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Synthesis, Antimicrobial Evaluation, Molecular Docking and ADME-Tox Prediction of Some New 1-Oxo-3,4-dihydro-1H-isochromene-3-carboxamides

Oksana Khropot [1], Viktoriia Matiichuk [2], Iryna Drapak [3], Yuliia Matiichuk [3], Vasyl Matiychuk [2,*]

[1] Sole proprietor Khropot O. S.; Danylo Halytsky Lviv National Medical University, 69 Pekarska Street, Lviv, 79010, Ukraine; kaf_genchemistry@meduniv.lviv.ua;
[2] Ivan Franko National University of Lviv, 6 Kyryla and Mefodia, Lviv, 79005, Ukraine; viktoriia.matiichuk@lnu.edu.ua (V.M.); v_matiychuk@ukr.net (V.M.);
[3] Danylo Halytsky Lviv National Medical University, 69 Pekarska Street, Lviv, 79010, Ukraine; iradrapak@ukr.net (I.D.); kaf_genchemistry@meduniv.lviv.ua (Y.M.);
* Correspondence: v_matiychuk@ukr.net;

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Abstract: 1-Oxo-3,4-dihydro-1*H*-isochromene-3-carboxamides were synthesized by the acylation reaction of substituted aromatic amines and 2-aminothiazole derivatives with 1-oxo-3,4-dihydro-1*H*-isochromene-3-carbonyl chloride in dry dioxane at room temperature in the presence of triethylamine. 7-chloro-1-oxo-*N*-[3-(2-oxopropyl)-1,2,4-thiadiazol-5-yl]-3,4-dihydro-1*H*-isochromene-3-carboxamide was formed in the following sequence of reaction of 7-chloro-1-oxo-3,4-dihydro-1*H*-isochromene-3-carbonyl chloride with potassium isothiocyanate and 5-methyl-isoxazol-3-ylamine. These new compounds were characterized by ^{1}H NMR spectroscopy and elemental analysis. Antimicrobial activity was investigated for prepared compounds. *N*-[5-(4-methoxybenzyl)-1,3-thiazol-2-yl]-1-oxo-3,4-dihydro-1*H*-isochromene-3-carboxamide **7c** and 7-chloro-1-oxo-*N*-[3-(2-oxopropyl)-1,2,4-thiadiazol-5-yl]-3,4-dihydro-1*H*-isochromene-3-carboxamide **11** exhibit significant antibacterial activity against *S. aureus* ATCC 43300 (MIC values ≤ 8 μg/mL). The drug-likeness and ADME-Tox (absorption, distribution, metabolism, excretion, and toxicity) parameters of prepared compounds were examined and reported. Molecular docking studies of the hit-compounds **7c**, **11** to GlcN-6-P synthase were conducted using AutoDock Vina software. Hit compounds can be used for further investigation with the aim of developing novel antibacterial agents.

Keywords: isochromene; acylation; thiazole; antimicrobial activity; ADME-Tox; molecular docking.

1. Introduction

Isocoumarin derivatives are one of the most important classes of biologically active compounds. They are present in a variety of natural and synthetic products with a broad range of biological activities [1-4]. It is an imported precursor for the biosynthesis of many carbo- and heterocyclic natural products, including isochromenes, isoquinolines, and various aromatic compounds [1-4]. The isocoumarin framework represents one of the privileged structures for the development of new medicines [5].

The aim of our research is the synthesis and evaluation of antimicrobial activity1-oxo-3,4-dihydro-1*H*-isochromene-3-carboxamides molecular docking, and ADME-Tox prediction was also investigated.

It should be noted that natural coumarins, in particular those derived from fungi, are currently the subject of intensive research on antibacterial agents [6-8].

Amides are an important class of secondary metabolites that are involved in helping plants develop and defend themselves against environmental stress [9].

The amide group is most often found in medicines and biologically active substances. It is present in over 40% of this type of compound [10]. In view of the above, the synthesis and study of the biological activity of isocomarincarboxamides is an urgent task today. Due to the presence of a labile lactone cycle and an amide group, such compounds are easily metabolized and excreted from the body.

2. Materials and Methods

2.1. Chemistry.

1-Oxo-3,4-dihydro-1H-isochromene-3-carboxylic acids (3a, b) were prepared according to previously reported procedures [11, 12].

1-Oxo-3,4-dihydro-1H-isochromene-3-carboxylic acid (3a). Yield 87%. Melting point 153-154°C (H_2O) [11]. ^{1}H NMR (400 MHz, DMSO-d_6), δ ppm: 7.94 (d, J = 7.2 Hz, 1H, 8H), 7.58 (t, J = 8.1 Hz, 1H, 6H), 7.41 (t, J = 8.0 Hz, 1H, Ar), 7.35 (d, J = 8.1 Hz, 1H, Ar), 5.20 (t, J = 5.4 Hz, 1H, CH), 3.48 (dd, J = 16.8, 5.4 Hz, 1H, CH_2), 3.25 (dd, J = 16.8, 4.8 Hz, 1H, CH_2). Anal. calcd for $C_{10}H_8O_4$, %: C, 62.50; H 4.20. Found, %: C, 62.61; H 4.14.

7-Chloro-1-oxo-3,4-dihydro-1H-isochromene-3-carboxylic acid (3b). Yield 65%. Melting point 195-196°C (EtOH). ^{1}H NMR (400 MHz, DMSO-d_6), δ ppm: 13.47 – 13.53 (bs, 1H, COOH), 7.86 (s, 1H, 8H), 7.69 (d, J = 7.6 Hz, 1H, 6H), 7.48 (d, J = 7.7 Hz, 1H, 5H), 5.36 (s, 1H, CH), 3.49 (dd, J = 16.8, 5.8 Hz, 1H, CH_2), 3.23 (dd, J = 16.4, 5.8 Hz, 1H, CH_2). Anal. calcd for $C_{10}H_7ClO_4$, %: C, 53.00; H 3.11. Found, %: C, 53.07; H 3.28.

General method of synthesis of 1-oxo-3,4-dihydro-1H-isochromene-3-carbonyl chlorides (4a, b). 0.05 mol of acid and thionyl chloride were dissolved in 50 mL of benzene and refluxed for 3 hours. The benzene was removed. The residue was distilled in a vacuum and used without purification.

General method of synthesis of 1-oxo-3,4-dihydro-1H-isochromene-3-carboxamides (5a-d, 6, 7a-c). A solution of 0.03 g of acid chlorohydride in dioxane is mixed with equimolar amounts of the corresponding aromatic amine or 2-aminithiazole and triethylamine in dioxane (10 mL). The mixture is kept for 30 min and poured into 100 mL of water. Filter off and recrystallize from an appropriate solvent.

7-Chloro-N-(4-methylphenyl)-1-oxo-3,4-dihydro-1H-isochromene-3-carboxamide (5a). Yield 84%. Melting point 184-185°C. ^{1}H NMR (400 MHz, DMSO-d_6), δ ppm: 10.08 (s, 1H, NH), 7.94 (d, J = 8.3 Hz, 1H, ArH), 7.44 (dd, J = 8.6, 6.5 Hz, 4H, ArH), 7.05 (d, J = 8.3 Hz, 2H, ArH), 5.26 (t, J = 5.9 Hz, 1H, $CHCH_2$), 3.46 (dd, J = 17.0, 5.0 Hz, 1H, $CHCH_2$), 3.31 (dd, J = 17.0, 6.7 Hz, 1H, $CHCH_2$), 3.14 (s, 3H, CH_3). Anal. calcd for $C_{17}H_{14}ClNO_3$, %: C, 64.67; H 4.47; N 4.44. Found, %: C, 64.59; H 4.39; N 4.48.

7-Chloro-N-(3-chlorophenyl)-1-oxo-3,4-dihydro-1H-isochromene-3-carboxamide (5b). Yield 81%. Melting point 191-192°C. ^{1}H NMR (400 MHz, DMSO-d_6), δ ppm: 10.38 (s, 1H, NH), 7.94 (d, J = 8.3 Hz, 1H, ArH), 7.75 (s, 1H, ArH), 7.49 – 7.40 (m, 3H, ArH), 7.27 (t, J = 8.1 Hz, 1H, ArH), 7.05 (d, J = 8.0 Hz, 1H), 5.29 (t, J = 5.9 Hz, 1H, $CHCH_2$), 3.49 (dd, J = 17.2, 5.2 Hz, 1H, $CHCH_2$), 3.31 (dd, J = 17.0, 6.5 Hz, 1H, $CHCH_2$). Anal. calcd for $C_{16}H_{11}Cl_2NO_3$, %: C, 57.17; H 3.30; N 4.17. Found, %: C, 57.23; H 3.17; N 4.21.

7-Chloro-N-(3-methoxyphenyl)-1-oxo-3,4-dihydro-1H-isochromene-3-carboxamide (5c). Yield 75%. Melting point 200-201°C. ^{1}H NMR (400 MHz, DMSO-d_6), δ ppm: 10.16 (s, 1H,NH), 7.90 (d, J = 2.1 Hz, 1H, ArH), 7.59 (dd, J = 8.1, 2.3 Hz, 1H, ArH), 7.41 (d, J = 8.1 Hz, 1H, ArH), 7.26 (s, 1H, ArH), 7.18 – 7.05 (m, 2H, ArH), 6.58 (d, J = 8.0 Hz, 1H, ArH), 5.29 (t, J = 5.8 Hz, 1H, $CHCH_2$), 3.74 (s, 3H, CH_3O), 3.47 (dd, J = 16.9, 5.1 Hz, 1H, $CHCH_2$), 3.30 (dd, J = 17.1, 6.5 Hz, 1H, $CHCH_2$). Anal. calcd for $C_{17}H_{14}ClNO_4$, %: C, 61.55; H 4.25; N 4.22. Found, %: C, 61.49; H 4.31; N 4.33.

7-Chloro-1-oxo-N-[2-(trifluoromethyl)phenyl]-3,4-dihydro-1H-isochromene-3-carboxamide (5d). Yield 83%. Melting point 167-169°C (EtOH-DMFA). ^{1}H NMR (400 MHz, DMSO-d_6), δ ppm: 9.81 (s, 1H, NH), 7.87 (d, J = 2.2 Hz, 1H, ArH), 7.71 – 7.55 (m, 4H, ArH), 7.52 (d, J = 7.9 Hz, 1H), 7.42 (t, J = 8.9 Hz, 2H, ArH), 5.43 (t, J = 5.7 Hz, 1H, $CHCH_2$), 3.51 (dd, J = 16.9, 5.4 Hz, 1H, $CHCH_2$), 3.28 (dd, J = 16.9, 6.1 Hz, 1H, $CHCH_2$). Anal. calcd for $C_{17}H_{11}ClF_3NO_3$, %: C, 55.23; H 3.00; N 3.79. Found, %: C, 55.31; H 2.91; N 3.78.

7-Chloro-1-oxo-N-1,3-thiazol-2-yl-3,4-dihydro-1H-isochromene-3-carboxamide (6). Yield 74%. Melting point 189-190°C (EtOH-DMFA). ^{1}H NMR (400 MHz, DMSO-d_6), δ ppm: 12.53 (s, 1H, NH), 7.90 (d, J = 2.3 Hz, 1H, ArH), 7.58 (dd, J = 8.2, 2.3 Hz, 1H, ArH), 7.41 (d, J = 3.4 Hz, 3H, ArH), 7.11 (d, J = 3.5 Hz, 1H, ArH), 5.45 (t, J = 5.6 Hz, 1H, $CHCH_2$), 3.50 (dd, J = 17.1, 5.5 Hz, 1H, $CHCH_2$), 3.34 (dd, J = 17.2, 5.5 Hz, 1H, $CHCH_2$). Anal. calcd for $C_{13}H_{10}N_2O_3S$, %: C, 56.92; H 3.67; N 10.21. Found, %: C, 57.01; H 3.75; N 10.33.

N-[5-(3-methylbenzyl)-1,3-thiazol-2-yl]-1-oxo-3,4-dihydro-1H-isochromene-3-carboxamide (7a). Yield 86%. Melting point 215-216°C. ^{1}H NMR (400 MHz, DMSO-d_6), δ ppm: 12.32 (s, 1H, NH), 7.92 (d, J = 8.0 Hz, 1H, ArH), 7.53 (t, J = 8.1 Hz, 1H, ArH), 7.40 (t, J = 7.9 Hz, 2H, ArH), 7.31 (d, J = 8.0 Hz, 1H, ArH), 7.10 – 7.14 (m, 2H, ArH), 7.03 – 7.10 (m, 3H, ArH), 5.34 (t, J = 5.8 Hz, 1H, $CHCH_2$), 3.50 (dd, J = 16.8, 5.5 Hz, 1H, $CHCH_2$), 3.23 (dd, J = 16.8, 6.1 Hz, 1H, $CHCH_2$), 2.28 (s, 3H, CH_3). Anal. calcd for $C_{21}H_{18}N_2O_3S$, %: C, 66.65; H 4.79; N 7.40. Found, %: C, 66.78; H 4.84; N 7.32.

N-[5-(4-methylbenzyl)-1,3-thiazol-2-yl]-1-oxo-3,4-dihydro-1H-isochromene-3-carboxamide (7b). Yield 79%. Melting point 224-225°C. ^{1}H NMR (400 MHz, DMSO-d_6), δ ppm: 12.31 (s, 1H, NH), 7.92 (d, J = 8.1 Hz, 1H, ArH), 7.52 (t, J = 4.1 Hz, 1H, ArH), 7.39 (t, J = 4.1 Hz, 1H, ArH), 7.30 (d, J = 8.0 Hz, 1H, ArH), 7.11 – 7.00 (m, 5H, ArH), 5.45 (t, J = 5.7 Hz, 1H, $CHCH_2$), 3.50 (dd, J = 16.9, 5.4 Hz, 1H, $CHCH_2$), 3.24 (dd, J = 16.9, 6.1 Hz, 1H, $CHCH_2$), 2.98 (s, 3H, CH_3). Anal. calcd for $C_{21}H_{18}N_2O_3S$, %: C, 66.65; H 4.79; N 7.40. Found, %: C, 66.59; H 4.71; N 7.45.

N-[5-(4-methoxybenzyl)-1,3-thiazol-2-yl]-1-oxo-3,4-dihydro-1H-isochromene-3-carboxamide (7c). Yield 85%. Melting point 232-233°C. ^{1}H NMR (400 MHz, DMSO-d_6), δ ppm: 12.31 (s, 1H, NH), 7.92 (d, J = 8.0 Hz, 1H, ArH), 7.53 (t, J = 4.0 Hz, 1H, ArH), 7.39 (t, J = 8.2 Hz, 1H, ArH), 7.30 (d, J = 8.0 Hz, 1H, ArH), 7.06 – 7.10 (m, 3H, ArH), 7.78 (d, 2H, J = 12.0 Hz,ArH), 5.34 (t, J = 5.6 Hz, 1H, $CHCH_2$), 3.95 (s, 3H, OCH_3), 3.48 (dd, J = 16.7, 5.5

Hz, 1H, $CHCH_2$), 3.24 (dd, J = 16.7, 6.2 Hz, 1H, $CHCH_2$). Anal. calcd for $C_{21}H_{18}N_2O_4S$, %: C, 63.95; H 4.60; N 7.10. Found, %: C, 64.01; H 4.51; N 7.01.

The synthesis of 5-R-benzyl-1,3-thiazol-2-amines, which are used in the synthesis of compound 7a-c, is described in [13, 14].

7-Chloro-N-[3-(2-methylprop-2-en-1-yl)-1,2,4-thiadiazol-5-yl]-1-oxo-3,4-dihydro-1H-isochromene-3-carboxamide (11). To 10 mL of dry acetonitrile, add 0.48 g of chloroanhydride 4b and 0.2 g of dry potassium isothiocyanate. The mixture is heated under stirring for 30 min. To the resulting solution of compound 8, 0.2 g of 3-amino-5-methylisoxazole 9 is added and continued heating for another 3 hours. The reaction mixture is cooled and diluted with water, and the precipitate is filtered off, washed with water, and recrystallized from EtOH-DMFA. Yield 52%. Melting point 183-184°C. ^{1}H NMR (400 MHz, DMSO-d_6), δ ppm: 13.39 (s, 1H, NH), 7.90 (s, 1HArH), 7.60 (d, J = 8.2 Hz, 1H, ArH), 7.42 (d, J = 8.1 Hz, 1H, ArH), 5.56 (t, J = 5.5 Hz, 1H, $CHCH_2$), 3.94 (s, 2H, CH_2), 3.55 (dd, J = 17.3, 5.6 Hz, 1H, $CHCH_2$), 3.37 (dd, J = 17.2, 5.8 Hz, 1H, $CHCH_2$), 3.12 (s, 3H, CH_3). Anal. calcd for $C_{15}H_{12}ClN_3O_4S$, %: C, 49.25; H 3.31; N 11.49. Found, %: C, 49.34; H 3.37; N 11.55.

2.2. Antimicrobial activity.

Antimicrobial activity was screened according to the CO-ADD (Community for Open Antimicrobial Drug Discovery) research program [15].

2.3. Druglikeness and pharmacokinetic prediction.

Druglikeness and pharmacokinetic properties were analyzed using the following Internet resources: SwissADME [16], pkCSM pharmacokinetics [17], ProTox 3.0 – Prediction Of Toxicity Of Chemicals program [18], OSIRIS Property Explorer [19].

2.4. Docking.

The docking studies were performed using the Autodock Vina [20] and Discovery Studio [21] software packages.

3. Results and Discussion

*3.1. Synthesis and drug-likeness prediction of carboxamides **5a-d**, **6**, **7a-c** and **11**.*

All the investigated 1-oxo-3,4-dihydro-1H-isochromene-3-carboxamides derivatives 5a-d, 6, and 7a-c were synthesized following the synthetic procedures illustrated in Figure 1. Acids 3a and b were prepared using previously described synthetic methods [11, 12]. The diazonium salts 1a and b, which were obtained from the corresponding methylanthranilates, react with methylacrylate under the Meyerwein reaction to form methyl 1-oxo-3,4-dihydro-1H-isochromene-3-carboxylate 2a, b [11, 12]. Their hydrolysis in an aqueous-alcoholic medium yielded the corresponding acids 3a, b, which were converted to 1-oxo-3,4-dihydro-1H-isochromene-3-carbonyl chlorides 4a, b under the action of thionyl chloride. Target amides 5a-d, 6, 7a-c were prepared by acylation of the corresponding amines with the acyl chloride 4a, b in dry dioxane at room temperature in the presence of triethylamine.

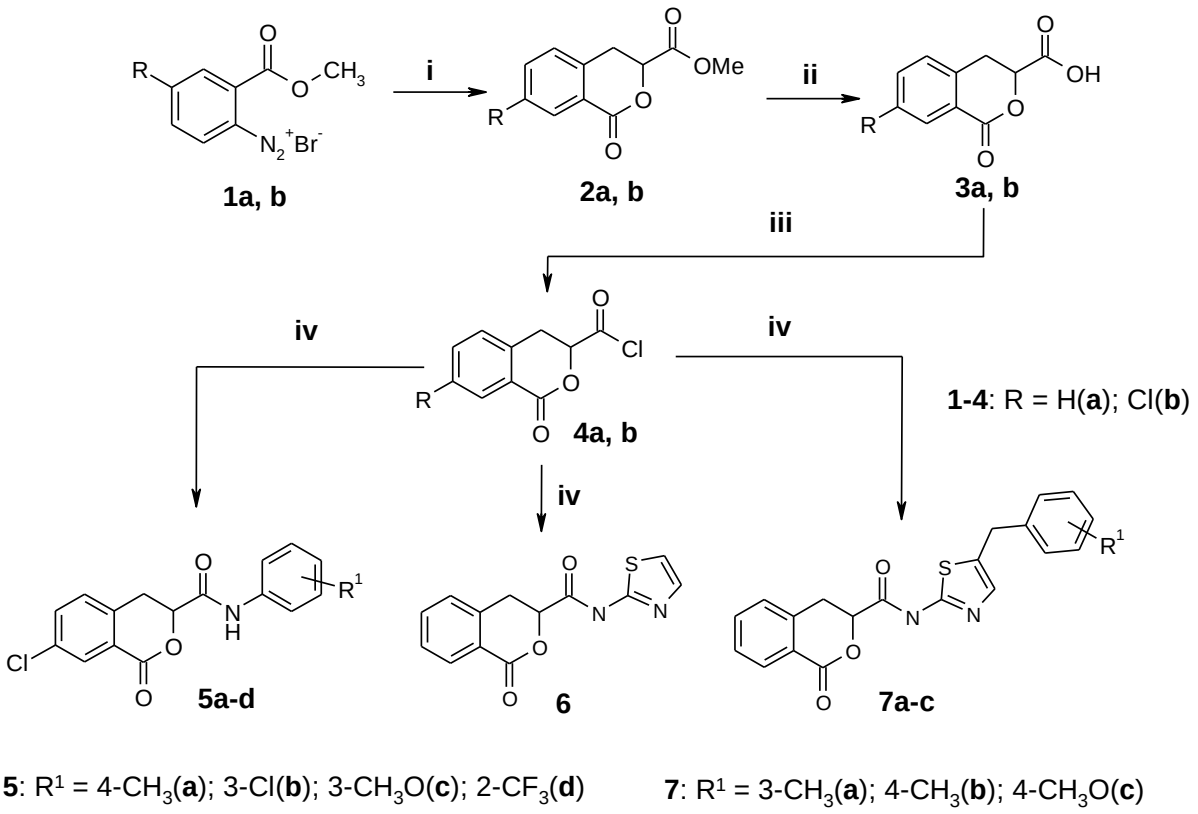


Figure 1. Synthesis of target compounds **5a-d, 6,** and **7a-c**. Reagents and conditions: **(i)** methylacrylate, $CuCl_2$, acetone/H_2O; **(ii)** NaOH, EtOH/H_2O 1:1, refluxing; **(iii)** $SOCl_2$, benzene, refluxing; **(iv)** corresponding aromatic amine (for compounds **5a-d**), or 2-amino-1,3-thiazole (for compounds **6**, **7a-c**), dioxane, NEt_3, r.t.

Chloroanhydride 4b was used for the construction of the 1,2,4-thiodiazole cycle (Figure 2). It was reacted with potassium isothiocyanate to give the acyl isothiocyanate 8, which, without isolation, was converted into acyl thiourea 10 by the reaction with the aminooxazole 9. Intermediate 10 spontaneously recycles in the 1,2,4-thiadiazole derivative 11.

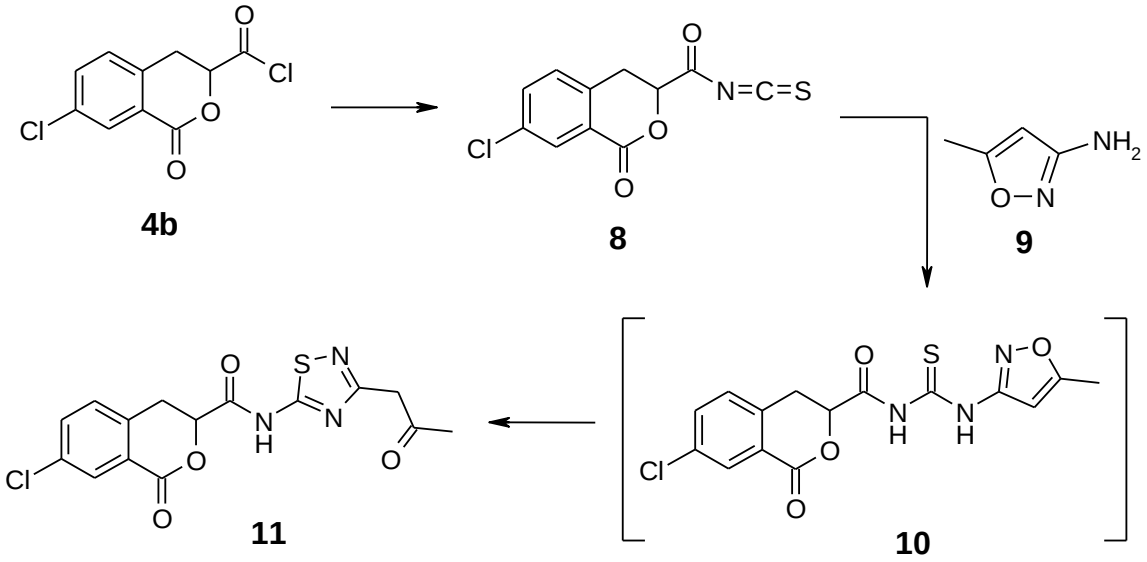


Figure 2. Synthesis of 7-chloro-1-oxo-*N*-[3-(2-oxopropyl)-1,2,4-thiadiazol-5-yl]-3,4-dihydro-1*H*-isochromene-3-carboxamide **11**.

All synthesized compounds 5a-d, 6, 7a-c, and 11 followed the rules of Lipinski [22], Ghose [23], Egan [24], Veber [25], and Muegge [26], which can be used as potential drug

candidate molecules. None of the mentioned compounds trigger the PAINS (Pan Assay Interference Structures) [27] and Brenk filters [28].

We used the BOILED-Egg method to predict blood-brain barrier penetration and gastrointestinal absorption [29]. The boiled egg plot showed that N-aryl amides 5a-d can be absorbed in the gastrointestinal tract and capable of crossing the blood-brain barrier (yellow area in Figure 3). The ability to penetrate the blood-brain barrier is not predicted for 1,3-thiazolyl 6, 7a-c, and 1,2,4-thiadiazolyl 11 derivatives (white area in Figure 3). Additionally, all compounds were not effluxed by P-glycoprotein, represented as (PGP-), which is indicated by the red color [29].

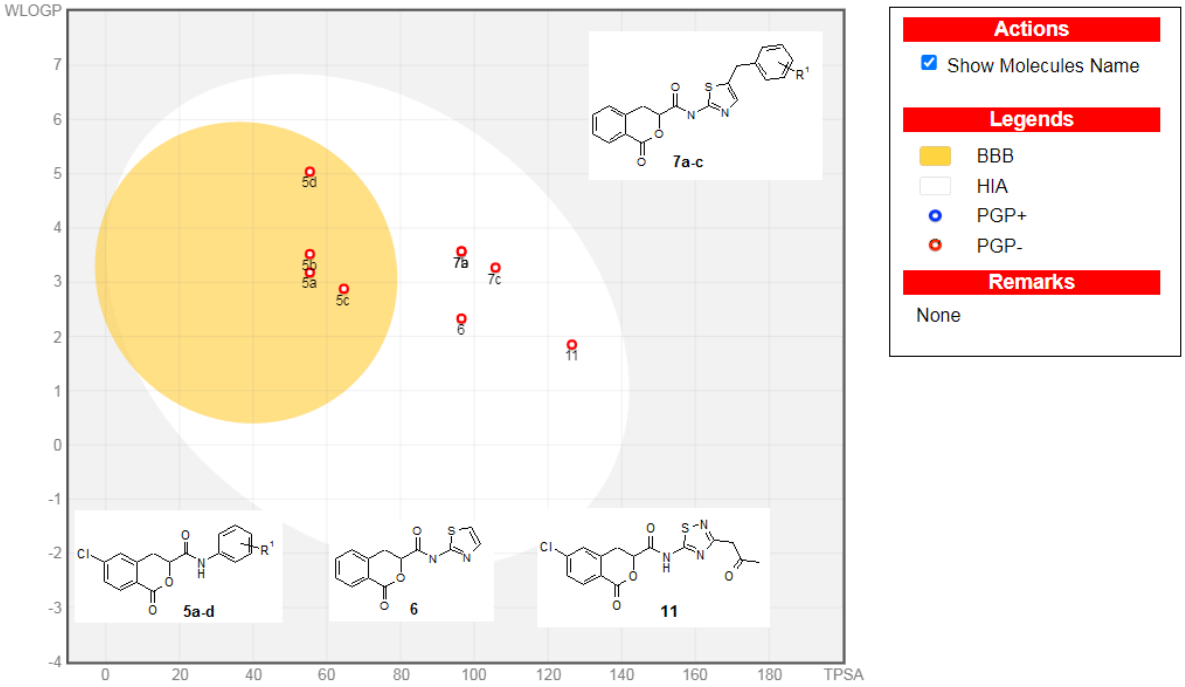


Figure 3. The BOILED-Egg plot of synthesized compounds **5a-d**, **6**, **7a-c** and **11**.

3.2. Antimicrobial activity.

Compounds were screened for their antimicrobial activity according to the CO-ADD protocol [15] against five ESKAPE bacterial pathogens: Escherichia coli, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and methicillin-resistant *Staphylococcus aureus* (MRSA) and two fungi (*Candida albicans* and *Cryptococcus neoformans*). They were initially screened at 32 μg mL^{-1} for %-inhibition before being tested again in a dose-responsive manner to determine more accurate MICs. The results of the research are shown in Table 1. Compounds 7c and 11 showed significant antibacterial activity against methicillin-resistant strains of *S. aureus* ATCC 43300. This species was also sensitive to compounds 6, and 7a, b. Compounds 6, 7a-c, and 11 were moderately active against the tested Gram-negative bacteria. Amides 5a-d showed no antibacterial effect. Also, antifungal activity is not observed for all compounds.

Table 1. Antibacterial and antifungal activity of synthesized compounds **5a-b**, **6**, **7a-c**, and **11** (GI, %).

Compound	*S. aureus* ATCC 43300	*E. coli* ATCC 25922	*K. pneumoniae* ATCC 700603	*P. aeruginosa* ATCC 27853	*A. baumannii* ATCC 19606	*C. albicans* ATCC 90028	*C. neoformans* ATCC 208821
5a	25.1; 21.2	1.5; 2.6	10.8; 19.4	8.5; -4.1	-8.4; 21.1	18.2; 5.2	11.2; 13.2
5b	25.4; 27.8	31.1; 24.5	1.2;1.3	11.4; 2.2	15.1; 8.5	12.3; 11.5	14.2; 22.3
5c	7.7; 8.1	11.5; 3.3	-11.1; 4.4	12.1; 4.5	4.8; 6.2	5.5; 4.3	22.1; 12.5

Compound	*S. aureus* ATCC 43300	*E. coli* ATCC 25922	*K. pneumoniae* ATCC 700603	*P. aeruginosa* ATCC 27853	*A. baumannii* ATCC 19606	*C. albicans* ATCC 90028	*C. neoformans* ATCC 208821
5d	22.3; 5.1	4.4; 3.1	2..5; 6.1	11.9; 12.3	10.1; 10.5	12.3; 10.5	14.2; 12.5
6	42.3; 34.5	-5.1; -3.3	14.1; 12.5	17.3; 4.4	-6.2; 11.5	23.1; 10.8	14.5; 16.1
7a	44.5; 55.5	57.3; 44.1	22.5; 33.9	34.6; 53.2	17.4; 11.7	22.3; 10.1	12.5; 10.2
7b	54.5; 47.1	33.1; 32.2	45.8; 37.8	41.8; 42.4	14.5; 11.3	14.3; 10.1	15.1; 16.2
7c	95.5; 98.9	27.6; 5.4	11.4; 17.0	44.3; 41.1	27.1; 21.4	7.0; 8.4	10.8; 15.1
11	87,9; 94.7	52,.9; 62.3	45.6; 66.4	28.3; 45.3	53.1; 35.6	-1.5; -3.4	14.2; 11.3

For the perspective compounds **7c** and **11**, the minimum inhibitory concentration value against *S. aureus* ATCC 43300 was determined, as well as cytotoxicity against human embryonic kidney cells (Hk CC_{50}) and human erythrocytes (Hm HC_{10}) (Table 2). The antimicrobial activity of classic antibiotics ceftriaxone and vancomycin hydrochloride is also shown for comparison. The attractive candidates for antimicrobial therapy – Tamoxifen [30] and Melittin [31] were used as reference drugs for cytotoxicity investigation.

Table 2. Results of the determination of the minimum inhibitory concentration (MIC) and cytotoxicity against human embryonic kidney cells and human erythrocytes of compounds **7c** and **11** and reference drugs (μg/mL).

Сполука	MIC *S. aureus* ATCC 43300	Hk CC_{50} [1]	Hm HC_{10} [2]	SI = HC_{10} / MIC
7c	8; 8	>32; >32	>32; >32	>4; >4
11	8; 4	>32; >32	>32; >32	>4; >8
Ceftriaxone	32	NT [3]	NT	NT
Vancomycin hydrochloride	0.625	NT	NT	NT
Tamoxifen	NT	9	NT	NT
Melittin	NT	NT	2.7	NT

[1] Hk CC_{50} is the concentration at 50% cytotoxicity (cytotoxicity against a human embryonic kidney cell line); [2] Hm HC_{10} is the concentration at 10% hemolysis (hemolysis of human red blood cells); [3] NT means Not Tested.

The *S. aureus* ATCC 43300 strain was found to be susceptible with a minimum inhibitory concentration (MIC) ≤ 8 μg/mL without cytotoxicity (or hemolysis) to human red blood cells and embryonic kidney (HEK-293) cells.

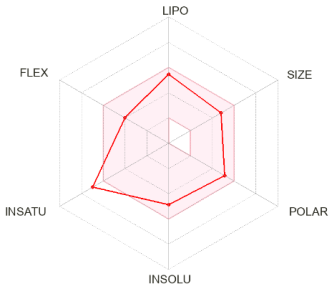
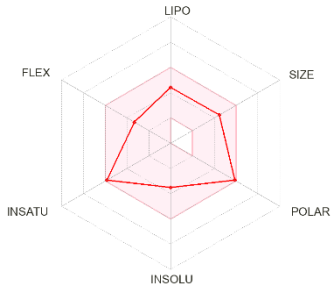
*3.3. Pharmacokinetic profiling of hit compounds **7c** and **11**.*

Statistically, almost 90% of the drugs under development have unsatisfactory physical and chemical properties. In order to evaluate the drug-likeness properties of prepared compounds, we used bioavailability radar (Table 3). According to Daina et al. [32], the pink area represents the optimal range for each property (lipophilicity: XLOGP3 between -0.7 and +5.0; size: MW between 150 and 500 g/mol; polarity: TPSA between 20 and 130 $Å^2$; solubility: log S not higher than 6; saturation: fraction of carbons in the sp3 hybridization not less than 0.25; and flexibility: no more than 9 rotatable bonds). Compound 11 is predicted to have excellent oral bioavailability (the above-mentioned parameters are optimal). In the case of 7c, the Csp^3 fraction is 0.19, which is slightly different from the optimal value.

Prediction using the Caco-2 cell monolayer model showed that the compounds 7c and 11 have high intestinal drug permeability. The good skin permeability of these compounds is also expected. Compound 7c (log VDss -0.156) will be mainly distributed in the tissues, whereas compound 11 (log VDss -0.362) will be mainly distributed in the plasma. The compounds are not expected to penetrate the central nervous system. It is assumed that these molecules are not Renal OCT2 substrates (Table 3) [17, 33].

As for the toxicological profile of the analyzed compounds, according to the ProTox 3.0 - Prediction Of Toxicity Of Chemicals program [18, 34], they belong to the 3rd (compound 7c) and 4th (compound 11) toxicity classes. According to the pkCSM web tool [17], no Ames toxicity is predicted for them, which means that these ligands are probably not mutagenic and, therefore, not carcinogenic. These compounds will also not affect skin sensitivity. No Flathead Minnow toxicity is predicted. However, they are expected to be hepatotoxic. According to the OSIRIS Property Explorer [19], the compounds tested are not predicted to be mutagenic, tumorigenic, irritant, or reprotoxic (Table 3), except for compound 11, which is expected to be irritant (Table 3).

Table 3. Predicted drug-likeness and toxicity profiles of the top hits 7c and 11 using web tools [16-19].

Parameters	Unit	Compound 7c	Compound 11
Bioavailability radar	Radar plot		
Water solubility	log mol/L	-4.634	-3.698
LogP		3.4627	2.0432
Rotatable Bonds		5	4
Acceptors		6	7
Donors		1	1
Surface Area	Å^2	165.689	145.709
Absorption and distribution			
Caco2 permeability	log Papp in 10^{-6} cm/s	1.391	0.992
Intestinal absorption (human)	% Absorbed	93.6	77.046
Skin Permeability	log Kp	-2.802	-2.903
VDss (human)	log L/kg	-0.156	-0.362
CNS permeability	log PS	-2.314	-3.006
Renal OCT2 substrate	Yes/No	No	No
Toxicity profiles			
AMES toxicity	Yes/No	No	No
Max. tolerated dose (human)	log mg/kg/day	0.101	0.622
Predicted Toxicity Class	Class	3 LD_{50}: 300mg/kg	4 LD_{50}: 800mg/kg
Hepatotoxicity	Yes/No	Yes	Yes
Skin Sensitisation	Yes/No	No	No
Minnow toxicity	log mM	0.35	0.434
Mutagenicity		Low risk	Low risk
Tumorgenicity		Low risk	Low risk
Irritant effects		Low risk	High risk
Reproductive effects		Low risk	Low risk

*3.4. Docking study of hit compounds **7c** and **11**.*

Since the compounds showed high or moderate antibacterial activity against all tested bacteria, the enzyme GlcN-6-P synthase was selected as a potential target for the *in silico* docking study. GlcN-6-P synthase is a ubiquitous protein found in all known organisms. This enzyme is involved in the biosynthesis of *N*-acetylglucosamine (NAG), glycoproteins, and mucopolysaccharides, which are essential for bacterial growth and function. The results are shown in Table 4 and Figures 4 and 5.

Table 4. Binding scores and amino acids involved in interactions of the docked compounds 7c and 11 on the active sites of GlcN-6-P synthase (PDBID: 4AG7).

Compound	Binding energy, kcal/mol		Interacting residues					
			Pi-Sulfur		Pi-Alkyl		Other	
			Amino acid	Distance Å	Amino acid	Distance Å	Amino acid	Distance Å
7c	GlcN-6-P synthase (PDBID: 4AG7)	-8.3	Phe B:148	4.92	Phe B:152 Ala B:119 Ala B:119 Phe B:148 Val B:108 Arg B:113 Cys B:141 Val B:142	6.60 4.45 5.65 4.91 5.47 5.40 5.59 5.55	Phe B:148 (Pi-Pi T-shaped) Phe B:148 (Pi-Pi T-shaped)	5.51 5.54
11	GlcN-6-P synthase (PDBID: 4AG7)	-7.8	Cys B:141	6.72	Leu B:145 Lys B:116 Val B:108 Arg B:114	5.60 4.07 4.94 5.63	Arg B:134 (Pi-Donor Hydrogen Bond) Phe B:148 (Pi-Pi Stacked)	5.99 3.90

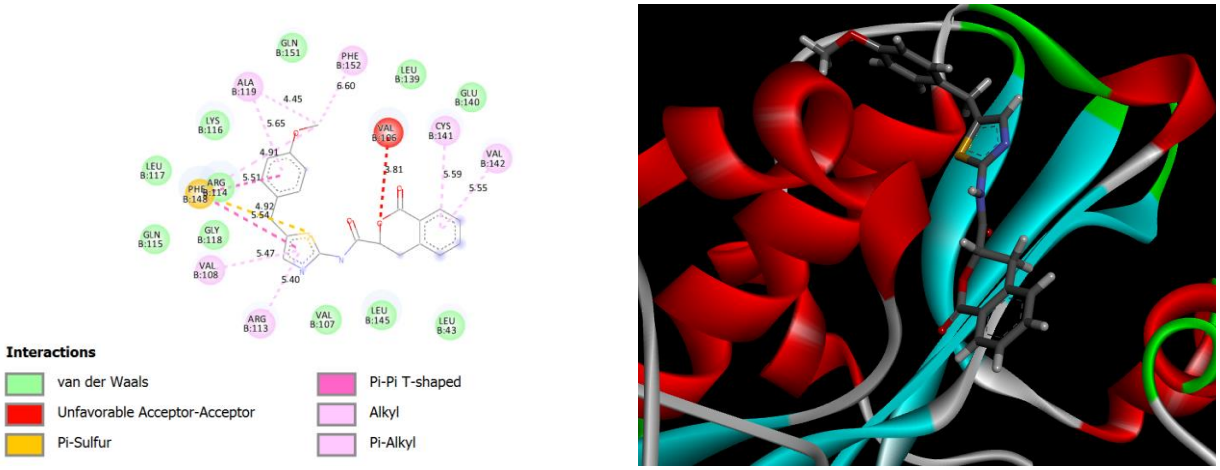


Figure 4. Binding mode of amide **7c** on the active sites of GlcN-6-P synthase (PDBID: 4AG7).

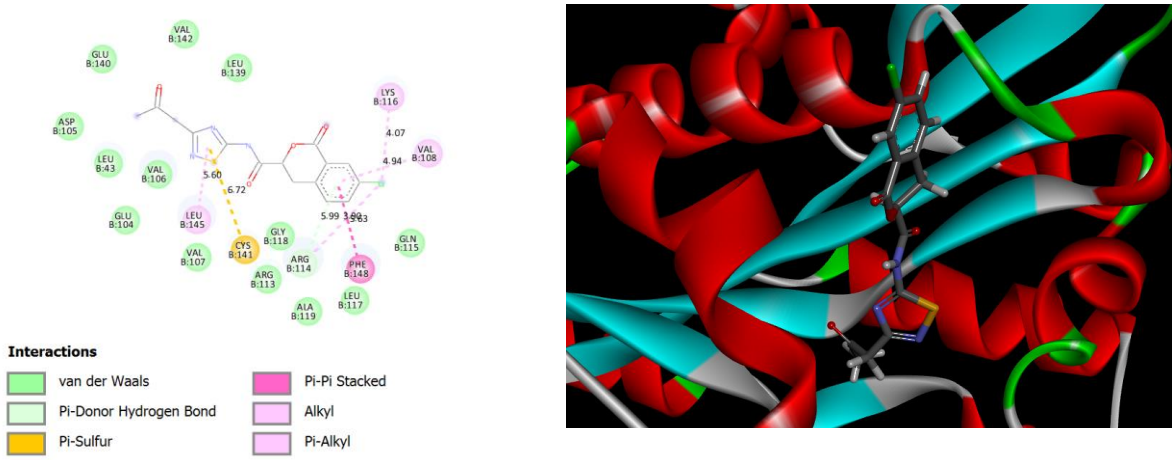


Figure 5. Binding mode of amide 11 on the active sites of GlcN-6-P synthase (PDBID: 4AG7).

In the case of compound 11, the binding is realized with the participation of the aromatic system of the thiadiazole cycle through the formation of a pi-sulfur bond with cysteine and a Pi-Alkyl bond with leucine. The Pi-Pi Stacked interaction involves the benzene cycles of phenylalanine B:148 and isochromene rings. The isochromene cycle also forms a Pi-Donor Hydrogen Bond with arginine B:114 and a Pi-Alkyl bond with valine B:108. The chlorine in this Pi-Alkyl cycle forms bonds with lysine B:116 and arginine B:114.

The orientation of amide 7c in the active site of GlcN-6-P synthase is opposite, and the aromatic cycle of phenylalanine B:148 forms Pi-Pi T-shaped and Pi-Sulfur non-covalent

bonds. It also binds to the methoxybenzyl fragment through Pi-Pi and Pi-Alkyl bonds. Alanine B:119 and phenylalanine B:152 interact with this fragment in a similar way. The thiazole cycle forms Pi-Alkyl bonds with valine B:108 and arginine B:113, and the benzene cycle with cysteine B:141 and valine B:142. The carbonyl oxygen of the valine carbonyl group of B:106 rescue is involved in an unfavorable acceptor-acceptor interaction between the p-orbital of the oxygen in the second position of the isochromene cycle. It affects the stability of the compound activity. Despite this, compound 7c forms eleven different bonds with the active site of GlcN-6-P synthase and possesses a good binding affinity with -8.3 kcal.mol^{-1}.

4. Conclusions

Some novel 1-oxo-3,4-dihydro-1*H*-isochromene-3-carboxamides were synthesized and were characterized by the ^{1}H NMR spectra and elemental analyses, which were completely fit with the assigned structures. These prepared compounds were screened against gram-positive bacteria (*S. aureus* ATCC 43300), gram-negative bacteria (*E. coli* ATCC 25922, *K. pneumoniae* ATCC 700603, *P. aeruginosa* ATCC 27853 and *A. baumannii* ATCC 19606) and fungi (*C. albicans* ATCC 90028, *C. neoformans* ATCC 208821). *N*-[5-(4-methoxybenzyl)-1,3-thiazol-2-yl]-1-oxo-3,4-dihydro-1*H*-isochromene-3-carboxamide **7c** and 7-chloro-1-oxo-*N*-[3-(2-oxopropyl)-1,2,4-thiadiazol-5-yl]-3,4-dihydro-1*H*-isochromene-3-carboxamide **11** show high antibacterial activity against *S. aureus* ATCC 43300 (MIC values ≤ 8 μg/mL). *In silico* molecular docking studies were carried out to examine interactions of compounds **7c** and **11** with GlcN-6-P synthase as the most promising target for antimicrobial agents. They showed a good binding energy of -8.3 and -7.8 kcal/mol, respectively. The drug-likness and ADME-Tox parameters of synthesised compounds were calculated.

Author Contributions

Synthesis and identification of organic compounds, O.K. and Y.M.; docking studies, I.D. and V.M. (Viktoriia Matiichuk); project administration, I.D. and V.M. (Vasyl Matiychuk); funding acquisition, V.M. (Vasyl Matiychuk). All authors have read and agreed to the published version of the manuscript.

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Data supporting the findings of this study are available upon reasonable request from the corresponding author.

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Conflicts of Interest

The authors declare no conflict of interest.

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