# Substituted (cycloalkylcarbonylthioureido)aryl-(benzyl-) carboxylic(sulfonic) acids: synthesis, antimicrobial and growth-regulating activity

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A – research concept and design; B – collection and/or assembly of data; C – data analysis and interpretation; D – writing the article; E – critical revision of the article; F – final approval of the article

Acylisothiocyanates are a promising class of organic compounds that are present in the plant world and can be used in the synthesis of disubstituted thioureas and various heterocycles. These derivatives are characterized by growth-regulating, antibacterial, fungicidal, cytotoxicity, and other activities. Modification of acylisothiocyanates by fragments of substituted aminoarylcarboxylic (sulfo) acids is promising, as some of them (anthranilic, *p*-aminobenzoic acids) are precursors for the auxins and other natural compounds synthesis. Their combined activity is also an important aspect. Namely the simultaneous manifestation of both fungicidal and restrictive activity. Based on this, the synthesis of new substituted (cycloalkylcarbonylthioureido)aryl-(benzyl-)carboxylic (sulfonic) acids is relevant as promising regulators of plant growth with antibacterial activity.

**The aim** of this work is to search for effective compounds with growth-regulating and antimicrobial activity among substituted (cycloalkylcarbonylthioureido)aryl-(benzyl-)carboxylic (sulfonic) acids.

**Materials and methods.** Methods of organic synthesis, physical and physical-chemical methods of analysis of organic compounds (IR, NMR $^1$H-spectroscopy, chromato-mass spectrometry, elemental analysis). Antimicrobial activity studies were performed on standard strains of bacteria and fungi (*S. aureus* ATCC 25923, *E. coli* ATCC 25922, *P. aeruginosa* ATCC 27853 and *C. albicans* ATCC 885-653). The effect of synthesized compounds on growth rates was evaluated on wheat (variety Grom).

**Results.** An "*in situ*" method for the synthesis of substituted (cycloalkylcarbonylthioureido)aryl-(benzyl-)carboxylic (sulfonic) acids was proposed. It was shown that the latter could be easily synthesized by the sequential interaction of cyclopropanecarbonyl chloride, ammonium isothiocyanate, and aminoaryl-(benzyl-)carboxylic, sulfanilic acids or sulfamide. Data of $^1$H NMR spectra showed the peculiarities of the structure of the synthesized compounds, namely the presence of singlet signals of protons of urea, thioamide and carboxyl groups, multiple signals of methine and methylene protons of cyclopropane fragment. It was found that the synthesized compounds showed moderate antimicrobial activity against *S. aureus* and *P. aeruginosa* (MIC 50 μg/ml, MBC 100 μg/ml) and significant antifungal activity against *C. albicans* (MIC 25–50 μg/ml, MFC 25–50 μg/ml). A number of compounds were identified as effective regulators of wheat growth and exceed the natural analogue – heteroauxin (3-indolylacetic acid) in terms of auxin-like activity.

**Conclusions.** A one-step method for the synthesis of substituted (cyclopropanecarbonylthioureido)aryl-(benzyl-)carboxylic (sulfonic) acids was developed. The physical-chemical properties of the synthesized compounds were studied using a set of methods (IR, $^1$H NMR spectroscopy, chromato-mass spectrometry, elemental analysis) and the features of the structure were discussed. The synthesized compounds reveal moderate antimicrobial, high antifungal activity, and growth-promoting activity.

**Key words:** synthesis, disubstituted thioureas, aminoaryl-(benzyl-)carboxylic acids, sulfanilic acid and its amide, antimicrobial activity, growth-regulating activity.

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## Заміщені (циклоалкілкарбонілтіоуреїдо)арил-(бензил-)карбонові (сульфонові) кислоти: синтез, антимікробна та рістрегулююча активність

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Ацилізотіоціанати – перспективний клас органічних сполук, який представлений у рослинному світі та може бути використаний у синтезі дизаміщених тіосечовин і різних гетероциклів. Для цих похідних характерні рістрегулююча, антибактеріальна, фунгіцидна, цитотоксична та інші види активності. Модифікація ацилізотіоціанатів фрагментами заміщених аміноарилкарбонових (сульфо)

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**Key words:** synthesis, disubstituted thioureas, aminoaryl-(benzyl-)carboxylic acids, sulfanilic acid and its amide, antimicrobial activity, growth-regulating activity.

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кислот перспективна, оскільки деякі з них (антранілова, *п*-амінобензойна кислоти) є попередниками синтезу ауксинів та інших природних сполук. Важливий аспект – їхня комбінована дія, як-от одночасний прояв фунгіцидної та ріст-регулювальної активності. Отже, актуальним є синтез нових заміщених (циклоалкілкарбонілтіоуреїдо)арил-(бензил-)карбонових (сульфонових) кислот як перспективних регуляторів росту рослин з антибактеріальною активністю.

**Мета роботи** – пошук ефективних сполук із рістрегулюючою та протимікробною активністю серед заміщених (циклоалкілкарбонілтіоуреїдо)арил-(бензил-)карбонових (сульфонових) кислот.

**Матеріали та методи.** Використали методики органічного синтезу, фізичні й фізико-хімічні методи аналізу органічних сполук (ІЧ-, ЯМР $^1$Н-спектроскопія, хромато-мас-спектрометрія, елементний аналіз). Дослідження на протимікробну активність виконали на стандартних штамах бактерій і грибів (*S. aureus* ATCC 25923, *E. coli* ATCC 25922, *P. aeruginosa* ATCC 27853 та *C. albicans* ATCC 885-653). Вплив синтезованих сполук на показники росту оцінювали на пшениці (сорт Гром).

**Результати.** Запропоновано «*in situ*» метод формування заміщених (циклоалкілкарбонілтіоуреїдо)арил-(бензил-)карбонових (сульфонових) кислот. Показано, що останні легко формуються послідовною взаємодією циклопропанкарбоніл хлориду, амонію ізотіоціанату та аміноарил-(бензил-)карбонових, сульфанілової кислот або сульфаміду. Дані $^1$Н ЯМР-спектрів показали особливості будови синтезованих сполук: наявність синглетних сигналів протонів карбамідної, тіоамідної та карбоксильної груп, мультиплетних сигналів метинового та метиленових протонів циклопропанового фрагмента. Встановили, що синтезовані сполуки характеризуються помірною антимікробною активністю щодо *S. aureus* і *P. aeruginosa* (МІК 50 мкг/мл, МБК 100 мкг/мл), чималою протигрибковою активністю проти *C. albicans* (МІК 25–50 мкг/мл, МФК 25–50 мкг/мл). Виявили ряд сполук, що є ефективними регуляторами росту пшениці, за ауксиноподібною дією перевершують природний аналог гетероауксин (3-індолілоцтову кислоту).

**Висновки.** Розробили одностадійний метод синтезу заміщених (циклопропанкарбонілтіоуреїдо)арил-(бензил-)карбонових(сульфонових) кислот. Дослідили фізико-хімічні властивості синтезованих сполук, використавши комплекс методів (ІЧ-, $^1$Н ЯМР-спектроскопія, хромато-мас-спектрометрія, елементний аналіз), виявили особливості їхньої будови. Синтезовані сполуки мають помірну антимікробну, високу протигрибкову активність і рістстимулюючу активності.

**Ключові слова:** синтез, дизаміщені тіосечовини, аміноарил-(бензил-)карбонові кислоти, сульфанілова кислота та її аміди, протимікробна активність, рістрегулююча активність.

**Актуальні питання фармацевтичної і медичної науки та практики. 2021. Т. 14, № 1(35). С. 4–11**

## Замещенные (циклоалкилкарбонилтиоуреидо)арил(бензил-)карбоновые (сульфоновые) кислоты: синтез, антимикробная и рострегулирующая активность

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Ацилизотиоцианаты – перспективный класс органических соединений, представленный в растительном мире, может быть использован в синтезе дизамещенных тиомочевин и различных гетероциклов. Для этих производных характерны рострегулирующая, антибактериальная, фунгицидная, цитотоксическая и другие виды активности. Модификация ацилизотиоцианатов фрагментами замещенных аминоарилкарбоновых (сульфо) кислот перспективна, так как некоторые из них (антраниловая, *п*-аминобензойная кислоты) – предшественники синтеза ауксинов и других природных соединений. Важный аспект – их комбинированное действие, а именно одновременное проявление фунгицидной и рострегулирующей активности. Исходя из этого, актуальным является синтез новых замещенных (циклоалкилкарбонилтиоуреидо)арил-(бензил-)карбоновых (сульфоновых) кислот как перспективных регуляторов роста растений с антибактериальной активностью.

**Цель работы** – поиск эффективных соединений с рострегулирующей и противомикробной активностью среди замещенных (циклоалкилкарбонилтиоуреидо)арил-(бензил-)карбоновых (сульфоновых) кислот.

**Материалы и методы.** Использованы методики органического синтеза, физические и физико-химические методы анализа органических соединений (ИК, ЯМР $^1$Н-спектроскопия, хромато-масс-спектрометрия, элементный анализ). Исследования противомикробной активности проведены на стандартных штаммах бактерий и грибов (*S. aureus* ATCC 25923, *E. coli* ATCC 25922, *P. aeruginosa* ATCC 27853 и *C. albicans* ATCC 885-653). Влияние синтезированных соединений на показатели роста оценивали на пшенице (сорт Гром).

**Результаты.** Предложен «*in situ*» метод формирования замещенных (циклоалкилкарбонилтиоуреидо)арил-(бензил-)карбоновых(сульфоновых) кислот. Показано, что последние легко формируются последовательным взаимодействием циклопропанкарбонил хлорида, аммония изотиоцианата и аминоарил-(бензил-)карбоновых, сульфаниловой кислот или сульфамидом. Данные $^1$Н ЯМР-спектров показали особенности строения синтезированных соединений, а именно наличие синглетных сигналов протонов карбамидной, тиоамидной и карбоксильной групп, мультиплетных сигналов метинового и метиленовых протонов циклопропанового фрагмента. Установлено, что синтезированные соединения проявляют умеренную антимикробную активность в отношении *S. aureus* и *P. aeruginosa* (МИК 50 мкг/мл, МБК 100 мкг/мл) и значительную противогрибковую активность в отношении *C. albicans* (МИК 25–50 мкг/мл, МФК 25–50 мкг/мл). Установлен ряд соединений, эффективных регуляторов роста пшеницы, по ауксиноподобному действию превышающие природный аналог гетероауксин (3-индолилуксусную кислоту).

**Выводы.** Разработан одностадийный метод синтеза замещенных (циклопропанкарбонилтиоуреидо)арил-(бензил-)карбоновых (сульфоновых) кислот. Исследованы физико-химические свойства синтезированных соединений с использованием комплекса методов (ИК-, $^1$Н ЯМР-спектроскопия, хромато-масс-спектрометрия, элементный анализ) и отмечены особенности строения. Показано, что синтезированные соединения проявляют умеренную антимикробную, высокую противогрибковую и ростстимулирующую активности.

**Ключевые слова:** синтез, дизамещенные тиомочевины, аминоарил-(бензил-)карбоновые кислоты, сульфаниловая кислота и ее амиды, противомикробная активность, рострегулирующая активность.

**Актуальные вопросы фармацевтической и медицинской науки и практики. 2021. Т. 14, № 1(35). С. 4–11**

Common plant growth regulators are auxins, gibberellins, cytokinins, brassinosteroids, etc. [1–3]. In addition to phytohormones, plants also produce secondary metabolic products, which also are growth regulators (flavonoids, amino acids, lipids, carboxylic acids, alkaloids, unsaturated lactones, terpenoids, etc.) [4]. However, the use of substances with direct hormonal activity is likely to give way to the use of chemical agents. The latter should modify the metabolism or transport of plant hormones [5]. This approach is advantageous because it increases yields, and synthetic growth regulators are more metabolically stable and cheaper than those with direct hormonal activity. Among the synthetic growth regulators, today are analogues of auxins and cytokinins; antiauxins and cytokinin antagonists; inhibitors of auxin transport and gibberellin biosynthesis; substances that emit ethylene or promote its biosynthesis in plants [3].

Aryl and aryloxyaliphatic acids, onium salts, heterocyclic compounds, etc. are of practical importance among synthetic plant growth regulators. Thus, 3-indolylbutyric, 3-indolyl- and *N*-naphthylacetic acids, chlormequat, mepiquat, 2,6-dimethylpyridine *N*-oxide, dimethyl sulfoxide, etc. are used to improve plant growth. Such compounds are used for growth retardation: onium compounds (chlormequat chloride, bromholin bromide, iodolin iodide, etc.), maleic and succinic acid hydrazides, 1,2,4-triazole derivatives (paclobutrazol, uniconazole, etc.), ethylene producers (dextrel hydrel, ectdrel and ether), dichloroisobutyrate (sodium dichloroisobutyrate), etc. It should be noted that these retardants are not universal, not all of them are able to inhibit all forms of growth (for example, rooting cuttings and distortion of coleoptiles), don't show a strong effect compared to phytohormones, are toxic, difficult to metabolize and accumulate in plants. Therefore, the development of new effective environmentally friendly plant growth regulators based on low molecular weight compounds is of great theoretical and practical interest. Their combined activity is also an important aspect. Namely the simultaneous manifestation of both fungicidal and restrictive activity.

Acylisothiocyanates are interesting objects in terms of antimicrobial and growth-regulating agents. Firstly, they are low-toxic compounds, widely represented in the plant world (plants of the cruciferous family) [6,7]. Secondly, the chemistry of cycloalkane carbonylisocyanates is diverse and can be used in the synthesis of functionalized acylthioureas and acylthiosemicarbazides [8–12], as well as various heterocycles [13] which are characterized by growth-regulating, antibacterial, fungicidal and cytotoxicity activity. Thirdly, the modification of acyl isothiocyanates by fragments of substituted aminoarylcarboxylic (sulfo) acids is promising, as some of them (anthranilic, *p*-aminobenzoic acids) are important precursors of the synthesis of auxins and other natural compounds [14,15].

## Aim

Therefore, the aim of the study is to search of effective compounds with growth-regulating and antimicrobial activity among substituted (cycloalkylcarbonylthioureido)aryl-(benzyl-)carboxylic (sulfonic) acids.

## Materials and methods

Cyclopropanecarbonyl chloride (**1.1**) was synthesized by known method [16]. Other starting materials and solvents were obtained from commercially available sources and were used without additional purification.

Melting points were determined in open capillary tubes in a 'Mettler Toledo MP 50" apparatus and were uncorrected. The elemental analyses (C, H, N, S) were performed using the ELEMENTAR vario EL cube analyzer (USA). Analyses were indicated by the symbols of the elements or functions within ±0.3 % of the theoretical values. IR spectra (4000–600 $cm^{-1}$) were recorded on a Bruker ALPHA FT-IR spectrometer (Bruker Bioscience, Germany) using a module for measuring attenuated total reflection (ATR). $^1H$ NMR spectra (400 MHz) were recorded on a Varian-Mercury 400 spectrometer (Varian Inc., Palo Alto, CA, USA) with TMS as internal standard in DMSO-$d_6$ solution. LC-MS were recorded using chromatography/mass spectrometric system which consists of high-performance liquid chromatography Agilent 1100 Series (Agilent, Palo Alto, CA, USA) equipped with diode-matrix and mass-selective detector Agilent LC/MSD SL (atmospheric pressure chemical ionization – APCI).

**Antimicrobial test.** The sensitivity of the microorganisms to the synthesized compounds was evaluated according to the described methods [17]. The assay was conducted on Mueller–Hinton agar by two-fold serial dilution of the compound in 1 ml. After that, 0.1 ml of microbial seeding (106 cells/ml) was added. Minimal inhibit concentration of the compound was determined by the absence of visual growth in the test tube with a minimal concentration of the substance, minimal bactericide/fungicide concentration was determined by the absence of growth on agar medium after inoculation of the microorganism from the transparent test-tubes. DMSO was used as a solvent, initial solution concentration was 1 mg/ml. For preliminary screening, the mentioned ahead standard test cultures were used: *S. aureus* ATCC 25923, *E. coli* ATCC 25922, *P. aeruginosa* ATCC 27853 and *C. albicans* ATCC 885-653 standard test cultures. All test strains were received from bacteriological laboratory in Zaporizhzhia Regional Laboratory Center of State Sanitary and Epidemiological Service of Ukraine. Nitrofurazone and Ketoconazole were used as reference compounds with proved antibacterial/antifungal activity. Additional quality control of the culture media and solvents was conducted by commonly used methods [17].

**Influence of synthesized compounds on growth rates.** The effect of synthesized compounds on growth rates was assessed on wheat (variety Grom) at the laboratory of the State Enterprise "State Center for Certification and Examination of Agricultural Products" (Zaporizhzhia) by a known method [18]. The concentration of aqueous solutions of "Heteroauxin" (3-indolylacetic acid) and test compounds was 0.00002 %. To achieve that concentration a 0.2 g of substances was

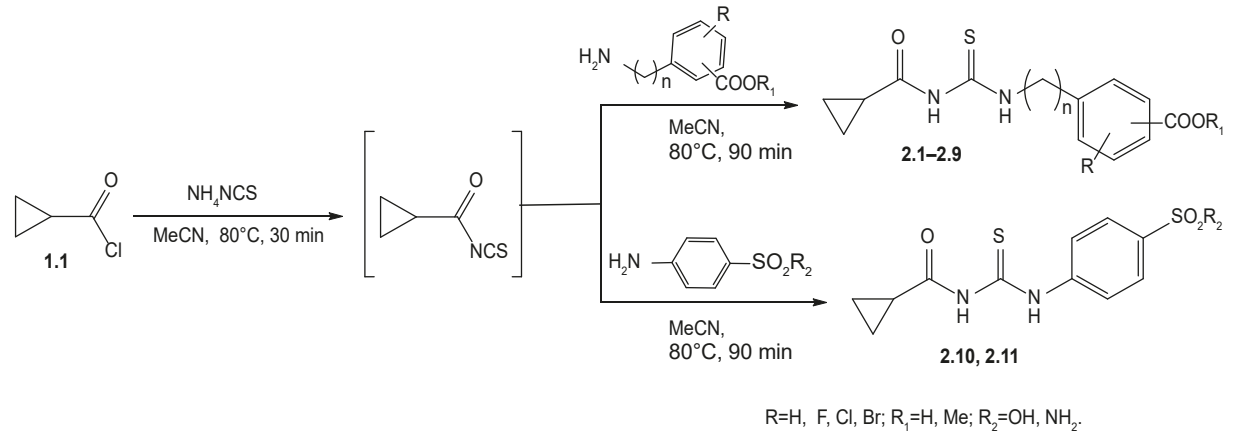


Fig. 1. Methods for the synthesis of the substituted (cyclopropanecarbonylthioureido)aryl-(benzyl-)carboxylic(sulfonic) acids.

emulsified in 1 ml of Twin-80 and adjusted with water to 1 l. Then from the resulting solution, 1 ml was taken and again adjusted with water to 1 l. Irrigation with a solution of test substances was performed every other day, the rate of water consumption – 40 ml per Petri dish. Control seeds were watered with water and an emulsifier. Grain germination on days 4 and 8, the total number and length of roots, total mass of aboveground part (considering the weight of the grain) were evaluated during liquidation of the experiment.

## Results

The synthesis of the original cyclopropanecarbonyl isothiocyanate was carried out according to a known synthetic approach, which included the interaction of cyclopropanecarbonyl chloride (**1.1**) with ammonium isothiocyanate (acetonitrile medium) (*Fig. 1*) [12]. Cyclopropanecarbonyl isothiocyanate without isolation from the reaction medium (*in situ* method) regioselectively and easily has attached aminoaryl-(benzyl-)carboxylic acids, 4-aminobenzenesulfonic acid, and its amide. This was produced individual compounds **2.1–2.11** with satisfactory yields (48–74 %).

The structure and individuality of compounds 2 were proved using elemental analysis, chromato-mass, IR and $^1$H NMR spectra.

### Experimental section

*The general method for the synthesis of the substituted (cyclopropanecarbonylthioureido)aryl-(benzyl-)carboxylic(sulfonic) acids* (**2.1–2.11**). To a solution of corresponding 1.04 g (0.01 mol) cyclopropanecarbonyl chloride (**1.1**) in 20 mL of acetonitrile 0.76 g (0.01 mol) of ammonium isothiocyanate was added and stirred at 80 ºC for 30 min. The mixture was cooled down to r.t. and 0.01 mol of corresponding aminoaryl(carboxylic)sulfonic acids was added and stirred at 80 ºC for 90 min. The solution was cooled down, poured into the water. The formed precipitate was filtrated, dried and recrystallized from ethanol.

**4-((3-(Cyclopropanecarbonyl)thioureido)methyl)benzoic acid (2.1).** Yield: 48 %; Mp.: 218–225 °C; IR (cm$^{-1}$): 3772, 3763, 3713, 3693, 3679, 3663, 3631, 3591, 3571, 3531, 3415, 3284, 3012, 2924, 2892, 2558, 2370, 2348, 2272, 2083, 1928, 1874, 1851, 1834, 1804, 1785, 1754, 1738, 1722, 1685, 1658, 1627, 1612, 1579, 1563, 1536, 1502, 1483, 1463, 1428, 1407, 1346, 1321, 1291, 1233, 1215, 1181, 1148, 1127, 1101, 1065, 1042, 1019, 982, 941, 894, 879, 858, 823, 798, 781, 760, 738, 702, 673, 636, 625, 617; $^1$H NMR (400 MHz, DMSO-$d_6$) δ 12.50 (s, 1H, -COOH), 11.44 (s, 1H, -C(S)N*H*-), 11.05 (s, 1H, -C(O)N*H*-), 7.86 (d, $J$ = 7.7 Hz, 2H, Ph H-3,5), 7.33 (dd, $J$ = 8.0 Hz, 2H, Ph H-2,6), 4.30 (d, $J$ = 5.9 Hz, 1H, -$CH_2$), 2.53–2.42 (m, 1H, cyclopropyl H-1), 0.91–0.79 (m, 4H, cyclopropyl cyclopropyl H-2$_{eq}$, 2$_{ax}$, 3$_{eq}$, 3$_{ax}$); LC-MS, m/z = 279 [M+1]; Anal. Calcd. for $C_{13}H_{14}N_2O_3S$: C, 56.10; H, 5.07; N, 10.07; S, 11.52; Found: C, 54.59; H, 4.67; N, 10.69; S, 11.89.

**2-(3-(Cyclopropanecarbonyl)thioureido)benzoic acid (2.2).** Yield: 59 %; Mp.: 158–161 °C; IR (cm$^{-1}$): 3955, 3906, 3888, 3870, 3835, 3844, 3824, 3807, 3753, 3715, 3693, 3678, 3653, 3632, 3591, 3571, 3472, 3186, 3004, 2924, 2543, 2369, 2348, 2277, 1874, 1851, 1835, 1804, 1785, 1755, 1738, 1722, 1688, 1641, 1630, 1607, 1581, 1530, 1503, 1484, 1468, 1444, 1422, 1390, 1312, 1284, 1244, 1169, 1148, 1102, 1087, 1068, 1031, 952, 928, 877, 855, 800, 776, 758, 745, 713, 698, 675, 659, 642, 627, 611; $^1$H NMR (400 MHz, DMSO-$d_6$) δ: 13.01 (s, 1H, -C(S)N*H*-), 11.48 (s, 1H, -C(O)N*H*-), 8.18 (d, $J$ = 8.2 Hz, 1H, Ph H-6), 7.88 (dd, $J$ = 7.9 Hz, 1H, Ph H-3), 7.47 (t, $J$ = 7.5 Hz, 1H, Ph H-5), 7.23 (t, $J$ = 7.6 Hz, 1H, Ph H-4), 2.14–2.06 (m, 1H, cyclopropyl H-1), 0.90–0.81 (m, 4H, cyclopropyl H-2$_{eq}$, 2$_{ax}$, 3$_{eq}$, 3$_{ax}$); LC-MS, m/z = 265 [M+1]; Anal. Calcd. for $C_{12}H_{12}N_2O_3S$: C, 54.53; H, 4.58; N, 10.60; S, 12.13; Found: C, 54.60; H, 4.64; N, 10.69; S, 12.26.

**2-(3-(Cyclopropanecarbonyl)thioureido)-6-fluorobenzoic acid (2.3).** Yield: 59 %; Mp.: 186–190 °C; IR (cm$^{-1}$): 3887, 3833, 3810, 3746, 3718, 3694, 3678, 3564, 3632, 3572, 3187, 3013, 2847, 2686, 2543, 2372, 2348, 2277, 1851, 1835, 1817,

1785, 1754, 1737, 1722, 1688, 1641, 1617, 1580, 1532, 1470, 1447, 1419, 1391, 1307, 1281, 1243, 1203, 1176, 1164, 1151, 1133, 1103, 1069, 1028, 942, 917, 899, 889, 831, 812, 781, 747, 709, 676, 660, 609; $^1$H NMR (400 MHz, DMSO-$d_6$) δ 13.45 (s, 1H, -COOH), 12.67 (s, 1H, -C(S)NH-), 11.67 (s, 1H, -C(O)NH-), 7.82 (d, $J$ = 8.3 Hz, 1H, Ar H-3), 7.41 (td, $J$ = 8.3, 5.9 Hz, 1H, Ar H-5), 7.03 (t, $J$ = 8.9 Hz, 1H, Ar H-4), 2.13-2.05 (m, 1H, cyclopropyl H-1), 0.98–0.85 (m, 4H, cyclopropyl H-$2_{eq}$, $2_{ax}$, $3_{eq}$, $3_{ax}$); LC-MS, m/z = 283 [M+1]; Anal. Calcd. for $C_{12}H_{11}FN_2O_3S$: C, 51.06; H, 3.93; F, 6.73; N, 9.92; S, 11.36; Found: C, 51.21; H, 4.03; N, 10.01; S, 11.46.

**5-Chloro-2-(3-(cyclopropanecarbonyl)thioureido)benzoic acid (2.4).** Yield: 52 %; Mp.: 172–175 °C; IR (cm$^{-1}$): 3660, 3632, 3622, 3611, 3591, 3571, 3473, 3414, 3358, 3184, 3006, 2858, 2624, 2483, 2371, 2347, 2258, 2117, 1974, 1928, 1900, 1874, 1852, 1834, 1817, 1803, 1785, 1765, 1754, 1738, 1722, 1690, 1679, 1658, 1641, 1630, 1599, 1573, 1547, 1513, 1502, 1478, 1468, 1441, 1412, 1385, 1321, 1298, 1282, 1241, 1201, 1163, 1146, 1113, 1062, 1027, 949, 926, 885, 868, 816, 789, 756, 732, 699, 675, 663, 617; $^1$H NMR (400 MHz, DMSO-$d_6$) δ 13.06–12.95 (br.s, Hz, 2H, -COOH, -C(S)NH-), 11.60 (s, 1H, -C(O)NH-), 8.25 (dd, $J$ = 8.7 Hz, 1H, Ar H-3), 7.89–7.80 (m, 1H, Ar H-6), 7.49 (m, 1H, Ar H-4), 2.14–2.12 (m, 1H, cyclopropyl H-1), 1.00–0.88 (m, 4H, cyclopropyl H-$2_{eq}$, $2_{ax}$, $3_{eq}$, $3_{ax}$); LC-MS, m/z = 299 [M+1]; Anal. Calcd. for $C_{12}H_{11}ClN_2O_3S$: C, 48.25; H, 3.71; Cl, 11.87; N, 9.38; S, 10.73; Found: C, 48.33; H, 3.78; N, 9.42; S, 10.81.

**3-(3-(Cyclopropanecarbonyl)thioureido)benzoic acid (2.5).** Yield: 66 %; Mp.: 223–228 °C; IR (cm$^{-1}$): 3855, 3734, 3609, 3010, 2813, 2532, 2310, 1681, 1606, 1590, 1525, 1452, 1416, 1392, 1298, 1250, 1164, 1116, 1101, 1081, 1069, 1031, 1003, 942, 925, 913, 877, 817, 797, 766, 744, 726, 696, 676, 666, 636, 619; $^1$H NMR (400 MHz, DMSO-$d_6$) δ: 12.95 (s, 1H, -COOH), 11.73 (s, 1H, -C(S)NH-), 11.33 (s, 1H, -C(O)NH-), 8.14 (s, 1H, Ph H-2), 7.85–7.74 (m, 2H, Ph H-4,6), 7.41 (t, J = 7.9 Hz, 1H, Ph H-5), 2.14–2.05 (m, 1H, cyclopropyl H-1), 1.00–0.86 (m, 4H, cyclopropyl H-$2_{eq}$, $2_{ax}$, $3_{eq}$, $3_{ax}$); LC-MS, m/z = 265 [M+1]; Anal. Calcd. for $C_{12}H_{12}N_2O_3S$: C, 54.53; H, 4.58; N, 10.60; S, 12.13; Found: C, 54.58; H, 4.64; N, 10.66; S, 12.18.

**4-Chloro-3-(3-(cyclopropanecarbonyl)thioureido)benzoic acid (2.6).** Yield: 52 %; Mp.: 161–165 °C; IR (cm$^{-1}$): 3196, 3018, 1707, 1679, 1600, 1571, 1513, 1503, 1403, 1332, 1246, 1197, 1167, 1107, 920, 879, 821, 775, 751, 735, 701, 677; $^1$H NMR (400 MHz, DMSO-$d_6$) δ 12.89 (s, 1H, -COOH), 11.74 (s, 1H, -C(S)NH-), 11.04 (s, 1H, -C(O)NH-), 8.33 (s, 1H, Ar H-2), 7.83 (q, $J$ = 8.7, 1H, Ar H-6), 7.57 (d, $J$ = 8.7, 1H, Ar H-5), 2.43–2.38 (m, 1H, cyclopropyl H-1), 1.00–0.88 (m, 4H, cyclopropyl H-$2_{eq}$, $2_{ax}$, $3_{eq}$, $3_{ax}$); LC-MS, m/z = 299 [M+1]; Anal. Calcd. for $C_{12}H_{11}ClN_2O_3S$: C, 48.25; H, 3.71; Cl, 11.87; N, 9.38; S, 10.73; Found: C, 48.31; H, 3.78; N, 9.42; S, 10.78.

**4-Bromo-3-(3-(cyclopropanecarbonyl)thioureido)benzoic acid (2.7).** Yield: 51 %; Mp.: 163–166 °C; IR (cm$^{-1}$): 3191, 3012, 1694, 1527, 1417, 1243, 1169, 1097, 937, 823, 763, 716, 673; $^1$H NMR (400 MHz, DMSO-$d_6$) δ 13.13 (s, 2H, -COOH, -C(S)N<u>H</u>-), 11.60 (s, 1H, -C(O)N<u>H</u>-), 8.53 (s, 1H, Ar H-2), 7.80 (d, J = 8.4 Hz, 1H, Ar H-6), 7.37 (d, J = 8.5 Hz, 1H, Ar H-5), 2.14-2.05 (m, 1H, cyclopropyl H-1), 0.98–0.80 (m, 4H, cyclopropyl H-$2_{eq}$, $2_{ax}$, $3_{eq}$, $3_{ax}$); LC-MS, m/z = 342 [M+1], 345 [M+4]; Anal. Calcd. for $C_{12}H_{11}BrN_2O_3S$: C, 42.00; H, 3.23; Br, 23.28; N, 8.16; S, 9.34; Found: C, 42.09; H, 3.31; N, 8.23; S, 9.38.

**4-(3-(Cyclopropanecarbonyl)thioureido)benzoic acid (2.8).** Yield: 74 %; Mp.: 230–233° C; IR (cm$^{-1}$): 3121 ($n_{NH}$), 3004, 2986, 1679, 1512, 1288, 1252, 1157, 860, 778, 742, 728, 694; $^1$H NMR (400 MHz, DMSO-$d_6$) δ 12.92 (s, 1H, -COOH), 12.48 (s, 1H, -C(S)N<u>H</u>-), 11.69 (s, 1H, -C(O)NH-), 7.91 (d, J = 8.4 Hz, 2H, Ph H-2,6), 7.77 (d, J = 8.4 Hz, 2H, Ph H-3,5), 2.10–2.06 (m, cyclopropyl H-1), 1.00–0.86 (m, 4H, cyclopropyl H-$2_{eq}$, $2_{ax}$, $3_{eq}$, $3_{ax}$); LC-MS, m/z = 265 [M+1]; Anal. Calcd. for $C_{12}H_{12}N_2O_3S$: C, 54.53; H, 4.58; N, 10.60; S, 12.13; Found: C, 54.58; H, 4.62; N, 10.65; S, 12.19.

**Dimethyl 2-(3-(cyclopropanecarbonyl)thioureido)terephthalate (2.9).** Yield: 74 %; Mp.: 208–211 °C; IR (cm$^{-1}$): 3121, 3004, 2986, 1715, 1686, 1525, 1432, 1390, 1281, 1241, 1222, 1192, 1158, 1130, 1098, 1065, 949, 937, 883, 755, 709, 674, 613; $^1$H NMR (400 MHz, DMSO-$d_6$) δ 13.02 (s, 1H, -C(S)N<u>H</u>-), 11.71 (s, 1H, -C(O)N<u>H</u>-), 8.71 (s, 1H, Ar H-3), 7.95 (d, $J$ = 8.1 Hz, 1H, Ar H-6), 7.82 (d, $J$ = 8.2 Hz, 1H, Ar H-5), 5.75 (s, 6H, $CH_3$), 2.16–2.07 (m, 1H, cyclopropyl H-1), 1.00–0.88 (m, 4H, cyclopropyl H-$2_{eq}$, $2_{ax}$, $3_{eq}$, $3_{ax}$); LC-MS, m/z = 338 [M+1]; Anal. Calcd. for $C_{15}H_{16}N_2O_5S$: C, 53.56; H, 4.79; N, 8.33; S, 9.53; Found: C, 53.62; H, 4.84; N, 8.39; S, 9.61.

**4-(3-(Cyclopropanecarbonyl)thioureido)benzenesulfonic acid (2.10).** Yield: 98.9 %; Mp.: 239–242 °C; IR (cm$^{-1}$): 3590, 3510, 3488, 3415, 3393, 3357, 3121, 3012, 2966, 2927, 2799, 2624, 2512, 2482, 2372, 2345, 2259, 2171, 2044, 1971, 1927, 1899, 1874, 1851, 1835, 1818, 1803, 1785, 1777, 1765, 1754, 1738, 1722, 1709, 1690, 1677, 1658, 1641, 1630, 1589, 1563, 1547, 1528, 1512, 1501, 1480, 1462, 1441, 1401, 1391, 1327, 1308, 1165, 1123, 1034, 1008, 951, 927, 890, 859, 832, 788, 765, 735, 693, 671, 640, 625; $^1$H NMR (400 MHz, DMSO-$d_6$) δ 12.73 (s, 1H, -C(S)NH-), 11.66 (s, 1H, -C(O)NH-), 7.72–7.52 (m, 4H, Ph H-2,3,5,6), 2.13–2.10 (m, 1H, cyclopropyl H-1), 1.04–0.70 (m, 4H, cyclopropyl H- H-$2_{eq}$, $2_{ax}$, $3_{eq}$, $3_{ax}$); Anal. Calcd. for $C_{11}H_{12}N_2O_4S_2$: C, 43.99; H, 4.03; N, 9.33; S, 21.35; Found: C, 44.09; H, 4.11; N, 9.39; S, 21.43.

**N-((4-Sulfamoylphenyl)carbamothioyl)cyclopropanecarboxamide (2.11).** Yield: 61.7 %; Mp.: 213–216 °C; IR (cm$^{-1}$): 3357, 3286, 3143, 3000, 1673, 1526, 1329, 1147, 767, 724, 687; $^1$H NMR (400 MHz, DMSO-$d_6$) δ 12.88 (s, 1H, -C(S)N<u>H</u>-), 11.74 (s, 1H, -C(O)N<u>H</u>-), 7.84–7.75 (m, 4H, Ar H-2,3,5,6), 7.12 (s, 2H, -$SO_2N\underline{H_2}$-), 2.15–2.05 (m, 1H, cyclopropyl H-1), 1.00–0.88 (m, 4H, cyclopropyl H-$2_{eq}$, $2_{ax}$, $3_{eq}$, $3_{ax}$); LC-MS, m/z = 300 [M+1]; Anal. Calcd. for $C_{11}H_{13}N_3O_3S_2$: C, 44.13; H, 4.38; N, 14.04; S, 21.42; Found: C, 44.19; H, 4.41; N, 14.09; S, 21.49.

The results of the study of the antibacterial activity of the synthesized compounds and their effect on growth rates (germination, total number and length of roots, total weight of the aboveground part) are shown in *Tables 1* and *2*.

**Table 1.** The antimicrobial activity of disubstituted thioureas **2.1–2.11**

| Compound | Strains | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | *E. coli* | | *S. aureus* | | *P. aeruginosa* | | *C. albicans* | |
| | MIC, μg/ml | MBC, μg/ml | MIC, μg/ml | MBC, μg/ml | MIC, μg/ml | MBC, μg/ml | MIC, μg/ml | MFC, μg/ml |
| **2.1** | 100 | 200 | 50 | 100 | 50 | 100 | 50 | 50 |
| **2.2** | 100 | 200 | 50 | 100 | 50 | 100 | 25 | 50 |
| **2.3** | 100 | 200 | 25 | 50 | 50 | 100 | 50 | 50 |
| **2.4** | 100 | 200 | 50 | 100 | 50 | 100 | 50 | 50 |
| **2.5** | 100 | 200 | 50 | 100 | 50 | 100 | 25 | 50 |
| **2.6** | 100 | 200 | 50 | 100 | 50 | 100 | 25 | 50 |
| **2.7** | 100 | 200 | 50 | 100 | 50 | 100 | 50 | 50 |
| **2.8** | 100 | 200 | 50 | 100 | 50 | 100 | 25 | 50 |
| **2.9** | 100 | 200 | 50 | 100 | 50 | 100 | 50 | 50 |
| **2.10** | 100 | 200 | 50 | 100 | 50 | 100 | 25 | 50 |
| **2.11** | 100 | 200 | 50 | 100 | 50 | 100 | 25 | 50 |
| Nitrofurazone | 1.50 | – | 6.25 | – | 6.25 | – | – | – |
| Ketoconazole | – | – | – | – | – | – | 25 | 50 |

**Table 2.** Influence of synthesized compounds on wheat growth rates (n = 10)

| Compound | Grain germination, % | | Total number of roots, pcs | Total length of roots, mm | Total mass of the aboveground part, g |
|---|---|---|---|---|---|
| | Day 4 | Day 8 | | | |
| Control | 95 | 95 | 30 | 298 | 0.102 ± 0.008 |
| **2.1** | 95 | 95 | 30 | 421 | 0.118 ± 0.004 |
| **2.2** | 95 | 95.5 | 38 | 387 | 0.136 ± 0.004 |
| **2.3** | 94.5 | 96.5 | 33 | 325 | 0.115 ± 0.005 |
| **2.4** | 94 | 94 | 31 | 462 | 0.132 ± 0.005 |
| **2.5** | 94.5 | 94.5 | 36 | 367 | 0.128 ± 0.006 |
| **2.6** | 94 | 95 | 35 | 312 | 0.116 ± 0.005 |
| **2.7** | 96 | 96 | 40 | 410 | 0.137 ± 0.006 |
| **2.9** | 93.5 | 94 | 30 | 348 | 0.111 ± 0.002 |
| **2.10** | 91.5 | 92 | 38 | 331 | 0.118 ± 0.005 |
| **2.11** | 95 | 95 | 34 | 328 | 0.117 ± 0.005 |
| IAAs | 96 | 96 | 41 | 311 | 0.125 ± 0.004 |

## Discussion

Synthesized compounds (**2.1–2.11**) were white or light-yellow crystalline compounds, insoluble in water, soluble in organic solvents.

Analysis of these chromato-mass spectra showed that compounds **2** had a characteristic quasimolecular ion [M+1], which corresponds to the calculated mass and confirms their structure and individuality.

The structure and individuality of compounds **2** were proved using elemental analysis, chromato-mass, IR, and $^1$H NMR spectra. The structure and individuality of compounds **2** were confirmed by chromato-mass spectra, in which the quasimolecular ion [M+1] corresponded to the calculated mass. In the $^1$H NMR spectra of compounds **2**, singlet or broad singlet signals of protons of the -COO*H* group were registered at the 13.45–12.50 ppm, the -C(S)N*H* groups – at the 13.02–11.44 ppm, and the C(O)N*H* groups – at the 11.69–11.04 ppm. In compounds **2.2, 2.10, 2.11** signals of protons -COO*H*-group were absent, due to deuteroexchange with DMSO.

In the $^1$H NMR spectra of compounds **2,** there were proton signals of the cyclopropane fragment, which appear in a strong field as wide multiplets of sequentially arranged signals of axial and equatorial protons. Thus, for com-

pounds **2**, the methine proton of the cyclopropane fragment was registered as a broad multiplet in the range of 2.53–2.05 ppm, and the methylene proton was observed as multiplets at the 1.00–0.70 ppm. Aromatic protons of compounds **2** in $^1$H NMR spectra have "classical" multiplicity and chemical shifts, which were in accordance with the proposed structures [19].

Analysis of the IR spectra of compounds **2** showed the presence of wide bands of valence vibrations of the associated NH-groups in the range of 3995–3121 $cm^{-1}$, which indicated the presence of secondary amide and thioamide groups in the molecule. Compounds **2** were also characterized by vibrations of two $\upsilon_{CO}$ groups (band "Amide I") at the 1693–1657 $cm^{-1}$ and 1674–1602 $cm^{-1}$, mixed valence-strain vibrations of N-H and C-N bonds ("Amide II") at the 1591–1504 $cm^{-1}$. In addition, characteristic contours of low-intensity vibrations of $\upsilon_{C=C}$-bond of the aromatic ring at the 1486–1424 $cm^{-1}$, non-planar vibrations γ (=C-H) at the 850–666 $cm^{-1}$ and intense bands of symmetric and antisymmetric vibrations of $\upsilon_{CH2}$-groups at the 2994–2304 $cm^{-1}$ (cyclopropane fragment) were present in the spectrum [20].

The conducted microbiological screening showed that compounds **2.1–2.11** inhibited the growth of *S. aureus* and *P. aeruginosa* at a concentration of 50.0 μg/ml and exhibited bactericidal activity at a concentration of 100 μg/ml (*Table 1*). However, compounds **2.1–2.11** weren't effective against *E. coli* (MIC 100.0 μg/ml, MBC 200 μg/ml). It is important to note, that the antimicrobial activity of the studied compounds was significantly lower than the reference drug Nitrofurazone. Better results were obtained when the antifungal activity against *C. albicans* (*Table 1*) was studied. For example, compounds **2.1–2.**11 inhibited the growth of the *C. albicans* strain at the concentration of 25–100 μg/ml and exhibited fungicidal activity at the concentration of 50 μg/ml. Levels of antifungal activity of the compounds competed with the reference drug Ketoconazole (MIC 25 μg/ml, MFC 50 μg/ml).

Analysis of the results of the study of the effect of synthesized compounds on growth rates showed (*Table 2*) that compounds **2.1–2.11**, like IAAs, have virtually no effect on grain germination. However, auxin-stimulating activity (number of roots) of most tested compounds have exceeded the control, and compound **2.**7 competes with IAAs. In addition, all compounds stimulated their growth (root length) exceeding the IAAs by 0.3–48.5 %. This figure was also confirmed by the total weight of the roots and, importantly, compounds **2.2**, **2.4,** and **2.7** also exceed IAAs.

Analysis of the structure-activity relationship didn't reveal a correct relationship between the studied compounds. The combination of aryl moiety with a carboxyl group in the molecule is the key factor in its manifestation. This provides both antibacterial and growth-promoting activity. Moreover, compounds **2.2**, **2.4,** and **2.7** with high growth activity contain fragments of 2-amino- and 4-aminobenzoic acids, which are precursors for the synthesis of auxins and other natural compounds.

## Conclusions

A one-step method for the synthesis of the substituted (cyclopropanecarbonylthioureido)aryl-(benzyl-)carboxylic (sulfonic) acids was developed. The physical-chemical properties of the synthesized compounds were studied using a set of methods (IR, $^1$H NMR spectroscopy, chromato-mass spectrometry, elemental analysis) and the peculiarities of the structure were discussed. The synthesized compounds showed moderate antimicrobial activity against *S. aureus* and *P. aeruginosa* (MIC 50 μg/ml, MBC 100 μg/ml) and significant antifungal activity against *C. albicans* (MIC 25–50 μg/ml, MFC 25–50 μg/ml). Compounds **2.2, 2.4,** and **2.7** of combined activity (high antifungal and growth-stimulating) exceeding the natural growth stimulator (heteroauxin) were found.

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