.26 (m,8H) | 144.4 &131.8(thiophene), 140.7, 127.1, 128.2 & 129.3 (phenyl) | C, 49.26(49.20); H, 4.79(4.70); N, 9.96(9.89) |
| 7b | $C_{30}H_{35}N_5O_6S_5$ | 168-170 | 75 | 3478(N-H), 3135(Ar-H), 1710(C=O of thiazolidine), 1322 & 1182(asymmetric and symmetric stretching O=S=O),1245(C-N of thiazolidine) | 8.38(s,1H), 7.65-7.15(m, 6H),7.26-7.10(m,4H), 6.14(s,1H), 3.31(s,2H), 4.25(m,2H), 3.79(s,2H),3.10&2.88 (m,4H), 3.24(m,2H), 2.19(s,3H), 0.51&0.26 (m,8H) | 144.4 &131.8(thiophen), 137.5, 128.1, 129.2 & 138.1 (phenyl), 21.1 (-$CH_3$) | C, 49.86(49.91); H, 4.96(4.89); N, 9.79(9.70) |
| 7c | $C_{30}H_{35}N_5O_7S_5$ | 162-164 | 68 | 3476(N-H), 3135(Ar-H), 1708(C=O of thiazolidine), 1322 & 1182(asymmetric and symmetric stretching O=S=O),1243(C-N of thiazolidine) | 8.39(s,1H), 7.65-7.15(m, 6H), 7.22-6.88(m,4H), 6.14(s,1H), 4.25(m,2H), 3.81(s,3H), 3.79(s,2H), 3.31(s,2H), 3.24(m,2H), 3.10&2.88 (m,4H), , 0.51&0.26 (m,8H) | 144.4 &131.8(thiophen), 130.0, 129.0, 113.6 & 160.0(phenyl), 56.0 (-$OCH_3$) | C, 48.87(48.83); H, 4.71(4.78); N, 9.42(9.49) |
| 7d | $C_{29}H_{32}ClN_5O_6S_5$ | 158-160 | 71 | 3487(N-H), 3135(Ar-H), 1715(C=O of thiazolidine), 1322 & 1182(asymmetric and symmetric stretching O=S=O),1253(C-N of thiazolidine) | 8.39(s,1H), 7.65-7.15(m, 6H), 7.37-7.23(m,4H), 6.14(s,1H), 3.31(s,2H), 4.25(m,2H), 3.79(s,2H),3.10&2.88 (m,4H), 3.24(m,2H), 2.19(s,3H), 0.51&0.26 (m,8H) | 144.4 &131.8(thiophene), 138.6, 129.1, 128.0 & 133.3 (phenyl) | C, 46.99(46.92); H, 4.40(4.34); N, 9.42(9.49) |
| 7e | $C_{29}H_{32}BrN_5O_6S_5$ | 170-172 | 73 | 3485(N-H), 3135(Ar-H), 1712(C=O of thiazolidine), 1322 & 1182(asymmetric and symmetric stretching O=S=O),1250(C-N of thiazolidine) | 8.37(s,1H), 7.65-7.15(m, 6H), 7.78-7.33(m,4H), 6.14(s,1H), 3.31(s,2H), 4.25(m,2H),3.79(s,2H ),3.10&2.88(m,4H), 3.24(m,2H), 2.19(s,3H), 0.51&0.26 (m,8H) | 144.4 &131.8(thiophen), 138.5, 130.0, 131.0 & 121.3 (phenyl) | C, 44.39(44.27); H, 4.19(4.10); N, 8.99(8.90) |
| 7f | $C_{29}H_{32}N_6O_8S_5$ | 178-180 | 65 | 3489(N-H), 3135(Ar-H), 1720(C=O of thiazolidine), 1322 & 1182) (asymmetric and symmetric stretching O=S=O),1256(C-N of thiazolidine) | 8.41(s,1H), 7.65-7.15(m, 6H), 8.20-7.71 (m,4H), 6.14(s,1H), 3.31(s,2H), 4.25(m,2H), 3.79(s,2H),3.10&2.88 (m,4H), 3.24(m,2H), 2.19(s,3H, 0.51&0.26 (m,8H) | 144.4 &131.8(thiophen), 146.9, 127.7, 124.0 & 148.4 (phenyl) | C, 46.35(46.26); H, 4.35(4.28); N, 11.22(11.16) |



**4a-f** **6a-f** **7a-f**

**R = -H, $-CH_3$, $-OCH_3$, -Cl, -Br, $-NO_2$**

azetidine), 3.97 (d, 1H, C-H of azetidine), 3.21 (s, 2H, $N-CH_2$-), 3.52 (m, 2H, -SO2-N-CH-), 3.16 (m, 2H, $H_a$ protons of pyrrolidine), 2.69 (m, 2H, cyclopropyl C-H attached to N), 2.18 (m, 2H, $H_b$ protons of pyrrolidine), 0.51&0.26 (m, 8H, $-CH_2$- of cyclopropyl ring). The $^{13}C$ NMR (75 MHz, $^{\delta}$ppm) spectrum has the peaks at 144.4 & 131.8 (thiophene), 142.7, 128.1, 129.2 & 130.3 (phenyl). The spectral and physical characterization data of compounds **6a-6f** are shown in Table 1.

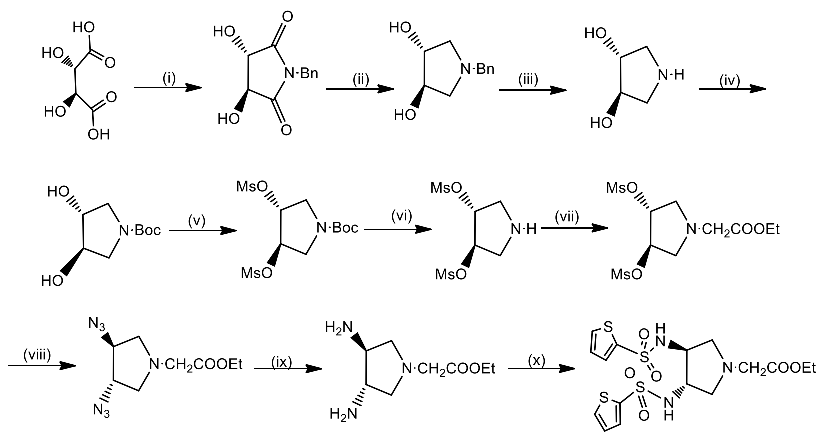
*2.3.6 Synthesis of 2-((3S,4S)-3,4-bis(N-cyclopropylthiophene-2-sulfonamido)pyrrolidin-1-yl)-N-(4-oxo-2-(4-substituted)phenylthiazolidin-3-yl)acetamide **(7a)***

A mixture of **5a** (0.64 g, 1.0 mmol) and mercaptoacetic acid (0.18 g, 2.0 mmol) was heated in an oil bath at 120-125°C for 12 h, cooled and treated with 10% sodium bicarbonate solution. The product was isolated and recrystallized from methanol-dioxane (4:1) mixture to give the desired compound. Compounds **7b-7f** were prepared from **7a** by using a procedure similar to that described for the synthesis of **7a**.

The IR (KBr) spectrum of compound **7a** showed peaks ($cm^{-1}$) around 3482 (N-H), 3135 (Ar-H), 1712 (C=O of thiazolidine), 1322 & 1182 (asymmetric and symmetric stretching), 1215 (C-N of thiazolidine). The $^1H$ NMR (300 MHz, $^{\delta}$ppm) spectrum shows the signals at 8.38, 1H, -CO-NH), 7.65-7.15 (m, 6H, thiophene), 7.36-7.23 (m, 5H, Ar-H), 6.14 (s, 1H, thiazolidine-N-CH-S-), 4.25 (m, 2H, $-SO_2$-N-CH-), 3.79 (s, 2H, -CO-CH-S- of thiazolidine), 3.31 (s, 2H, $N-CH_2$-), 3.10 (m, 2H, $H_a$ protons of pyrrolidine), 3.24 (m, 2H, cyclopropyl C-H attached to N), 2.88 (m, 2H, $H_b$ protons of pyrrolidine), 0.51&0.26 (m, 8H, $-CH_2$- of cyclopropyl ring. The $^{13}C$ NMR (75 MHz, $^{\delta}$ppm) spectrum has the peaks at 144.4 &131.8 (thiophene), 140.7, 127.1, 128.2 & 129.3 (phenyl). The spectral and physical characterization data of compounds **7a-f** are shown in Table 1.

### 3. Results and discussion

The strategy starts with the synthesis of starting material *ethyl 2-((3S,4S)-3,4-bis(thiophene-2-sulfonamido)pyrrolidin-1-yl)acetate* **(1)** from L-tartaric acid as shown in Figure 1 [25,26].

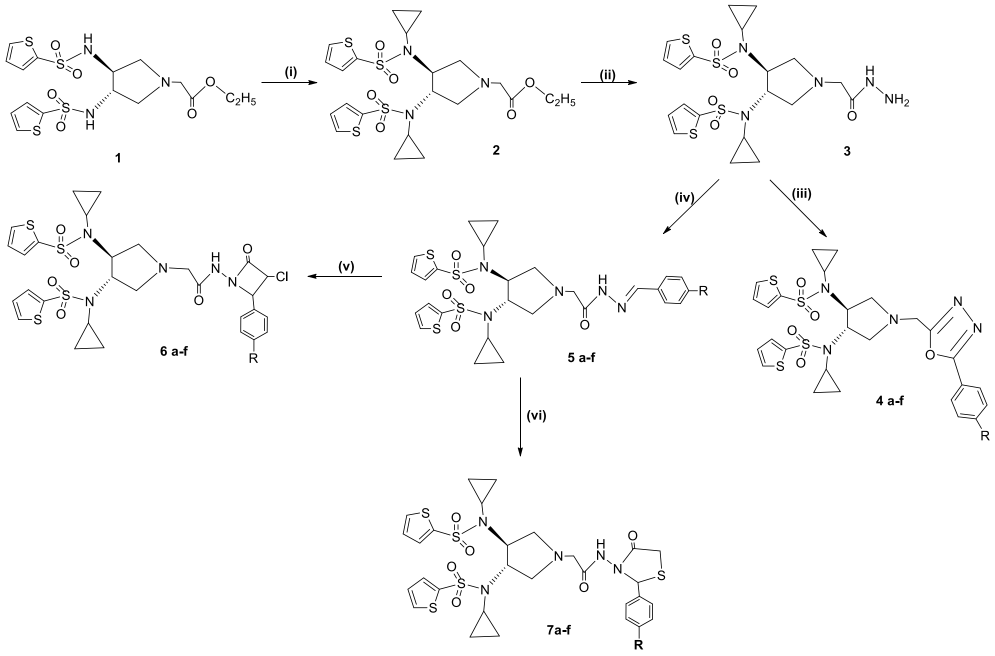


**Reagents & Conditions:** (i) Benzylamine, xylene, 190°C, 8h; (ii) $I_2$, $NaBH_4$, THF, r.t; (iii) Pd/C/$H_2$, MeOH, r.t.; (iv) $Boc_2O$, $NaHCO_3$, dioxane, r.t., 2h; EtOAc (v) MsCl, $Et_3N$, DCM (vi) $CF_3COOH$, $H_2O$; (vii) $ClCH_3COOC_2H_5$, $K_2CO_3$ (viii) $NaN_3$, DMF (ix) Pd/C/$H_2$, EtOAc; (x) $RSO_2Cl$, Py, reflux, 2h

**Figure 1** – Synthesis of ethyl-3,4-bis(thiophene-2-sulfonamido)pyrrolidinylacetate

Initially *ethyl 2-((3S,4S)-3,4-bis(thiophene-2-sulfonamido) pyrrolidin-1-yl)acetate* **(1)** was alkylated with cyclopropyl bromide to get N-alkylated sulfonamide **(2)**. This on treatment with hydrazine produces respective hydrazide **(3)**, which on reaction with substituted benzoic acid in presence of $POCl_3$ gives 1,3,4-oxadiazole derivatives **(4a-f)**. Again, the hydrazide **(3)** on treatment with substituted aldehydes gives benzylidene derivative **(5a-f)**, which on reaction with chloroacetylchloride and mercaptoacetic acid produces azitidinones **(6a-f)** and thiazolidinones **(7a-f)** respectively. The synthesis of target compounds is depicted in Figure 2.

The compound **1** undergoes N-alkylation at sulfonamide group and further on treatment with hydrazine produces hydrazide **(3)**. This hydrazide can be converted to 1,3,4-oxadiazole **(4a-f)** and substituted benzylidene hydrazinyl derivatives **(5a-f)** on reaction with substituted benzoic acid in the presence of $POCl_3$ and substituted benzaldehyde respectively. Finally, the cyclization of the compounds **5a-f** takes place in presence of chloroacetylchloride and mercaptoacetic acid to produce azetidinone derivatives **(6a-f)** and thiazolidinone derivatives **(7a-f)** respectively.




| Comp | 4a/5a/6a/7f | 4b/5b/6b/7f | 4c/5c/6c/7f | 4d/5d/6d/7f | 4e/5e/6e/7f | 4f/5f/6f/7f |
|---|---|---|---|---|---|---|
| R | -H | -CH3 | -OCH3 | -Cl | -Br | -NO2 |

**Reagents & Conditions:** (i) acetonitrile, potassium carbonate, cyclopropyl bromide, KI, reflux, 20h; (ii) ) Hydrazine hydrate, ethanol, reflux, 5h; (iii) 4-substituted benzoic acid, phosphoryl chloride, reflux, 5-8h; (iv) 4-substituted benzaldehyde, Glacial aceticacid, reflux, 4-8h; (v) chloroacetylchloride, triethylamine, dioxane, 0-5°C, 8h; (vi) Mercaptoaceticacid, 120-125°C, 12h

**Figure 2** – Synthesis of pyrrolidine-3,4-disubstitutedsulfonamides containing 1,3,4-oxadiazole, azetidinone and thiazolidinone

*3.1 Antimicrobial studies*

The antibacterial activities of titled compounds, **4a-f**, **6a-f** and **7a-f** have been conducted against gram positive *Staphylococcus aureus, Bacillus subtilis,* and gram negative *Escherichia coli, Proteus vulgaris*. The compounds belonging to 6a-f series are highly active against gram-positive and gram-negative bacteria showing the broad spectra of antibacterial activity. The activity of the rest of the compounds was found moderate to low against the tested microorganisms. This was expected because of the presence of ß-lactum ring in the **6a-f** series. The antibacterial activity of the tested compounds is shown in Table 2.

The antifungal activities of the series **4a-f**, **6a-f** and **7a-f** were tested against *Asperigillusflavus* and *Candida albicans*. The compounds **7a-f** exhibit privileged activity among the tested compounds and the others were found either moderately active or slightly active. 1,3,4-Oxadizole possessing pyrrolidine-3,4-diyl sulfonamide derivative bearing thiazolidin-4-one moiety **(4f)** showed moderate activity. The test results are presented in Table 2.

**Table 2** – Antimicrobial activity

| Comp (20 µg/mL) | Zone of inhibition (mm)* | | | | | |
|---|---|---|---|---|---|---|
| | Antibacterial activity | | | | Antifungal activity | |
| | Gm + ve | | Gm -ve | | *Asperigillus flavus* | *Candida albicans* |
| | *S. aureus* | *B. subtilis* | *E. coli* | *P. vulgaris* | | |
| 4a | 13 | 16 | 18 | 23 | 15 | 16 |
| 4b | 14 | 15 | 16 | 19 | 13 | 15 |
| 4c | 11 | 14 | 17 | 21 | 18 | 14 |
| 4d | 18 | 19 | 22 | 25 | 16 | 19 |
| 4e | 15 | 17 | 20 | 26 | 17 | 17 |
| 4f | 19 | 21 | 23 | 29 | 20 | 21 |
| 6a | 14 | 13 | 20 | 25 | 17 | 15 |
| 6b | 16 | 14 | 24 | 20 | 15 | 13 |
| 6c | 14 | 18 | 23 | 27 | 14 | 17 |
| 6d | 18 | 17 | 19 | 21 | 18 | 16 |
| 6e | 17 | 19 | 17 | 22 | 19 | 18 |
| 6f | 20 | 21 | 25 | 29 | 23 | 21 |
| 7a | 13 | 16 | 12 | 22 | 18 | 17 |
| 7b | 15 | 14 | 14 | 25 | 16 | 19 |
| 7c | 11 | 19 | 13 | 22 | 17 | 21 |
| 7d | 17 | 17 | 16 | 23 | 19 | 22 |
| 7e | 15 | 20 | 14 | 21 | 21 | 20 |
| 7f | 18 | 21 | 19 | 27 | 23 | 24 |
| Streptomycin | 22 | 24 | 28 | 32 | -- | -- |
| Clotrimazole | -- | -- | -- | -- | 25-30 | 25-30 |

* indicate diameter of inhibition in mm.

*3.2 In silico studies*

It was already evident that β-glucosidase and related proteins are prime controllers of apoptosis or programmed cell death concerned with human disease including diabetes. N-substituted pyrrolidines exhibit glycosidase inhibitory activity [27,28]. The synthesized compounds were screened for antidiabetic activity by choosing human β-glucosidase as the target protein. In a view to assessing the potential of the synthesized compounds for the β-glucosidase inhibitory activity, they were docked into the active site of the receptor (3VKK).

GOLD Score is a result of force field based scoring functions of protein-ligand hydrogen bond energy S(hb_ext), protein-ligand van der Waals energy S (vdw_ext), ligand internal van der Waals energy S(hb_int), ligand intramolecular hydrogen bond energy S(vdw_int). The total fitness score was computed by multiplying the external vdw score with 1.375, an empirical correction to encourage the hydrophobic protein-ligand contact. Ligand binding positions were predicted by optimizing the fitness function:

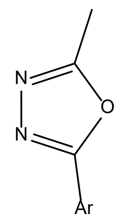
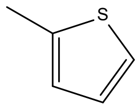
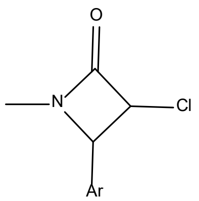
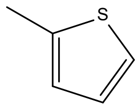
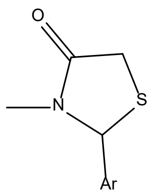
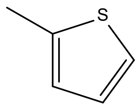
$$\text{GOLD Score} = S(hb\_ext) + S(vdw\_ext) + S(hb\_int) + S(vdw\_int)$$

It was evident that the docking results show the amino acid residues Tyr 18, Arg 98, Val 145, Glu 152, Gly 101 of the enzyme were involved in hydrogen bonding interaction with the top poses of compounds. The inhibitory interactions translate into therapeutic efficiency to be established by traditional clinical studies. The β-glucosidase inhibitory activity of the model compounds from series **4**, **6** and **7** in terms of GOLD Score fitness and bonding interactions were shown in Table 3.

The title compounds under investigation exhibited remarkable inhibitory action against ß-glucosidases. The fitness score of 44.99 indicated that the presence of 1,3,4-oxadiazole containing pyrrolidine sulfonamides exhibit higher inhibitory activity against β-glucosidase.

The negative binding energy values represent the highest potential for the binding sites of the target protein to the title compounds. The low $k_i$ values either in the micromolar or in nanomolar ranges of the title compounds are direct evidence

**Table 3** – β-glucosidase inhibitory activity and hydrogen bonding interactions of compounds **4f**, **6f** and **7f**

| Comp | G | Ar | $R^1$ | Number of hydrogen bonds | Atoms | | Bond length ($A^O$) | Fitness |
|---|---|---|---|---|---|---|---|---|
| | | | | | Protein | Comp | | |
| 4f |  | $C_6H_4NO_2$ |  | 5 | Val 145 | O27 | 2.226 | 44.99 |
| | | | | | Glu 152 | O39 | 2.052 | |
| | | | | | Tyr 18 | N9 | 1.485 | |
| | | | | | Tyr 18 | O7 | 2.513 | |
| | | | | | Gly 101 | O7 | 2.652 | |
| 6f |  | $C_6H_4NO_2$ |  | 3 | Tyr 18 | O19 | 2.217 | 37.12 |
| | | | | | Tyr 21 | O19 | 2.225 | |
| | | | | | Arg 98 | O26 | 2.310 | |
| 7f |  | $C_6H_4NO_2$ |  | 2 | Val 148 | O27 | 1.598 | 17.32 |
| | | | | | Arg 98 | O10 | 1.986 | |

for their high affinity interaction for the protein under investigation. The details are given in Table 4. The docking conformations of **4f**, **6f** and **7f** are shown in Figures 3-5 and represent the active site of the β-glucosidase protein.

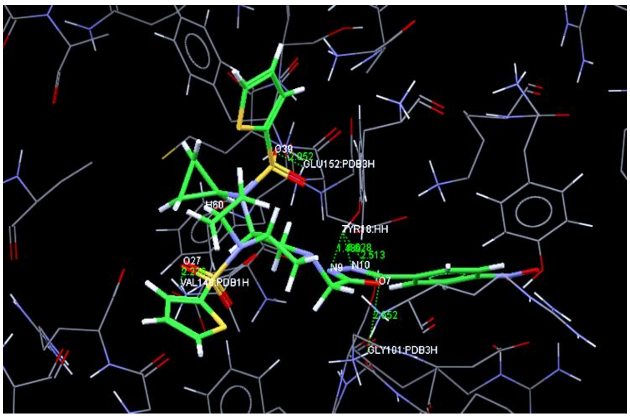



**Figure 3** – Docking result of compound **4f**

**Table 4** – Docking results and pharmacophore analysis of model compounds

| Parameter | 4f | 6f | 7f |
|---|---|---|---|
| Free energy of Binding (kcal/mol) | -9.22 | -8.24 | -7.92 |
| Inhibition constant $k_i$ at 298.15 K | 835.12 nM | 712.10 nM | 691.12 nM |
| Total Intermolecular Energy (kcal/mol) | -11.23 | -9.21 | -10.12 |
| vdW + Hbond + desolv Energy (kcal/mol) | -12.12 | -11.11 | -12.02 |
| GPCR ligand | -0.07 | -0.69 | 0.03 |
| Ion channel modulator | -0.69 | -1.56 | -0.82 |
| Kinase inhibitor | -0.49 | -1.06 | -0.46 |
| Nuclear receptor ligand | -0.79 | -0.59 | -1.10 |
| Protease inhibitor | 0.39 | 0.42 | -0.22 |
| Enzyme inhibitor | -0.25 | -1.21 | -0.21 |
| miLogP | -0.212 | 2.108 | 2.624 |
| Clogp | -2.16 | 1.09 | -0.51 |

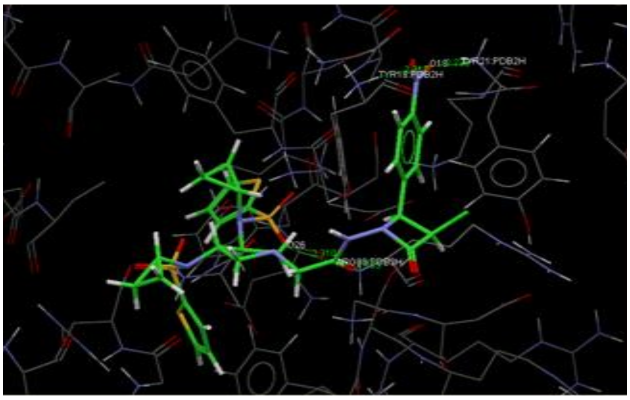


**Figure 4** – Docking result of compound **6f**

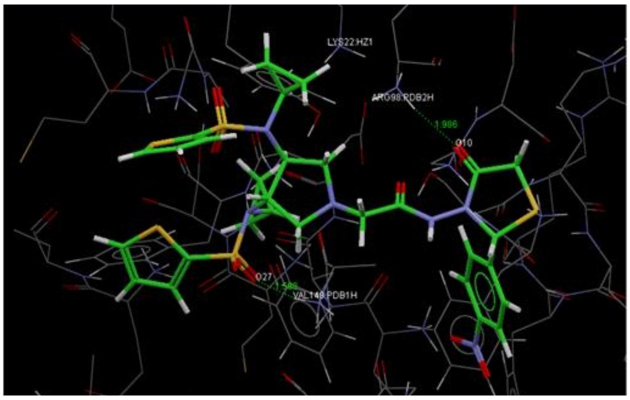


**Figure 5** – Docking result of compound **7f**

**4. Conclusions**

Title compounds were established as potent antibacterial and antifungal active by exhibiting comparable results with standard drugs. A series of 3,4-disubstitutedpyrrolidine sulfonamide compounds were synthesized. The compounds exhibit moderate inhibition against the ß-glucosidases enzyme. The 3,4-disubstitutedpyrrolidinesulfonamides containing 1,3,4-oxadiazole moiety is a higher potent ß-glucosidase inhibitor than that of azetidinone and thiazolidinone moieties. Structure activity relation (SAR) proved that the inhibition activity against ß-glucosidase was favored by the introduction of thiophensulfonyl group at the 3 & 4 positions and a five membered oxadiazole ring at the N1 position of the pyrrolidine ring. These SAR results are in good compatible with docking studies.

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**Conflicts of interest**

On behalf of all authors, the corresponding author states that there is no conflict of interest.

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МАЗМҰНЫ – СОДЕРЖАНИЕ

CONTENTS