Full Paper

# Synthesis, Cytotoxicity by Bioluminescence Inhibition, Antibacterial and Antifungal Activity of ([1,2,4]Triazolo[1,5-*c*]quinazolin-2-ylthio)carboxylic Acid Amides

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We report in this work the synthesis, cytotoxicity, and antimicrobial activity of ([1,2,4]triazolo[1,5-*c*]quinazolin-2-ylthio)carboxylic acid amides **4**–**7** in connection with our previous research in the preparation of triazoloquinazoline derivatives. Due to simplicity, general availability of starting materials, and high yields, the most reliable method of synthesis appeared to be the one with *N*,*N*-carbonyldiimidazole activation stage. The chemical structures of all obtained substances were deduced from FT-IR, $^1$H-NMR, EI-MS, and LC-MS spectral data. The results of cytotoxicity evaluated by bioluminescence inhibition of bacterium *Photobacterium leiognathi,* strain Sh1 showed that compounds **4.1**, **4.6**, and **6.1** were the most cytotoxic. Investigation of the antimicrobial and antifungal activity of amides **4**–**7** (concentration 5 mg/mL) was carried out by the stiff-plate agar-diffusion method. We found that the compounds possessed low (**4.1**, **4.7**) antifungal activity against *Candida tenuis* and strong (**4.21**, **5.1**, **5.9**) or inefficient (**4.7**, **4.12**, **4.16**) activity against *Aspergillus niger*. Substances **5.1** and **5.9** slightly affected *Mycobacterium luteum*. *Staphylococcus aureus* was resistant to all obtained substances, and only the *n*-butyramide derivatives **7.1** and **7.5** inhibited the growth of *Escherichia coli.* Hence, there was no strong correlation between bioluminescence inhibition and antimicrobial activity of the investigated substances.

**Keywords:** Antifungal / Antibacterial / Bioluminescence / Cytotoxicity / 2-Thio-[1,2,4]triazolo[1,5-*c*]quinazoline

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## Introduction

Nowadays, biotesting, a determination of *e.g.*, toxicity, by direct action on a living organism, is widely used as control of the biological system state on different levels of an ecosystem [1]. It is known that certain insects (*e.g.*, beetles known as fireflies) and a few species of bacteria (*Photobacterium phosphoreum, P. leiognathi, Vibrio fischeri, V. harveyi*) possess the ability to emit light [2]. The biochemistry and genetics of the light reaction (luciferin oxidation) have been widely studied, and now scientists are exploring other potential applications of luminescence in microbial quality control of soil, air, food products, and drinking water. Consequently, bioluminescence (BL) inhibition test is a simple rapid *in-vitro* method for the screening and monitoring of a wide range of organic xenobiotics [2]. Still, it should always be kept in mind that rigorous testing of the method should be carried out in comparison to accepted methods.

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**Abbreviation:** bioluminescence (BL)

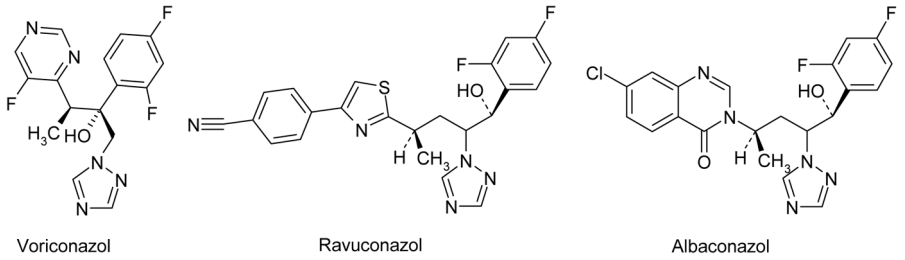



**Figure 1**. Chemical structures of the antifungals – 1,2,4-triazole derivatives.

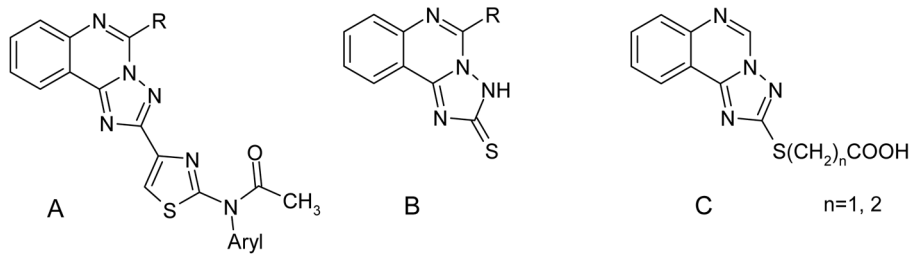



**Figure 2**. Chemical structures of the [1,2,4]triazolo[1,5-*c*]quinazoline derivatives with antimicrobial properties.

At the present time, medicine is faced with the real and frightening threat of entering the "postantibiotic era", when existing antibiotic arsenal will become largely ineffective against bacterial and fungal infections because of the accumulation of antibiotic resistance mechanisms: selection of mutants which produce modified low-affinity drug targets, acquirement of mobile genetic determinants of drug-inactivating enzymes, target protection and bypass system [3, 4]. The usage of broad-spectrum antibiotics increases the risk of therapeutic failure, drug side effects and excess costs of care. Confronting this danger effectively requires new strategies to be implemented across the healthcare such as synthesis of new, more selective and effective antimicrobial and antifungal substances.

More than that, 1,2,4-triazole derivatives are well known to be effective antifungal drugs [5]. Thus, second generation derivatives of fluconazol – voriconazol and ravuconazol – (Fig. 1), showed better *in-vitro* activity against *Aspergillus* spp., *Candida* spp., *Fusarium* spp., and *Scedosporium ariospermum* in comparison with the reference antimycotics [6]. Another derivative of itraconazol – albaconazol (Fig. 1), which contains 1,2,4-triazole and quinazoline rings in its structure, is in early Phase of clinical development and shows very potent activity against species of *Candida*, *Cryptococcus* and *Aspergillus* [7].

Fusion of the 1,2,4-triazole ring to quinazoline leads to formation of new antimicrobial *N*-aryl-(4-[1,2,4]triazolo[1,5-*c*]quinazolin-2-yl-thiazol-2-yl)acetamides **A** (Fig. 2) [8]. Introduction of a sulphur atom in the above-mentioned core formed [1,2,4]triazolo[1,5-*c*]quinazolin-2-thiones **B**, which also demonstrated antibacterial activity [9–12]. Moreover, in our previous research it was proved that ([1,2,4]triazolo[1,5-*c*]quinazolin-2-ylthio)acetic and -3-propionic acids **C** possess strong antifungal activity against *C. albicans* [13].

Consequently, it appeared to be interesting and useful to continue investigations in the field of 2-thio-[1,2,4]triazolo[1,5-*c*]quinazoline derivatives. The aim of this work was to estimate the cytotoxicity of novel ([1,2,4]triazolo[1,5-*c*]quinazolin-2-ylthio)carboxylic acid amides by measuring the light extinction of luminescent bacteria, and to evaluate their antimicrobial and antifungal properties.

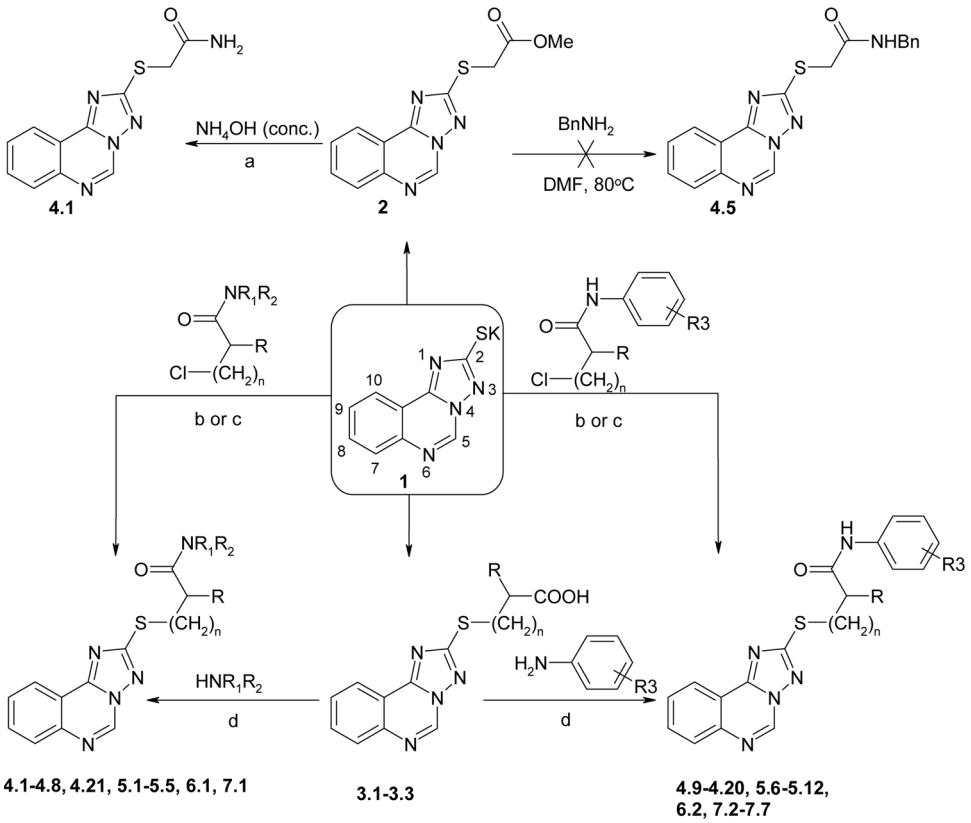
## Results and discussion

### Chemistry

The above-mentioned amides **4**–**7** were synthesized in several ways. Since the synthesis from esters usually works well, we started from ammonolysis of methyl ester **2** (Scheme 1). The latter was obtained by a reported procedure from the potassium salt **1** [13], yet, the resulting ([1,2,4]triazolo[1,5-*c*]quinazolin-2-ylthio)acetic acid amide **4.1** turned out to be contaminated with side products.

The attempt to conduct aminolysis of ester **2** by treatment with benzylamine at 80°C in DMF lead to degradation products instead of the anticipated amide **4.5** (Scheme 1, Table 1).




**Reactions and conditions**: (a) Propan-2-ol, r.t., 48 h; (b) propan-2-ol / $H_2O$, reflux, 2 h; (c) propan-2-ol, reflux, 2 h; (d) i) CDI, dioxane, 60–80°C, ii) amine, reflux, 1 h.

**Scheme 1**. Synthesis of the compounds **4–7**.

Taking into account the low electrophilicity of carboxylic group of ester **2**, we developed several alternative methods for the target compound preparation. The amides **4.1**, **4.6**, **4.9**, **4.12**, **4.14**, **4.15**, **4.20**, **5.4**, **5.6**, **5.9–5.12**, **6.1**, **6.2**, and **7.1–7.7** were obtained as the result of alkylation of the potassium salt **1** by halogenoacylamides under reflux in propan-2-ol (Scheme 1). Changing the reaction conditions by adding water did not greatly affect the yield of the products. However, the use of the given method was limited by the application of commercially inaccessible halogenoacylamides, which were synthesized through acylation of the corresponding amines according to the general procedure [14].

Our subsequent efforts were directed towards the development of one more efficient method of synthesis. We used *N*,*N*-carbonyldiimidazole for the activation of the carboxylic group of acids **3.1–3.3** with subsequent aminolysis of the resulting intermediates. The advantages of this reaction were purity and high yields of the products **4.2–4.5**, **4.7–4.11**, **4.13**, **4.16–4.19**, **4.21**, **5.1–5.3**, and **5.5–5.8** (Scheme 1).

The structures of all synthesized compounds were confirmed by their spectral data. Thus, the position of the carbonyl absorbtion band in FT-IR spectra was influenced by the physical state of the compounds as substances were taken in solid state. Hence, primary amides showed $\nu_s$ and $\nu_{as}$ NH vibration at 3026 and 2968 $cm^{-1}$. Free NH-stretching vibration of secondary amides was observed as multiple bands in the 3347 to 3042 $cm^{-1}$ region. Primary amides have a strong «Amide I» ($\nu_{C=O}$) band at 1686 $cm^{-1}$. The latter band appeared near 1683–1638 $cm^{-1}$ in the case of open chain secondary amides, and shifted to the 1697–1651 $cm^{-1}$ region for aryl amides because of the ring influence on the non-bondened electron pair of the nitrogen. The carbonyl frequency of tertiary amides occurred in the range of 1649–1634 $cm^{-1}$. The «Amide II» band ($\nu_{C-N}$ and $\delta_{N-H}$) was shown as sharp absorption band near 1623 $cm^{-1}$ for the primary amide, in the region 1558 to 1537 $cm^{-1}$ for acyclic amides, and 1590–1535 $cm^{-1}$ for aryl amides. The second weaker band near 1274–1240 $cm^{-1}$ also resulted from interaction between NH-bending and CH-stretching vibrations. The medium bands in the 796–610 $cm^{-1}$ region

**Table 1**. Physical data of ([1,2,4]triazolo[1,5-*c*]quinazolin-2-ylthio)carboxylic acid amides **4**–**7**.

| Compd. | n | R | $R_1$ | $R_2$ | $R_3$ | Yield (%) (method) | Recryst. Solvent* | M.p. (°C) |
|---|---|---|---|---|---|---|---|---|
| **4.1** | 0 | H | H | H | – | 50.0 (A)<br>70.7 (B) | § | 250–252 |
| **4.2** | 0 | H | H | Bu | – | 57.0 (D) | † | 156–158 |
| **4.3** | 0 | H | H | cyclopropyl | – | 33.3 (D) | # | 203–205 |
| **4.4** | 0 | H | H | $C(CH_3)_3$ | – | 22.1 (D) | # | 180–182 |
| **4.5** | 0 | H | H | Bn | – | 62.5 (D) | – | 207–209 |
| **4.6** | 0 | H | H | $CH_2$-COOEt | – | 34.3 (C) | – | 158–160 |
| **4.7** | 0 | H | -$(CH_2)_5$- | | – | 73.2 (D) | # | 156–158 |
| **4.8** | 0 | H | –$(CH_2)_2O(CH_2)_2$- | | – | 51.5 (D) | § | 230–232 |
| **4.9** | 0 | H | – | – | H | 50.6 (B)<br>53.2 (D) | # | 196–198 |
| **4.10** | 0 | H | – | – | 2-Me | 42.3 (D) | # | 166–168 |
| **4.11** | 0 | H | – | – | 3-Me | 43.1 (D) | # | 162–168 |
| **4.12** | 0 | H | – | – | 2-OMe | 36.7 (B) | # | 174–176 |
| **4.13** | 0 | H | – | – | 2-Me-4-Cl | 32.5 (D) | # | 224–226 |
| **4.14** | 0 | H | – | – | 2-$NO_2$ | 39.5 (C) | # | 158–160 |
| **4.15** | 0 | H | – | – | 4-$NO_2$ | 21.0 (B) | # | 221–223 |
| **4.16** | 0 | H | – | – | 3-Cl | 29.9 (D) | # | 154–156 |
| **4.17** | 0 | H | – | – | 4-Cl | 47.0(D) | # | 208–210 |
| **4.18** | 0 | H | - | - | 4-Br | 22.2(D) | # | 228–230 |
| **4.19** | 0 | H | - | - | 3-$CF_3$ | 40.0 (D) | † | 142–144 |
| **4.20** | 0 | H | H | | 4-COOEt | 54.5 (C) | # | 178–180 |
| **4.21** | 0 | H | Et | Et | – | 57.0 (D) | – | 138–140 |
| **5.1** | 0 | Me | H | Bu | – | 96.1 (D) | † | 122–124 |
| **5.2** | 0 | Me | H | $C(CH_3)_3$ | – | 69.0 (D) | – | 126–128 |
| **5.3** | 0 | Me | H | Bn | – | 61.8 (D) | # | 164–166 |
| **5.4** | 0 | Me | -$(CH_2)_5$- | | – | 31.9 (C) | † | 164–166 |
| **5.5** | 0 | Me | -$(CH_2)_2O(CH_2)_2$- | | - | 15.6 (D) | † | 169–171 |
| **5.6** | 0 | Me | H | – | H | 34.3 (C) | # | 172–174 |
| **5.7** | 0 | Me | H | – | 2-Me | 30.6 (D) | # | 169–171 |
| **5.8** | 0 | Me | H | – | 3-Me | 68.9 (D) | # | 142–144 |
| **5.9** | 0 | Me | H | – | 2-OMe | 42.9 (C) | † | 118–120 |
| **5.10** | 0 | Me | H | – | 4-$NO_2$ | 25.4 (C) | # | 230–232 |
| **5.11** | 0 | Me | H | – | 4-Cl | 62.5 (C) | # | 202–204 |
| **5.12** | 0 | Me | H | – | 3-$CF_3$ | 58.4 (C) | † | 176–178 |
| **6.1** | 1 | H | -$(CH_2)_2O(CH_2)_2$- | | – | 30.0 (C) | § | 124–126 |
| **6.2** | 1 | H | H | – | 3-Me | 36.7 (C) | § | 178–180 |
| **7.1** | 2 | H | H | Bn | – | 41.1 (C) | # | 196–198 |
| **7.2** | 2 | H | – | – | H | 17.0 (C) | # | 184–186 |
| **7.3** | 2 | H | – | – | 2-Me | 21.0 (C) | # | 186–188 |
| **7.4** | 2 | H | – | – | 2-OMe | 21.8 (C) | # | 140–142 |
| **7.5** | 2 | H | – | – | 2-OMe-4-Cl | 34.0 (C) | # | 156–158 |
| **7.6** | 2 | H | – | – | 4-Br | 36.2 (C) | # | 212–124 |
| **7.7** | 2 | H | – | – | 3-$CF_3$ | 46.4 (C) | † | 142–144 |

§ dioxane; # propan-2-ol / dioxane (2 : 1); † propan-2-ol.

appeared due to out-of-plane NH wagging. Also, FT-IR spectra showed frequencies of certain molecular vibration in terms of single bonds of 1,2,4-triazoloquinazoline, which own specific absorbtion influenced the intensity and frequency of one another.

In the $^1$H-NMR spectra, the [1,2,4]triazolo[1,5-*c*]quinazoline system was characterized by signals in the aromatic area (8.43–7.76 ppm) and a characteristic H-5 singlet at 9.54–9.48 ppm, in agreement with the literature data [13, 15]. The protons of the amide group of compound **4.1** showed up as two inequivalent one-proton singlets at 7.72 and 7.28 ppm, as a result of its hindered rotation around the anisotropic C=O group. For (aryl)alkyl amides, broadened one-proton triplet (for **4.2**, **4.5**, **4.6**, **5.1**, **5.3**, **7.1**) or a singlet (for **4.3**, **5.2**) of the amide group were observed in the low field (8.84–8.00 ppm). One-proton NH broadened singlet of the arylamides **4.9**–**4.20**, **5.6**–**5.12**, **6.2**, **7.2**–**7.7** shifted more to the low field (11.05–9.12 ppm). It is also interesting to notice that two-proton singlet of the $SCH_2$ group of acetic acid amides **4.2**–**4.4** resonated at

4.05–4.02 ppm, and protons of the SCH-group of α-propionic acid derivatives **5.1**–**5.12** appeared in the lower area of spectrum (5.05–4.56 ppm) as quadruplet. The latter group-triplet signal shifted upfield for β-propionic acid amides **6.1**, **6.2** (3.53 ppm) and for *n*-butyric acid amides **7.2**–**7.7** (3.38–3.32 ppm).

LC-MS confirmed the purity of obtained substances **4**–**7** and demonstrated their appropriately protonated molecular ions $[M + H]^+$. The EI-MS spectra of acetic acid derivatives (**4.1**, **4.6**, **4.17**, **4.21**) were characterized by successive fragmentation of the substances. Thus, during the cleavage ions $[HetarylSCH_2CO]^+$ (*m/z*: 243), $[HetarylSCH_2]^+$ (*m/z*: 215), $[HetarylSH]^+$ (*m/z*: 202) were formed through the appropriate split-off of the $CH_2$ fragments and the amide and acyl moiety. A similar cleavage took place for substances **5.2**, **5.4**, **5.12** with proper detection of the ions peaks with *m/z*: 257, 230, 202. The base peaks corresponded to the ion $[HetarylSCH\text{-}CH_3]^+$ (*m/z*: 229) for compounds **5.2**, **5.12** and $[HetarylSCH_2CH_2]^+$ for *n*-butyric acid derivatives (**7.1**, **7.6**). Above mentioned data were in accordance with literature reports for this heterocyclic system [13, 15].

## Pharmacology

The pharmacological investigations started from the estimation of cytoxicity of the novel ([1,2,4]triazolo[1,5-*c*]quinazolin-2-ylthio)carboxylic acids by measuring the inhibition of BL of *Photobacterium leiognathi* in acute (inhibiting BL, Table 2) and chronic action tests (inhibiting BL and growth, Table 3).

Thus, in the acute action test the strongest inhibition of BL was observed in the samples treated mostly with amides of acetic and less with those of 2-propionic acid (**4.2**, **4.4**, **4.6**, **4.7**, **4.11**, **4.14**, **4.20**, **5.1**, **5.6**, **6.1**). A moderate action was shown by almost 25% of all substances (**4.5**, **4.8**–**4.10**, **4.13**, **4.15**, **4.18**, **4.21**, **5.2**, **5.4**, **5.6**, **5.11**, **5.12**, **6.2**, **7.1**, **7.4**, **7.5**, **7.7**). Some of the obtained compounds revealed an inhibition effect only in concentrations of 0.025 and 0.1 mg/mL (**4.1**, **4.16**, **5.7**, **5.9**). It is remarkable that substances **4.19** and **5.10** were BL promoters.

It was established that opposite to the previous test, where substances did or did not inhibit BL, in chronic action test, substances either inhibited or potentiated BL (Table 3). Hence, amides **4.1**, **4.6**, **4.16**, **6.1**, and **7.7** have shown strong cytotoxic activity, and **4.7**, **4.12**, **4.18**, **4.13**, **5.10**, **5.5**, **5.9**, **7.2**, **7.4**, **7.5**, **7.6** show a moderate inhibitory effect, compared to the standard. All other compounds promoted BL. Among the latter, substances **4.4**, **4.5**, **4.15** were cytotoxic only in concentration 0.25 mg/mL.

Summing up the BL assay results, substances have **4.1**, **4.6**, **6.1** strongly, and **4.13**, **4.18**, **7.4**, **7.5** moderately inhibited both BL and the growth of bacteria, namely, they were the most toxic compounds among all investigated. It is worth mentioning that amides **4.1**, **4.16**, **5.7**, **5.9** (in the acute test) and **5.1**, **5.12**, **6.2** (in chronic test) inhibited BL or bacterial growth only in the first two concentrations (0.025 and 0.1 mg/mL), demonstrating the hormesis phenomenon of low-dose inhibition [16].

The results of the antimicrobial assay showed that some of synthesized substances (**4.1**, **4.7**, **4.12**, **4.16**, **4.21**, **5.1**, **5.9**, **5.12**) posessed antifungal activity against *Aspergillus niger*, comparable with nystatine activity, but had no

**Table 2**. Values of BL in acute action test (%).

| Compound* | Concentration (mg/mL) | | | |
|---|---|---|---|---|
| | **0** | **0.025** | **0.1** | **0.25** |
| **4.1** | 100.0 | 58.1 | 74.4 | 130.2 |
| **4.2** | 100.0 | 39.1 | 39.1 | 26.1 |
| **4.4** | 100.0 | 44.1 | 39.7 | 22.1 |
| **4.5** | 100.0 | 71.7 | 47.8 | 38.2 |
| **4.6** | 100.0 | 46.9 | 39.8 | 23.4 |
| **4.7** | 100.0 | 26.0 | 8.7 | 5.2 |
| **4.8** | 100.0 | 90.4 | 82.7 | 76.9 |
| **4.9** | 100.0 | 69.4 | 91.7 | 99.2 |
| **4.10** | 100.0 | 61.8 | 44.1 | 59.6 |
| **4.11** | 100.0 | 26.5 | 33.1 | 44.1 |
| **4.12** | 100.0 | 15.6 | 26.0 | 34.7 |
| **4.13** | 100.0 | 46.9 | 65.6 | 82.0 |
| **4.14** | 100.0 | 6.6 | 8.8 | 11.0 |
| **4.15** | 100.0 | 84.8 | 80.9 | 71.7 |
| **4.16** | 100.0 | 16.3 | 10.2 | 100.0 |
| **4.17** | 100.0 | 99.2 | 99.2 | 106.6 |
| **4.18** | 100.0 | 70.3 | 70.3 | 82.0 |
| **4.19** | 100.0 | 74.4 | 119.0 | 131.4 |
| **4.20** | 100.0 | 12.4 | 17.4 | 24.8 |
| **4.21** | 100.0 | 68.8 | 79.2 | 83.3 |
| **5.1** | 100.0 | 57.2 | 43.4 | 31.2 |
| **5.2** | 100.0 | 105.9 | 94.9 | 77.2 |
| **5.3** | 100.0 | 98.4 | 89.1 | 98.4 |
| **5.4** | 100.0 | 78.3 | 52.2 | 19.6 |
| **5.5** | 100.0 | 149.0 | 122.4 | 100.0 |
| **5.6** | 100.0 | 67.8 | 62.6 | 65.2 |
| **5.7** | 100.0 | 77.2 | 99.3 | 99.3 |
| **5.8** | 100.0 | 99.3 | 99.3 | 105.9 |
| **5.9** | 100.0 | 34.7 | 116.2 | 104.0 |
| **5.10** | 100.0 | 110.4 | 177.1 | 100.0 |
| **5.11** | 100.0 | 75.7 | 75.7 | 78.3 |
| **5.12** | 100.0 | 71.4 | 83.3 | 95.2 |
| **6.1** | 100.0 | 35.7 | 19.0 | 11.9 |
| **6.2** | 100.0 | 66.7 | 83.3 | 95.2 |
| **7.1** | 100.0 | 116.2 | 76.3 | 78.0 |
| **7.2** | 100.0 | 97.5 | 82.8 | 110.4 |
| **7.3** | 100.0 | 97.5 | 82.8 | 110.4 |
| **7.4** | 100.0 | 82.0 | 77.3 | 82.0 |
| **7.5** | 100.0 | 60.7 | 65.9 | 57.2 |
| **7.6** | 100.0 | 98.4 | 82.0 | 93.8 |
| **7.7** | 100.0 | 82.0 | 89.1 | 89.1 |
| **Tetracyclin** | 100.0 | 80.7 | 9.1 | 0 |
| **DMSO (control)** | 100.0 | 141.7 | 119.6 | 110.4 |

* substance **4.3** was not tested.

**Table 3**. Values of BL in chronic action test (%).

| Compound* | Concentration (mg/mL) | | | |
|---|---|---|---|---|
| | **0** | **0.025** | **0.1** | **0.25** |
| **4.1** | 100.0 | 36.8 | 14.7 | 5.9 |
| **4.2** | 190.0 | 268.1 | 383.0 | 287.2 |
| **4.4** | 100.0 | 200.0 | 216.7 | 50.0 |
| **4.5** | 100.0 | 135.0 | 120.0 | 82.5 |
| **4.6** | 100.0 | 35.3 | 31.8 | 8.8 |
| **4.7** | 100.0 | 67.5 | 105.0 | 82.5 |
| **4.8** | 100.0 | 263.2 | 228.1 | 175.4 |
| **4.9** | 100.0 | 315.8 | 180.5 | 180.5 |
| **4.10** | 100.0 | 183.3 | 183.3 | 250.0 |
| **4.11** | 100.0 | 266.7 | 200.0 | 200.0 |
| **4.12** | 100.0 | 67.5 | 82.5 | 60.0 |
| **4.13** | 100.0 | 97.1 | 97.1 | 70.6 |
| **4.14** | 100.0 | 166.7 | 166.7 | 133.3 |
| **4.15** | 100.0 | 255.3 | 127.7 | 25.5 |
| **4.16** | 100.0 | 56.3 | 0.0 | 0.0 |
| **4.17** | 100.0 | 106.0 | 157.9 | 338.3 |
| **4.18** | 100.0 | 70.6 | 88.2 | 70.6 |
| **4.19** | 100.0 | 103.8 | 191.7 | 315.8 |
| **4.20** | 100.0 | 45.1 | 101.5 | 157.9 |
| **4.21** | 100.0 | 265.6 | 250.0 | 125.0 |
| **5.1** | 100.0 | 55.6 | 55.6 | 111.1 |
| **5.2** | 100.0 | 166.7 | 233.3 | 116.7 |
| **5.3** | 100.0 | 105.9 | 105.9 | 105.9 |
| **5.4** | 100.0 | 191.5 | 223.4 | 255.3 |
| **5.5** | 100.0 | 83.3 | 58.3 | 25.0 |
| **5.6** | 100.0 | 191.5 | 255.3 | 255.3 |
| **5.7** | 100.0 | 183.3 | 250.0 | 133.3 |
| **5.8** | 100.0 | 116.7 | 116.7 | 133.3 |
| **5.9** | 100.0 | 45.0 | 82.5 | 37.5 |
| **5.10** | 100.0 | 76.9 | 76.9 | 96.2 |
| **5.11** | 100.0 | 95.7 | 140.4 | 300.0 |
| **5.12** | 100.0 | 44.1 | 7.4 | 88.2 |
| **6.1** | 100.0 | 51.5 | 14.7 | 44.1 |
| **6.2** | 100.0 | 44.1 | 5.9 | 80.9 |
| **7.1** | 100.0 | 93.8 | 104.2 | 97.9 |
| **7.2** | 100.0 | 85.1 | 85.1 | 117.0 |
| **7.3** | 100.0 | 74.5 | 85.1 | 12.8 |
| **7.4** | 100.0 | 35.3 | 61.8 | 61.8 |
| **7.5** | 100.0 | 52.1 | 83.3 | 62.5 |
| **7.6** | 100.0 | 88.2 | 79.4 | 52.9 |
| **7.7** | 100.0 | 35.3 | 52.9 | 52.9 |
| **Tetracyclin** | 100.0 | 0 | 0 | 0 |
| **DMSO (control)** | 100.0 | 74.5 | 127.7 | 127.7 |

* Compound **4.3** was not tested.

antibacterial activity against *Staphylococcus aureus* (Table 4).

The structure / activity study showed that antimicrobial activity was dependent on the nature of the substituents. Considering 2-propionamide ones **5.1** and **5.9**, they were more active against *Aspergillus niger* compared to the acetamide derivatives **4.7**, **4.12**, **4.16**, exept for **4.21**. The growth of *Candida tenuis* was non-sensitive to almost all investigated compounds, beside the aliphatic-substituted acetamides **4.1** and **4.7**. Only two *N*-phenyl-substituted propionamides **5.9**, **5.12** slightly inhibited the growth of *Mycobacterium luteum*. The *n*-butyric acid derivatives **7.1** and **7.5** demonstrated antimicrobial activity against *Escherichia coli*. The mentioned compounds **4.12, 5.9** and **7.5** had a 2-methoxy-phenyl substituent, **4.16** and **7.5** a chlorine.

In conclusion, 42 novel compounds, namely ([1,2,4]triazolo[1,5-*c*]quinazolin-2-ylthio)carboxylic acid amides were synthesized and evaluated for their cytotoxicity, antibacterial, and antimicrobial activity. Comparing the results of antimicrobial screening with bioluminescence tests, it was found that the compounds that didn't inhibit BL and growth of bacteria at all, appeared to have no antibacterial properties. The most active substances in the entire series appeared to be the derivatives of acetic acid, **4.1** and **4.7** against *Candida tenuis,* of *n*-butyric acid, **7.1** and **7.5** against *Escherichia coli*, and of 2-propionic and acetic acid, **5.1** and **5.9**, and **4.21**, respectively against *Aspergillus niger*. Almost all substances, except **4.21** and **7.1**, that were shown to be antimicotics or antifungals, were cytotoxic for bioluminescent bacteria. In spite of this fact, there was no strong correlation between BL inhibition and antimicrobial activity of the investigated substances.

## Experimental

### Chemistry

Melting points were determined in open capillary tubes and were uncorrected. IR spectra (4000–600 $cm^{-1}$) were recorded on a Bruker ALPHA FT-IR spectrometer (Bruker Bioscience, USA) using a module for measuring attenuated total reflection (ATR). $^1$H-NMR spectra (400 MHz and 500 MHz) were recorded on a Varian-Mercury 400 (Varian Inc., Palo Alto, CA, USA) and Bruker Avance DRX-500 spectrometers 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 chromatograph "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). Electron impact mass spectra (EI-MS) were recorded on a Varian 1200 L instrument at 70 eV (Varian). The purity of all obtained compounds was checked by $^1$H-NMR and LC-MS.

Substances **1**–**3** were synthesized according to the reported procedures [13, 15]. Other starting materials and solvents were obtained from commercially available sources and used without additional purification.

#### *General procedure for synthesis of ([1,2,4]triazolo[1,5-c]-quinazolin-2-ylthio)carboxylic acid amides **4–7** (Table 1)*

**Method A**: 25% Ammonia solution (10 mL) was added to a suspension of **2** (1.05 g, 3.8 mmol) in 2-propanol (5 mL). The mixture was left at room temperature for 48 h. The precipitate was filtered out, washed with water, recrystallized from suitable solvent, and dried.

**Method B**: A solution of the proper halogenoacylanilide (6 mmol) in 2-propanol (10 mL) was added to a solution of **1**

**Table 4**. Inhibitory zones of the investigated compounds (mm).

| Compound* | Concentration (mg/mL) | *Escherichia coli* | *Staphylococcus aureus* | *Mycobacterium luteum* | *Candida tenuis* | *Aspergillus niger* |
|---|---|---|---|---|---|---|
| **4.1** | 5.0 | – | – | – | 14 | – |
| **4.7** | 5.0 | – | – | – | 15 | 16 |
| **4.12** | 5.0 | – | – | – | – | 15 |
| **4.16** | 5.0 | – | – | – | – | 11 |
| **4.21** | 5.0 | – | – | – | – | 25 |
| **5.1** | 5.0 | – | – | – | – | 25 |
| **5.9** | 5.0 | – | – | 11 | – | 25 |
| **5.12** | 5.0 | – | – | 14 | – | – |
| **7.1** | 5.0 | 13 | – | – | – | – |
| **7.5** | 5.0 | 14 | – | – | – | – |
| Vancomicin | 0.1 | 16 | 18 | 58 | – | – |
| Nystatin | 0.1 | 0 | 11 | 15 | 24 | 25 |
| Oxacillin | 0.1 | 0 | 21 | – | – | – |

* Bacteria were resistant to all other tested compounds.

(1.2 g, 5 mmol) in $H_2O$ (10 mL). The mixture was refluxed for 2 hours and then cooled to room temperature. Water was added to the resulting mixture. The precipitate was filtered out, washed with water, recrystallized from suitable solvent, and dried.

**Method C**: The proper halogenoacylamide (6 mmol) was added to a suspension of **1** (1.2 g, 5 mmol) in 2-propanol (10 mL). Further work-up as in method B afforded the proper substances.

**Method D**: Carbonyldiimidazole (1.0 g, 6 mmol) was added to a solution of the acid **3.1**, **3.2**, or **3.3** (5 mmol) in anhydrous dioxane (15 mL). The resulting mixture was heated at 60–80°C until $CO_2$ evolution stopped. The corresponding amine (6 mmol) was added, refluxed for 1 hour, and then cooled to room temperature. Further work-up as in method B afforded the proper substances.

### *Compound* ***4.1***

IR ($cm^{-1}$): 3026, 2968, 1686, 1646, 1623, 1597, 1520, 1471, 1443, 1410, 1360, 1252, 1189, 1133, 1101, 1026, 968, 898, 820, 774, 739, 706, 622; $^1$H-NMR (400 MHz) δ: 9.52 (s, 1H, H-5), 8.40 (d, *J* = 8.0 Hz, 1H, H-10), 8.06 (d, *J* = 8.0 Hz, 1H, H-7), 7.95 (t, *J* = 8.0 Hz, 1H, H-8), 7.83 (t, *J* = 8.0 Hz, 1H, H-9), 7.72 (br.s, 2H, N$H_2$), 4.36 (t, *J* = 4.9 Hz, 2H, SC$H_2$); LC-MS *m/z*: 260 $[M + H]^+$, 262; EI-MS *m/z* (I,%): 260 (3.0), 259 (10.7) $[M + 1]^+$, 242 (5.3), 218 (4.7), 217 (10.4), 216 (100.0), 215 (55.4), 202 (9.2), 188 (8.2), 187 (11.0), 184 (4.7), 171 (8.0), 129 (18.7), 102 (5.8), 62 (4.7), 58 (11.1). Anal. calcd. for $C_{11}H_9N_5OS$: C, 50.96; H, 3.5; N, 27.01; S, 12.37. Found: C, 51.74; H, 3.34; N, 27.05; S, 12.30.

### *Compound* ***4.2***

IR ($cm^{-1}$): 3330, 3061, 2955, 2930, 2860, 1651, 1621, 1537, 1511, 1477, 1392, 1356, 1315, 1264, 1197, 1103, 958, 902, 782, 767, 734, 714, 731, 631; $^1$H-NMR (400 MHz) *d*: 9.49 (s, 1H, H-5), 8.38 (d, *J* = 7.8 Hz, 1H, H-10), 8.22 (m, 1H, N*H*), 8.05 (d, *J* = 7.8 Hz, 1H, H-7), 7.94 (t, *J* = 8.1 Hz, 1H, H-8), 7.82 (t, *J* = 7.8 Hz, 1H, H-9), 4.05 (s, 2H, SC$H_2$), 3.08 (q, *J* = 7.8 Hz, 2H, NC$H_2$), 1.37 (q, *J* = 7.8 Hz, 2H, NCH$_2$C$H_2$), 1.24 (sext, 2H, N(CH$_2$)$_2$C$H_2$), 0.80 (t, *J* = 7.8 Hz, 3H, CH$_2$C$H_3$); LC-MS *m/z*: 316 $[M + H]^+$. Anal. calcd. for $C_{15}H_{17}N_5OS$: C, 57.12; H, 5.43; N, 22.20; S, 10.17. Found: C, 57.07; H, 5.49; N, 22.28; S, 10.13.

### *Compound* ***4.3***

IR ($cm^{-1}$): 3305, 3083, 3009, 1651, 1620, 1535, 1511, 1478, 1392, 1325, 1266, 1198, 1104, 1000, 902, 766, 714, 669; $^1$H-NMR (400 MHz) δ: 9.49 (s, 1H, H-5), 8.38 (d, *J* = 7.8 Hz, 1H, H-10), 8.05 (d, *J* = 7.8 Hz, 1H, H-7), 7.93 (m, 2H, H-8, N*H*), 7.82 (t, *J* = 7.6 Hz, 1H, H-9), 4.03 (s, 2H, SC$H_2$), 1.26 (s, 9H, (C$H_3$)$_3$); LC-MS *m/z*: 300 $[M + H]^+$. Anal. calcd. for $C_{15}H_{17}N_5OS$: C, 57.12; H, 5.43; N, 22.20; S, 10.17. Found: C, 57.07; H, 5.49; N, 22.28; S, 10.13.

### *Compound* ***4.4***

IR ($cm^{-1}$): 3296, 3069, 2957, 2972, 2864, 1666, 1622, 1555, 1513, 1477, 1452, 1379, 1358, 1306, 1253, 1223, 1145, 1106, 898, 762, 717, 648; $^1$H-NMR (400 MHz) δ: 9.48 (s, 1H, H-5), 8.78 (t, *J* = 8.0 Hz, 1H, N*H*), 8.37 (d, *J* = 8.0 Hz, 1H, H-10), 8.06 (d, *J* = 8.0 Hz, 1H, H-7), 7.94 (t, *J* = 7.8 Hz, 1H, H-8), 7.82 (t, *J* = 7.8 Hz, 1H, H-9), 7.21 (m, 5H, Ph), 4.32 (d, *J* = 6.2 Hz, 2H, NC$H_2$), 4.14 (c, 2H, SC$H_2$); LC-MS *m/z*: 316 $[M + H]^+$. Anal. calcd. for $C_{15}H_{17}N_5OS$: C, 57.12; H, 5.43; N, 22.20; S, 10.17. Found: C, 57.07; H, 5.49; N, 22.28; S, 10.13.

### *Compound* ***4.5***

IR ($cm^{-1}$): 3324, 3029, 1668, 1621, 1551, 1478, 1396, 1385, 1264, 1210, 1199, 1120, 1078, 1049, 1017, 904, 781, 736, 719, 695, 645; $^1$H-NMR (400 MHz) δ: 9.49 (s, 1H, H-5), 8.37 (d, *J* = 6.8 Hz, 1H, H-10), 8.70 (m, 1H, N*H*), 8.03 (d, *J* = 8.0 Hz, 1H, H-7), 7.92 (t, *J* = 7.8 Hz, 1H, H-8), 7.80 (t, *J* = 7.8 Hz, 1H, H-9), 4.15 (s, 2H, SC$H_2$), 4.07 (q, *J* = 6.8 Hz, 2H, OC$H_2$), 3.89 (d, *J* = 7.8 Hz, 2H, NC$H_2$), 1.15 (t, *J* = 6.8 Hz, 3H, C$H_3$); LC-MS *m/z*: 250 $[M + H]^+$, 252. Anal. calcd. for $C_{18}H_{15}N_5OS$: C, 61.87; H, 4.33; N, 20.04; S, 9.18. Found: 61.78; H, 4.30; N, 20.10; S, 9.22.

### *Compound* ***4.6***

IR ($cm^{-1}$): 3292, 3056, 2971, 1742, 1683, 1657, 1624, 1600, 1558, 1518, 1477, 1395, 1372, 1304, 1263, 1186, 1104, 1022, 891, 768, 718, 642; $^1$H-NMR (400 MHz) δ: 9.49 (s, 1H, H-5), 8.37 (d, *J* = 8.0 Hz, 1H, H-10), 8.70 (m, 1H, N*H*), 8.03 (d, *J* = 8.0 Hz, 1H, H-7), 7.92 (t, *J* = 7.8 Hz, 1H, H-8), 7.80 (t, *J* = 7.8 Hz, 1H, H-9), 4.15 (s, 2H, SC$H_2$), 4.07 (q, *J* = 6.8 Hz, 2H, OC$H_2$), 3.89 (d, *J* = 7.8 Hz, 2H, NC$H_2$), 1.15 (t, *J* = 6.8 Hz, 3H, C$H_3$); LC-MS *m/z*: 346 $[M + H]^+$, 348; EI-MS *m/z* (I,%): 346 (13.3), 345 (47.3) $[M]^+$, 300 (6.4), 243 (30.2), 242 (19.5), 218 (5.7), 217 (14.7), 216 (100.0), 215 (69.2), 203 (6.8), 202 (17.9), 187 (8.2),

184 (8.0), 171 (20.8), 130 (12.1), 129 (33.0), 102 (23.1). Anal. calcd. for $C_{15}H_{15}N_5O_3S$: C, 52.16; H, 4.38; N, 20.28; S, 9.27. Found: C, 52.20; H, 4.55; N, 20.17; S, 9.29.

### *Compound* ***4.7***

IR ($cm^{-1}$): 3060, 2944, 2922, 2852, 2310, 1636, 1622, 1602, 1515, 1476, 1437, 1395, 1354, 1309, 1242, 1199, 1132, 1007, 899, 851, 816, 766, 715, 645, 616; $^1$H-NMR (400 MHz) δ: 9.51 (s, 1H, H-5), 8.38 (d, *J* = 7.8 Hz, 1H, H-10), 8.03 (d, *J* = 7.8 Hz, 1H, H-7), 7.92 (t, *J* = 7.8 Hz, 1H, H-8), 7.80 (t, *J* = 7.8 Hz, 1H, H-9), 4.42 (s, 2H, SC$H_2$), 3.51 (t, *J* = 5.1 Hz, 2H, C$H_2$-$6_{pip}$), 3.45 (t, *J* = 5.1 Hz, 2H, C$H_2$-$2_{pip}$), 1.59 (m, 4H, (C$H_2$)$_2$-3,5pip), 1.59 (m, 2H, C$H_2$-$4_{pip}$); LC-MS *m/z*: 328 [M + H]$^+$. Anal. calcd. for $C_{16}H_{17}N_5OS$: C, 58.70; H, 5.23; N, 21.37; S, 9.79. Found: C, 58.75; H, 5.29; N, 21.30; S, 9.72.

### *Compound* ***4.8***

IR ($cm^{-1}$): 3063, 2987, 2896, 2850, 1623, 1515, 1455, 1397, 1356, 1283, 1253, 203, 1183, 1104, 1066, 1048, 1030, 959, 900, 871, 841, 773, 716, 645; $^1$H-NMR (400 MHz) δ: 9.44 (s, 1H, H-5), 8.38 (d, *J* = 7.8 Hz, 1H, H-10), 8.04 (d, *J* = 7.8 Hz, 1H, H-7), 7.93 (t, *J* = 7.6 Hz, 1H, H-8), 7.81 (t, *J* = 7.6 Hz, 1H, H-9), 4.39 (s, 2H, SC$H_2$), 3.63 (m, 4H, (C$H_2$)$_2$-2,$6_{morph}$), 3.56 (m, 4H, (C$H_2$)$_2$-3,$5_{morph}$); LC-MS *m/z*: 330 [M + H]$^+$. Anal. calcd. for $C_{15}H_{15}N_5O_2S$: C, 57.70; H, 4.59; N, 21.26; S, 9.73. Found: C, 57.72; H, 4.55; N, 21.30; S, 9.68.

### *Compound* ***4.9***

IR ($cm^{-1}$): 3325, 3052, 3024, 1674, 1600, 1543, 1516, 1475, 1442, 1391, 1326, 1263, 1244, 1176, 1103, 965, 941, 941, 902, 778, 756, 718, 690, 642; $^1$H-NMR (400 MHz) δ: 10.38 (br.s, 1H, NH), 9.50 (s, 1H, H-5), 8.38 (d, *J* = 8.0 Hz, 1H, H-10), 8.05 (d, *J* = 8.0 Hz, 1H, H-7), 7.94 (t, *J* = 7.8 Hz, 1H, H-8), 7.81 (t, *J* = 7.8 Hz, 1H, H-9), 7.60 (d, *J* = 7.8 Hz, 2H, H-$2_{Ph}$,$6_{Ph}$), 7.31 (t, *J* = 7.8 Hz, 2H, H-$3_{Ph}$,$5_{Ph}$), 7.05 (t, *J* = 7.3 Hz, 1H, H-$4_{Ph}$), 4.32 (s, 2H, $SCH_2$); LC-MS *m/z*: 336 [M + H]$^+$, 338. Anal. calcd. for $C_{17}H_{13}N_5OS$: C, 60.88; H, 3.91; N, 20.88; S, 9.56. Found: C, 60.80; H, 3.96; N, 20.81; S, 9.49.

### *Compound* ***4.10***

IR ($cm^{-1}$): 3343, 3054, 2975, 2919, 2850, 1670, 1589, 1538, 1513, 1474, 1450, 1396, 1371, 1263, 1246, 1189, 1174, 1102, 1046, 955, 895, 852, 766, 716, 664; $^1$H-NMR (400 MHz) δ: 9.71 (br.s, 1H, N*H*), 9.50 (s, 1H, H-5), 8.38 (d, *J* = 8.1 Hz, 1H, H-10), 8.04 (d, *J* = 8.1 Hz, 1H, H-7), 7.93 (t, *J* = 8.1 Hz, 1H, H-8), 7.81 (t, *J* = 8.1 Hz, 1H, H-9), 7.43 (d, *J* = 7.8 Hz, 1H, H-$6_{Ph}$), 7.19 (d, *J* = 7.8 Hz, 1H, H-$3_{Ph}$), 7.15 (t, *J* = 7.6 Hz, 1H, H-$5_{Ph}$), 7.07 (t, *J* = 7.8 Hz, 1H, H-$4_{Ph}$), 4.34 (s, 2H, SC$H_2$) 3.32 (s, 3H, C$H_3$); LC-MS *m/z*: 350 [M + H]$^+$. Anal. calcd. for $C_{18}H_{15}N_5OS$: C, 61.87; H, 4.33; N, 20.04; S, 9.18. Found: 61.78; H, 4.30; N, 20.10; S, 9.22.

### *Compound* ***4.11***

IR ($cm^{-1}$): 3290, 3257, 3198, 3064, 2920, 1665, 1614, 1555, 1515, 1478, 1402, 1384, 371, 1328, 1306, 1253, 1197, 1168, 1130, 900, 786, 765, 714, 695, 615; $^1$H-NMR (400 MHz) δ: 10.31 (br.s, 1H, N*H*), 9.47 (s, 1H, H-5), 8.34 (d, *J* = 7.8 Hz, 1H, H-10), 8.00 (d, *J* = 7.8 Hz, 1H, H-7), 7.89 (t, *J* = 7.8 Hz, 1H, H-8), 7.77 (t, *J* = 7.8 Hz, 1H, H-9), 7.44 (s, 1H, H-$2_{Ph}$), 7.38 (d, *J* = 7.8 Hz, 1H, H-$6_{Ph}$), 7.17 (t, *J* = 7.8 Hz, 1H, H-$5_{Ph}$), 6.85 (d, *J* = 7.8 Hz, 1H, H-$4_{Ph}$), 4.31 (s, 2H, SC$H_2$), 2.25 (s, 3H, C$H_3$); LC-MS *m/z*: 350 [M + H]$^+$. Anal. calcd. for $C_{18}H_{15}N_5OS$: C, 61.87; H, 4.33; N, 20.04; S, 9.18. Found: 61.78; H, 4.30; N, 20.10; S, 9.22.

### *Compound* ***4.12***

IR ($cm^{-1}$): 3312, 3057, 2995, 2930, 2828, 1669, 1602, 1537, 1476, 1458, 1398, 1384, 1353, 1250, 1216, 1171, 1103, 1034, 959, 898, 851, 771, 754, 716, 703, 642; $^1$H-NMR (400 MHz) δ: 9.60 (br.s, 1H, N*H*), 9.51 (s, 1H, H-5), 8.39 (d, *J* = 7.8 Hz, 1H, H-10), 8.04 (d, *J* = 7.8 Hz, 1H, H-7), 8.01 (d, *J* = 7.8 Hz, 1H, H-$6_{Ph}$), 7.93 (t, *J* = 7.8 Hz, 1H, H-8), 7.81 (t, *J* = 7.8 Hz, 1H, H-9), 7.03 (m, 3H, H-$3_{Ph}$,$5_{Ph}$,$6_{Ph}$), 6.88 (t, *J* = 7.8 Hz, 1H, H-$4_{Ph}$), 4.34 (s, 2H, SC$H_2$), 3.77 (s, 3H, OC$H_3$); LC-MS *m/z*: 366 [M + H]$^+$. Anal. calcd. for $C_{18}H_{15}N_5O_2S$: C, 59.17; H, 4.14; N, 19.,17; S, 8.77. Found: C, 59.12; H, 4.17; N, 19.15; S, 8.73.

### *Compound* ***4.13***

IR ($cm^{-1}$): 3306, 3113, 3061, 2977, 1678, 1595, 1533, 1477, 1417, 1402, 1387, 1356, 1250, 1218, 1171, 1127, 1033, 959, 899, 873, 811, 774, 716, 642; $^1$H-NMR (400 MHz) δ: 9.70 (br.s, 1H, N*H*), 9.50 (s, 1H, H-5), 8.39 (d, *J* = 7.8 Hz, 1H, H-10), 8.13 (s, 1H, H-$3_{Ph}$), 8.05 (d, *J* = 7.8 Hz, 1H, H-7), 7.94 (t, *J* = 7.8 Hz, 1H, H-8), 7.82 (t, *J* = 7.8 Hz, 1H, H-9), 7.09 (d, *J* = 8.1 Hz, 1H, H-$5_{Ph}$), 7.04 (d, *J* = 8.1 Hz, 1H, H-$6_{Ph}$), 4.35 (s, 2H, SC$H_2$), 3.79 (s, 3H, OC$H_3$); LC-MS *m/z*: 400 [M + H]$^+$, 401. Anal. calcd. for $C_{18}H_{14}ClN_5O_2S$: C, 54.07; H, 5.53; N, 17.51; S, 8.02. Found: C, 54.14; H, 5.49; N, 17.56; S, 8.05.

### *Compound* ***4.14***

IR ($cm^{-1}$): 3362, 3071, 2996, 2939, 1697, 1607, 1585, 1502, 1452, 1398, 1373, 1338, 1266, 1145, 956, 900, 781, 750, 717, 643; $^1$H-NMR (400 MHz) δ: 10.75 (br.s, 1H, N*H*), 9.49 (s, 1H, H-5), 8.39 (d, *J* = 8.0 Hz, 1H, H-10), 8.04 (d, *J* = 8.0 Hz, 1H, H-7), 7.98 (d, *J* = 7.8 Hz, 1H, H-$6_{Ph}$), 7.93 (t, *J* = 8.0 Hz, 1H, H-8), 7.88 (d, *J* = 7.8 Hz, 1H, H-$3_{Ph}$), 7.81 (t, *J* = 8.0 Hz, 1H, H-9), 7.72 (t, *J* = 7.8 Hz, 1H, H-$5_{Ph}$), 7.36 (t, *J* = 7.8 Hz, 1H, H-$4_{Ph}$), 4.33 (s, 2H, SC$H_2$); LC-MS *m/z*: 381 [M + H]$^+$, 383. Anal. calcd. for $C_{17}H_{12}N_6O_3S$: C, 53.68; H, 3.18; N, 22.09; S, 8.43. Found: C, 53.61; H, 3.26; N, 22.10; S, 8.50.

### *Compound* ***4.15***

IR ($cm^{-1}$): 3321, 3078, 3053, 2919, 2310, 1673, 1612, 1545, 1499, 1451, 1392, 1331, 1248, 1164, 1106, 955, 934, 901, 851, 769, 749, 716, 684, 629; $^1$H-NMR (400 MHz) δ: 11.03 (br.s, 1H, N*H*), 9.50 (s, 1H, H-5), 8.36 (d, *J* = 7.8 Hz, 1H, H-10), 8.23 (d, *J* = 9.0 Hz, 2H, H-$2_{Ph}$,$6_{Ph}$), 8.04 (d, *J* = 7.8 Hz, 1H, H-7), 7.94 (t, *J* = 8.0 Hz, 1H, H-8), 7.85 (d, *J* = 9.0 Hz, 2H, H-$3_{Ph}$,$5_{Ph}$), 7.81 (t, *J* = 8.0 Hz, 1H, H-9), 4.39 (s, 2H, SC$H_2$); LC-MS *m/z*: 380 [M + H]$^+$. Anal. calcd. for $C_{17}H_{12}N_6O_3S$: C, 53.68; H, 3.18; N, 22.09; S, 8.43. Found: C, 53.58; H, 3.21; N, 22.20; S, 8.45.

### *Compound* ***4.16***

IR ($cm^{-1}$): 3296, 3255, 3187, 3065, 1682, 1594, 1546, 1516, 1479, 1404, 1334, 1255, 1191, 1131, 1101, 901, 767, 715, 685, 611; $^1$H-NMR (400 MHz) δ: 10.60 (br.s, 1H, NH), 9.50 (s, 1H, H-5), 8.37 (d, *J* = 6.0 Hz, 1H, H-10), 8.04 (d, *J* = 6.0 Hz, 1H, H-7), 7.93 (t, *J* = 7.8 Hz, 1H, H-8), 7.81 (t, *J* = 7.8 Hz, 1H, H-9), 7.80 (t, *J* = 7.6 Hz, 1H, H-$2_{Ph}$), 7.47 (d, *J* = 7.8 Hz, 1H, H-$6_{Ph}$), 7.35 (t, *J* = 7.8 Hz, 1H, H-$5_{Ph}$), 7.12 (t, *J* = 7.8 Hz, 1H, H-$4_{Ph}$), 4.33 (s, 2H, $SCH_2$); LC-MS *m/z*: 370 [M + H]$^+$, 372, 374. Anal. calcd. for $C_{15}H_{12}ClN_5OS$: C, 55.21; H, 3.27; N, 18.94; S, 8.67. Found: C, 55.23; H, 3.37; N, 19.02; S, 8.62.

### *Compound* ***4.17***

IR ($cm^{-1}$): 3302, 3060, 1666, 1621, 1593, 1512, 1490, 1478, 1393, 1318, 1264, 1238, 1168, 1093, 1013, 966, 903, 820, 770, 715,667, 648; $^1$H-NMR (400 MHz) δ: 10.44 (br.s, 1H, N*H*), 9.47 (s, 1H, H-5), 8.37 (d, *J* = 7.8 Hz, 1H, H-10), 8.04 (d, *J* = 7.8 Hz, 1H, H-7), 7.93 (t, *J* =

7.6 Hz, 1H, H-8), 7.81 (t, *J* = 7.6 Hz, 1H, H-9), 7.62 (d, *J* = 8.1 Hz, 2H, H-2$_{Ph}$,6$_{Ph}$), 7.35 (d, *J* = 8.1 Hz, 2H, H-3$_{Ph}$,5$_{Ph}$), 4.31 (s, 2H, SC$H_2$); LC-MS *m/z*: 370 $[M + H]^+$, 372, 374; EI-MS *m/z* (I,%): 369 (10.2) $[M]^+$, 245 (8.0), 244 (16.5), 243 (100.0), 242 (9.3), 217 (5.8), 216 (33.7), 215 (58.7), 171 (8.3), 130 (6.3), 129 (26.4), 127 (11.3), 102 (10.1), 99 (8.9). Anal. calcd. for $C_{15}H_{12}ClN_5OS$: C, 55.21; H, 3.27; N, 18.94; S, 8.67. Found: C, 55.23; H, 3.37; N, 19.02; S, 8.62.

### *Compound* ***4.18***

IR ($cm^{-1}$): 3309, 3052, 2918, 1664, 1619, 1591, 1531, 1518, 1474, 1391, 1303, 1262, 1243, 1119, 1072, 1008, 902, 818, 775, 717, 642; $^1$H-NMR (400 MHz) δ: 10.55 (br.s, 1H, N*H*), 9.51 (s, 1H, H-5), 8.37 (d, *J* = 8.0 Hz, 1H, H-10), 8.05 (d, *J* = 8.0 Hz, 1H, H-7), 7.94 (t, *J* = 7.8 Hz, 1H, H-8), 7.82 (t, *J* = 7.8 Hz, 1H, H-9), 7.58 (d, *J* = 8.0 Hz, 2H, H-2$_{Ph}$,6$_{Ph}$), 7.50 (d, *J* = 8.0 Hz, 2H, H-3$_{Ph}$,5$_{Ph}$), 4.33 (s, 2H, SC$H_2$); LC-MS *m/z*: 416 $[M + H]^+$, 417. Anal. calcd. for $C_{17}H_{12}N_5OS$: C, 49.29; H, 2.92; N, 16.90; S, 7.74. Found: C, 49.30; H, 2.95; N, 16.98; S, 7.65.

### *Compound* ***4.19***

IR ($cm^{-1}$): 3295, 3264, 3204, 3148, 3066, 1681, 1668, 1619, 1602, 1553, 1479, 1403, 1327, 1313, 1253, 1185, 1169, 1115, 1094, 1072, 900, 766, 703; $^1$H-NMR (400 MHz) δ: 10.73 (br.s, 1H, N*H*), 9.49 (s, 1H, H-5), 8.36 (d, *J* = 7.8 Hz, 1H, H-10), 8.09 (s, 1H, H-2$_{Ph}$), 8.04 (d, *J* = 7.8 Hz, 1H, H-7), 7.93 (t, *J* = 7.8 Hz, 1H, H-8), 7.80 (t, *J* = 7.8 Hz, 2H, H-9, H-6$_{Ph}$), 7.56 (t, *J* = 8.0 Hz, 1H, H-5$_{Ph}$), 7.41 (d, *J* = 8.0 Hz, 1H, H-4$_{Ph}$), 4.34 (s, 2H, SC$H_2$); LC-MS *m/z*: 404 $[M + H]^+$, 405. Anal. calcd. for $C_{18}H_{12}F_3N_5OS$: C, 53.60; H, 3.0; N, 17.33; S, 7.95. Found: C, 53.65; H, 2.97; N, 17.28; S, 7.98.

### *Compound* ***4.20***

IR ($cm^{-1}$): 3250, 3181, 3053, 2982, 2907, 2862, 1689, 1626, 1597, 1546, 1513, 1479, 1398, 1267, 1253, 1169, 1118, 1102, 1024, 901, 858, 765, 712, 639, 613; $^1$H-NMR (400 MHz) δ: 10.75 (br.s, 1H, NH), 9.50 (s, 1H, H-5), 8.36 (d, *J* = 7.8 Hz, 1H, H-10), 8.04 (d, *J* = 7.8 Hz, 1H, H-7), 7.92 (t, *J* = 8.6 Hz, 3H, H-8, H-2$_{Ph}$,6$_{Ph}$), 7.80 (t, *J* = 7.8 Hz, 1H, H-9), 7.74 (d, *J* = 8.6 Hz, 2H, H-3$_{Ph}$,5$_{Ph}$), 4.36 (s, 2H, SC$H_2$), 4.27 (q, *J* = 7.1 Hz, 2H, C$H_2$CH$_3$), 1.29 (t, *J* = 7.1 Hz, 3H, C$H_3$); LC-MS *m/z*: 408 $[M + H]^+$, 409. Anal. calcd. for $C_{20}H_{17}N_5O_3S$: C, 58.96; H, 4.21; N, 17.19; S, 7.87. Found: C, 58.65; H, 4.30; N, 17.29; S, 7.88.

### *Compound* ***4.21***

IR ($cm^{-1}$): 3058, 2964, 2929, 1638, 1554, 1515, 1477, 1453, 1396, 1310, 1269, 1228, 1133, 1105, 952, 899, 767, 715, 645, 614; $^1$H-NMR (400 MHz) δ: 9.47 (s, 1H, H-5), 8.37 (d, *J* = 8.0 Hz, 1H, H-10), 8.04 (d, *J* = 8.0 Hz, 1H, H-7), 7.93 (t, *J* = 7.8 Hz, 1H, H-8), 7.81 (t, *J* = 7.8 Hz, 1H, H-9), 4.38 (s, 2H, SC$H_2$), 3.47 (m, NC$H_2$), 3.33 (m, NC$H_2$), 1.24 (t, *J* = 7.8 Hz, 3H, C$H_3$), 1.05 (t, *J* = 7.8 Hz, 3H, C$H_3$); LC-MS *m/z*: 316 $[M + H]^+$; EI-MS *m/z* (I,%): 316 (15.9), 315 (47.2) $[M]^+$, 243 (10.1), 242 (5.9), 216 (12.6), 215 (31.5), 203 (9.5), 202 (37.8), 171 (8.3), 130 (7.6), 129 (20.4), 115 (10.8), 114 (100.0), 102 (15.4), 100 (92.0). Anal. calcd. for $C_{15}H_{17}N_5OS$: C, 57.12; H, 5.43; N, 22.20; S, 10.17. Found: C, 57.07; H, 5.49; N, 22.28; S, 10.13.

### *Compound* ***5.1***

IR ($cm^{-1}$): 3336, 3056, 2970, 2957, 2931, 2873, 1642, 1626, 1546, 1521, 1478, 1454, 1384, 1306, 1267, 1215, 1105, 1079, 992, 961, 900, 771, 716, 615; $^1$H-NMR (400 MHz) δ: 9.51 (s, 1H, H-5), 8.39 (d, *J* = 8.0 Hz, 1H, H-10), 8.29 (t, *J* = 6.8 Hz, 1H, N*H*), 8.05 (d, *J* = 7.80 Hz, 1H, H-7), 7.94 (t, *J* = 7.8 Hz, 1H, H-8), 7.82 (t, *J* = 7.8 Hz, 1H, H-9), 4.56 (q, *J* = 6.8 Hz, 1H, SC*H*), 3.07 (m, 2H, NC$H_2$), 1.60 (d, *J* = 6.8 Hz, 3H, SCHC$H_3$), 1.37 (q, *J* = 7.8 Hz, 2H, NCH$_2$C$H_2$), 1.23 (sext, 2H, N(CH$_2$)$_2$C$H_2$), 0.80 (t, *J* = 7.8 Hz, 3H, CH$_2$C$H_3$); LC-MS *m/z*: 330 $[M + H]^+$, 331. Anal. calcd. for $C_{16}H_{19}N_5OS$: C, 58.34; H, 5.81; N, 21.26; S, 9.73. Found: C, 58.28; H, 5.76; N, 21.28; S, 9.79.

### *Compound* ***5.2***

IR ($cm^{-1}$): 3318, 2965, 1656, 1625, 1545, 1521, 1478, 1455, 1398, 1358, 1272, 1249, 1226, 989, 901, 784, 769, 715; $^1$H-NMR (400 MHz) δ: 9.51 (s, 1H, H-5), 8.39 (d, *J* = 7.8 Hz, 1H, H-10), 8.06 (d, *J* = 7.8 Hz, 1H, H-7), 8.00 (br.s, 1H, N*H*), 7.94 (t, *J* = 7.8 Hz, 1H, H-8), 7.82 (t, *J* = 7.8 Hz, 1H, H-9), 4.57 (q, *J* = 6.8 Hz, 1H, SC*H*), 1.58 (d, *J* = 6.8 Hz, 3H, SCHC$H_3$), 1.25 (s, 9H, (C$H_3$)$_3$); LC-MS *m/z*: 330 $[M + H]^+$; EI-MS *m/z* (I,%): 257 (4.4), 232 (4.3), 231 (12.3), 230 (100.0), 229 (28.8), 215 (23.9), 202 (6.9), 198 (11.7), 197 (65.7), 171 (36.9), 170 (4.9), 145 (5.3), 130 (3.3), 129 (26.5), 102 (15.8), 101 (5.4), 75 (3.6), 72 (4.8), 60 (7.9), 59 (23.6), 58 (10.3), 57 (47.0), 55 (8.2). Anal. calcd. for $C_{16}H_{19}N_5OS$: C, 58.34; H, 5.81; N, 21.26; S, 9.73. Found: C, 58.28; H, 5.76; N, 21.28; S, 9.79.

### *Compound* ***5.3***

IR ($cm^{-1}$): 3292, 3056, 2932, 1634, 1620, 1542, 1510, 1477, 1453, 1386, 1372, 1251, 1203, 1079, 963, 900, 778, 746, 696, 643; $^1$H-NMR (400 MHz) δ: 9.49 (s, 1H, H-5), 8.84 (t, *J* = 6.6 Hz, 1H, N*H*), 8.36 (d, *J* = 7.8 Hz, 1H, H-10), 8.04 (d, *J* = 7.8 Hz, 1H, H-7), 7.93 (t, *J* = 7.8 Hz, 1H, H-8), 7.81 (t, *J* = 7.8 Hz, 1H, H-9), 7.21 (s, 5H, Ph), 7.16 (d, *J* = 7.8 Hz, 1H, H-3$_{Ph}$), 7.09 (t, *J* = 7.8 Hz, 1H, H-4$_{Ph}$), 4.65 (q, *J* = 6.8 Hz, 1H, SC*H*), 4.31 (d, *J* = 8.6 Hz, 2H, NC$H_2$), 1.65 (d, *J* = 6.8 Hz, 3H, SCHC$H_3$); LC-MS *m/z*: 364 $[M + H]^+$, 365. Anal. calcd. for $C_{19}H_{17}N_5OS$: C, 62.79; H, 4.71; N, 19.27; S, 8.82. Found: C, 62.78; H, 4.60; N, 19.18; S, 8.72.

### *Compound* ***5.4***

IR ($cm^{-1}$): 3067, 3009, 2984, 2941, 2860, 1623, 1518, 1476, 1440, 1395, 1350, 1301, 1253, 1135, 1104, 1056, 1012, 953, 922, 897, 850, 765, 714, 644, 622; $^1$H-NMR (400 MHz) δ: 9.52 (s, 1H, H-5), 8.39 (d, *J* = 7.8 Hz, 1H, H-10), 8.04 (d, *J* = 7.8 Hz, 1H, H-7), 7.93 (t, *J* = 7.8 Hz, 1H, H-8), 7.81 (t, *J* = 7.8 Hz, 1H, H-9), 5.05 (q, *J* = 6.8 Hz, 1H, SC*H*), 3.53 (m, 4H, (C$H_2$)$_2$-2,6$_{pip}$), 1.61 (m, 6H, (C$H_2$)$_3$-3$_{pip}$,4$_{pip}$,5$_{pip}$), 1.45 (d, *J* = 6.8 Hz, 3H, SCHC$H_3$); LC-MS *m/z*: 342 $[M + H]^+$; EI-MS *m/z* (I,%): 229 (10.3), 203 (8.3), 202 (6.9), 141 (8.6), 140 (100.0), 139 (21.2), 129 (9.4), 112 (50.4), 111 (9.3), 102 (7.4), 84 (31.7), 83 (5.9), 70 (6.8), 69 (62.8), 59 (13.1), 56 (14.2), 55 (13.2). Anal. calcd. for $C_{17}H_{19}N_5OS$: C, 59.80; H, 5.61; N, 20.51; S, 9.39. Found: C, 59.81; H, 5.64; N, 20.47; S, 9.31.

### *Compound* ***5.5***

IR ($cm^{-1}$): 3050, 2976, 2952, 2906, 2859, 2310, 1643, 1617, 1512, 1475, 1428, 1396, 1302, 1217, 1105, 1062, 1025, 968, 939, 896, 844, 779, 717, 644; $^1$H-NMR (400 MHz) δ: 9.53 (s, 1H, H-5), 8.40 (d, *J* = 7.8 Hz, 1H, H-10), 8.06 (d, *J* = 7.8 Hz, 1H, H-7), 7.95 (t, *J* = 7.8 Hz, 1H, H-8), 7.83 (t, *J* = 7.8 Hz, 1H, H-9), 5.04 (q, *J* = 6.8 Hz, 1H, SC*H*), 3.64 (m, 4H, (C$H_2$)$_2$-2$_{morph}$,6$_{morph}$), 3.52 (m, 4H, (C$H_2$)$_2$-3$_{morph}$,5$_{morph}$), 1.61 (d, *J* = 6,8 Hz, 3H, SCHC$H_3$); LC-MS *m/z*: 344 $[M + H]^+$. Anal. calcd. for $C_{16}H_{19}N_5O_2S$: C, 55.96; H, 4.99; N, 20.39; S, 9.34. Found: C, 56.01; H, 5.05; N, 20.29; S, 9.44.

### *Compound* ***5.6***

IR ($cm^{-1}$): 3274, 3165, 3131, 3042, 2982, 2935, 1686, 1600, 1550, 1516, 1476, 1442, 1371, 1339, 1300, 1252, 1173, 1074, 900, 778, 755, 717, 695, 642; $^1$H-NMR (400 MHz) δ: 10.42 (br.s, 1H, N*H*), 9.53

(s, 1H, H-5), 8.40 (d, *J* = 8.1 Hz, 1H, H-10), 8.06 (d, *J* = 8.1 Hz, 1H, H-7), 7.95 (t, *J* = 7.8 Hz, 1H, H-8), 7.83 (t, *J* = 7.8 Hz, 1H, H-9), 7.60 (d, *J* = 8.1 Hz, 2H, H-$2_{Ph}$,$6_{Ph}$), 7.31 (d, *J* = 7.3 Hz, 2H, H-$3_{Ph}$,$5_{Ph}$), 7.07 (t, *J* = 7.3 Hz, 1H, H-$4_{Ph}$), 4.80 (q, *J* = 6.6 Hz, 1H, SC*H*), 1.72 (d, *J* = 6.6 Hz, 3H, SCHC$H_3$); LC-MS *m/z*: 350 $[M + H]^+$. Anal. calcd. for $C_{18}H_{15}N_5OS$: C, 61.87; H, 4.33; N, 20.04; S, 9.18. Found: C, 61.83; H, 4.35; N, 20.10; S, 9.14.

### *Compound* ***5.7***

IR ($cm^{-1}$): 3260, 3068, 2964, 2843, 1655, 1623, 1590, 1537, 1517, 1478, 1453, 1392, 1374, 1347, 1302, 1264, 1185, 1085, 992, 901, 773, 754, 717, 677; $^1$H-NMR (400 MHz) δ: 9.78 (br.s, 1H, N*H*), 9.54 (s, 1H, H-5), 8.42 (d, *J* = 7.8 Hz, 1H, H-10), 8.07 (d, *J* = 7.8 Hz, 1H, H-7), 7.95 (t, *J* = 7.6 Hz, 1H, H-8), 7.83 (t, *J* = 7.6 Hz, 1H, H-9), 7.40 (d, *J* = 8.0 Hz, 1H, H-$6_{Ph}$), 7.20 (d, *J* = 8.0 Hz, 1H, H-$5_{Ph}$), 7.16 (d, *J* = 7.8 Hz, 1H, H-$3_{Ph}$), 7.09 (t, *J* = 7.8 Hz, 1H, H-$4_{Ph}$), 4.88 (q, *J* = 6.8 Hz, 1H, SC*H*), 3.33 (s, 3H, C$H_3$), 1.74 (d, *J* = 6.8 Hz, 3H, SCHC$H_3$); LC-MS *m/z*: 364 $[M + H]^+$, 366. Anal. calcd. for $C_{19}H_{17}N_5OS$: C, 62.79; H, 4.71; N, 19.27; S, 8.82. Found: C, 62.74; H, 4.65; N, 19.23; S, 8.78.

### *Compound* ***5.8***

IR ($cm^{-1}$): 3307, 3055, 3032, 2970, 2920, 1662, 1624, 1543, 1518, 1477, 1451, 1392, 1303, 1262, 1197, 1074, 997, 901, 770, 717, 690, 640; $^1$H-NMR (400 MHz) δ: 10.38 (br.s, 1H, N*H*), 9.53 (s, 1H, H-5), 8.40 (d, *J* = 8.0 Hz, 1H, H-10), 8.06 (d, *J* = 8.0 Hz, 1H, H-7), 7.94 (t, *J* = 7.8 Hz, 1H, H-8, Ph), 7.82 (t, *J* = 7.8 Hz, 1H, H-9), 7.46 (s, 1H, H-$2_{Ph}$), 7.40 (d, *J* = 7.6 Hz, 1H, H-$6_{Ph}$), 7.19 (t, *J* = 7.6 Hz, 1H, H-$5_{Ph}$), 6.89 (d, *J* = 7.6 Hz, 1H, H-$4_{Ph}$), 4.80 (q, *J* = 6.8 Hz, 1H, SC*H*), 2.27 (s, 3H, C$H_3$), 1.72 (d, *J* = 6.8 Hz, 3H, SCHC$H_3$); LC-MS *m/z*: 364 $[M + H]^+$, 366. Anal. calcd. for $C_{19}H_{17}N_5OS$: C, 62.79; H, 4.71; N, 19.27; S, 8.82. Found: C, 62.74; H, 4.65; N, 19.23; S, 8.78.

### *Compound* ***5.9***

IR ($cm^{-1}$): 3305, 3050, 2981, 2933, 1679, 1604, 1538, 1513, 1480, 1460, 1453, 1396, 1377, 1264, 1251, 1220, 1173, 1107, 1026, 921, 899, 770, 736, 718, 701; $^1$H-NMR (400 MHz) δ: 9.61 (br.s, 1H, N*H*), 9.54 (s, 1H, H-5), 8.43 (d, *J* = 8.1 Hz, 1H, H-10), 8.07 (d, *J* = 8.1 Hz, 1H, H-7), 7.99 (d, *J* = 7.8 Hz, 1H, H-$6_{Ph}$), 7.96 (t, *J* = 7.6 Hz, 1H, H-8), 7.85 (t, *J* = 7.6 Hz, 1H, H-9), 7.06 (t, *J* = 7.6 Hz, 1H, H-$5_{Ph}$), 6.99 (d, *J* = 7.8 Hz, 1H, H-$3_{Ph}$), 6.89 (t, *J* = 7.8 Hz, 1H, H-$4_{Ph}$), 7.09 (t, *J* = 7.8 Hz, 1H, H-$4_{Ph}$), 4.95 (q, *J* = 6.8 Hz, 1H, SC*H*), 3.71 (s, 3H, OC$H_3$), 1.67 (d, *J* = 6.8 Hz, 3H, SCHC$H_3$); LC-MS *m/z*: 380 $[M + H]^+$. Anal. calcd. for $C_{19}H_{17}N_5O_2S$: C, 60.14; H, 4.52; N, 18.46; S, 8.45. Found: C, 60.18; H, 4.42; N, 18.37; S, 8.49.

### *Compound* ***5.10***

IR ($cm^{-1}$): 3273, 3218, 3089, 1696, 1625, 1597, 1569, 1500, 1442, 1393, 1338, 1262, 1210, 1172, 1116, 1075, 900, 851, 768, 750, 716, 689; $^1$H-NMR (400 MHz) δ: 11.05 (br.s, 1H, N*H*), 9.53 (s, 1H, H-5), 8.38 (d, *J* = 8.6 Hz, 1H, H-10), 8.06 (d, *J* = 8.6 Hz, 1H, H-7), 8.23 (d, *J* = 9.0 Hz, 2H, H-$2_{Ph}$,$6_{Ph}$), 7.95 (t, *J* = 7.8 Hz, 1H, H-8), 7.87 (d, *J* = 9.0 Hz, 2H, H-$3_{Ph}$,$5_{Ph}$), 7.82 (t, *J* = 7.8 Hz, 1H, H-9), 4.82 (q, *J* = 7.1 Hz, 1H, SC*H*), 1.73 (d, *J* = 7.1 Hz, 3H, SCHC$H_3$); LC-MS *m/z*: 395 $[M + H]^+$. Anal. calcd. for $C_{18}H_{14}N_6O_3S$: C, 54.82; H, 3.58; N, 21.31; S, 8.13. Found: C, 54.71; H, 3.52; N, 21.36; S, 8.18.

### *Compound* ***5.11***

IR ($cm^{-1}$): 3307, 3269, 3197, 3062, 2972, 2931, 2310, 1687, 1610, 1550, 1516, 1490, 1477, 1453, 1392, 1375, 1343, 1300, 1251, 1174, 1087, 1010, 898, 831, 808, 773, 717, 659; $^1$H-NMR (400 MHz) δ: 10.56 (br.s, 1H, N*H*), 9.52 (s, 1H, H-5), 8.39 (d, *J* = 7.8 Hz, 1H, H-10), 8.06 (d, *J* = 7.8 Hz, 1H, H-7), 7.94 (t, *J* = 7.8 Hz, 1H, H-8), 7.82 (t, *J* = 7.6 Hz, 1H, H-9), 7.64 (d, *J* = 8.6 Hz, 2H, H-$2_{Ph}$,$6_{Ph}$), 7.37 (d, *J* = 8.0 Hz, 2H, H-$3_{Ph}$,$5_{Ph}$), 4.77 (q, *J* = 6.8 Hz, 1H, SC*H*), 1.72 (d, *J* = 6.8 Hz, 3H, SCHC$H_3$); LC-MS *m/z*: 384 $[M + H]^+$, 385. Anal. calcd. for $C_{18}H_{14}ClN_5OS$: C, 56.32; H, 3.68; N, 18.24; S, 8.35. Found: C, 56.24; H, 3.65; N, 18.18; S, 8.30.

### *Compound* ***5.12***

IR ($cm^{-1}$): 3298, 3090, 2982, 1662, 1623, 1547, 1518, 1478, 1455, 1396, 1374, 1328, 1265, 1223, 1160, 1116, 991, 877, 796, 771, 695, 651; $^1$H-NMR (400 MHz) δ: 10.79 (br.s, 1H, N*H*), 9.52 (s, 1H, H-5), 8.38 (d, *J* = 7.8 Hz, 1H, H-10), 8.1 (s, 1H, H-$2_{Ph}$), 8.05 (d, *J* = 7.8 Hz, 1H, H-7), 7.94 (t, *J* = 7.8 Hz, 1H, H-8), 7.81 (t, *J* = 7.8 Hz, 2H, H-9, H-$6_{Ph}$), 7.56 (t, *J* = 7.8 Hz, 1H, H-$5_{Ph}$), 7.42 (d, *J* = 7.8 Hz, 1H, H-$4_{Ph}$), 4.78 (q, *J* = 6.8 Hz, 1H, SC*H*), 1.73 (d, *J* = 6.8 Hz, 3H, SCHC$H_3$); LC-MS *m/z*: 418 $[M + H]^+$; EI-MS *m/z* (I,%): 418 (8.2) $[M+1]^+$, 258 (6.9), 257 (56.7), 232 (4.3), 231 (13.6), 230 (100.0), 229 (54.2), 215 (19.2), 212 (5.0), 203 (3.4), 202 (13.6), 198 (8.4), 197 (69.3), 171 (26.5), 170 (5.0), 161 (5.8), 145 (8.1), 130 (3.0), 129 (27.2), 102 (16.6), 101 (6.6), 75 (5.0), 69 (11.9), 59 (29.9), 55 (8.9). Anal. calcd. for $C_{19}H_{14}F_3N_5OS$: C, 54.67; H, 3.38; N, 16.78; S, 7.68. Found: C, 54.60; H, 3.29; N, 16.80; S, 7.61.

### *Compound* ***6.1***

IR ($cm^{-1}$): 3284, 3195, 3136, 3058, 2983, 2947, 2918, 1661, 1623, 1603, 1539, 1519, 1487, 1476, 1454, 1412, 1394, 1386, 1373, 1353, 1301, 1257, 1191, 1149, 1127, 1103, 1042, 988, 965, 925, 900, 880, 797, 772, 755, 717, 700, 641; $^1$H-NMR (500 MHz) δ: 9.53 (s, 1H, H-5), 8.38 (d, *J* = 7.8 Hz, 1H, H-10), 8.04 (d, *J* = 7.9 Hz, 1H, H-7), 7.93 (t, *J* = 8.0 Hz, 1H, H-8), 7.80 (t, *J* = 8.0 Hz, 1H, H-9), 3.53 (m, 4H, S$CH_2$, H-$2_{morph}$,$6_{morph}$), 3.44 (m, 4H, H-$3_{morph}$,$5_{morph}$), 2.88 (t, *J* = 6.8 Hz, 2H, $SCH_2CH_2$); LC-MS *m/z*: 334 $[M + H]^+$, 336. Anal. calcd. for $C_{19}H_{17}N_5OS$: C, 62.79; H, 4.71; N, 19.27; S, 8.82. Found: C, 62.74; H, 4.65; N, 19.23; S, 8.78.

### *Compound* ***6.2***

IR ($cm^{-1}$): 3059, 2295, 2966, 2860, 1649, 1624, 1513, 1479, 1449, 1392, 1371, 1353, 1299, 1244, 1208, 1186, 1111, 1069, 1028, 984, 959, 913, 897, 852, 817, 781, 764, 715, 640; $^1$H-NMR (500 MHz) δ: 9.88 (br.s, 1H, N*H*), 9.54 (s, 1H, H-5), 8.39 (d, *J* = 7.8 Hz, 1H, H-10), 8.04 (d, *J* = 7.8 Hz, 1H, H-7), 7.93 (t, *J* = 7.8 Hz, 1H, H-8), 7.80 (t, *J* = 7.8 Hz, 1H, H-9), 7.40 (s, 1H, H-$2_{Ph}$), 7.34 (d, *J* = 7.9 Hz, 1H, H-$6_{Ph}$), 7.14 (t, *J* = 7.9 Hz, 1H, H-$5_{Ph}$), 6.83 (d, *J* = 7.8 Hz, 1H, H-$4_{Ph}$), 3.53 (t, *J* = 6.9 Hz, 2H, S$CH_2$), 2.90 (t, *J* = 6.8 Hz, 2H, $SCH_2CH_2$), 2.24 (s, 3H, C$H_3$); LC-MS *m/z*: 344 $[M + H]^+$, 346. Anal. calcd. for $C_{16}H_{17}N_5O_2S$: C, 55.96; H, 4.99; N, 20.39; S, 9.34. Found: C, 55.76; H, 5.09; N, 20.35; S, 9.62.

### *Compound* ***7.1***

IR ($cm^{-1}$): 3303, 3058, 2932, 1637, 1535, 1515, 1479, 1457, 1395, 1333,1266, 1200, 1002, 902, 782, 768, 738, 715, 695, 644; $^1$H-NMR (500 MHz) δ: 9.50 (s, 1H, H-5), 8.37 (d, *J* = 7.9 Hz, 1H, H-10), 8.04 (d, *J* = 7.8 Hz, 1H, H-7), 7.92 (t, *J* = 8.0 Hz, 1H, H-8), 7.79 (t, *J* = 7.8 Hz, 1H, H-9), 7.40 (m, 1H, N*H*), 7.24 (m, 5H, H-$_{Ph}$), 3.32 (m, 2H, S$CH_2$), 2.48 (t, *J* = 3.8 Hz, 2H, $SCH_2CH_2$), 2.03 (q, *J* = 7.5 Hz, 2H, $S(CH_2)_2CH_2$); LC-MS *m/z*: 378 $[M + H]^+$, 380, 381; EI-MS *m/z* (I,%): 378 (2.1), 377 (9.9) $[M]^{+\bullet}$, 270 (4.3), 257 (1.6), 243 (10.1), 231 (5.2), 230 (11.7), 229 (100.0), 216 (12.2), 215 (3.1), 203 (7.1), 202 (6.0), 197 (4.6), 176 (4.5), 171 (3.2), 149 (12), 148 (5.1), 130 (4.0), 129 (13.6), 107 (6.1), 106 (36.1), 102 (93.1), 92 (7.8), 91 (72.9), 85 (4.4), 83 (6.6),

79 (4.9), 77 (5.6), 69 (11.9), 59 (5.4). Anal. calcd. for $C_{20}H_{19}N_5OS$: C, 62.64; H, 5.07; N, 18.55; S, 8.49. Found: C, 62.73; H, 4.85; N, 18.53; S, 8.69.

### *Compound* ***7.2***

IR ($cm^{-1}$): 3323, 3060, 2966, 2918, 1665, 1621, 1599, 1515, 1478, 1442, 1393, 1329, 1265, 1183, 1035, 963, 901, 782, 765, 744, 714, 682, 610; $^1$H-NMR (500 MHz) δ: 9.90 (br.s, 1H, N*H*), 9.45 (s, 1H, H-5), 8.34 (d, *J* = 7.8 Hz, 1H, H-10), 8.02 (d, *J* = 7.8 Hz, 1H, H-7), 7.90 (t, *J* = 7.8 Hz, 1H, H-8), 7.76 (t, *J* = 7.8 Hz, 1H, H-9), 7.75 (d, *J* = 7.8 Hz, 2H, H-$2_{Ph}$,$6_{Ph}$), 7.25 (t, *J* = 7.8 Hz, 2H, H-$3_{Ph}$,$5_{Ph}$), 6.98 (t, *J* = 7.8 Hz, 1H, H-$4_{Ph}$), 3.35 (t, *J* = 6.9 Hz, 2H, SC$H_2$), 2.51 (t, *J* = 7.2 Hz, 2H, $SCH_2CH_2$), 2.09 (q, *J* = 7.2 Hz, 2H, $S(CH_2)_2CH_2$); LC-MS *m/z*: 364 [M + H]$^+$, 366. Anal. calcd. for $C_{19}H_{17}N_5OS$: C, 62.79; H, 4.71; N, 19.27; S, 8.82. Found: C, 62.76; H, 4.61; N, 19.27; S, 8.71.

### *Compound* ***7.3***

IR ($cm^{-1}$): 3295, 3053, 2972, 2934, 1662, 1623, 1604, 1519, 1478, 1454, 1393, 1324, 1287, 1256, 1181, 1104, 903, 769, 745, 717, 966, 692,645; $^1$H-NMR (500 MHz) δ: 9.50 (s, 1H, H-5), 9.31 (br.s, 1H, N*H*), 8.37 (d, *J* = 7.8 Hz, 1H, H-10), 8.04 (d, *J* = 7.8 Hz, 1H, H-7), 7.92 (t, *J* = 7.8 Hz, 1H, H-8), 7.78 (t, *J* = 7.8 Hz, 1H, H-9), 7.36 (d, *J* = 7.6 Hz, 1H, H-$6_{Ph}$), 7.05 (d, *J* = 7.6 Hz, 2H, H-$3_{Ph}$,$5_{Ph}$), 7.17 (d, *J* = 7.6 Hz, 1H, H-$4_{Ph}$), 3.36 (t, *J* = 7.0 Hz, 2H, SC$H_2$), 3.32 (s, 3H, C$H_3$), 2.53 (t, *J* = 7.2 Hz, 2H, $SCH_2CH_2$), 2.11 (m, 2H, $S(CH_2)_2CH_2$); LC-MS *m/z*: 378 [M + H]$^+$, 380. Anal. calcd. for $C_{20}H_{19}N_5OS$: C, 62.64; H, 5.07; N, 18.55; S, 8.49. Found: C, 62.71; H, 4.89; N, 18.56; S, 8.55.

### *Compound* ***7.4***

IR ($cm^{-1}$): 3057, 2973, 2927, 1621, 1721, 1595, 1515, 1477, 1453, 1370, 1240, 1175, 1031, 899, 773, 747, 714, 698, 639; $^1$H-NMR (400 MHz) δ: 9.50 (s, 1H, H-5), 9.12 (br.s, 1H, N*H*), 8.37 (d, *J* = 7.8 Hz, 1H, H-10), 8.04 (d, *J* = 7.8 Hz, 1H, H-7), 7.93 (t, *J* = 7.6 Hz, 2H, H-8, H-$5_{Ph}$), 7.79 (t, *J* = 7.6 Hz, 1H, H-9), 7.02 (m, 2H, H-$3_{Ph}$,$6_{Ph}$), 6.88 (t, *J* = 7.6 Hz, 1H, H-$4_{Ph}$), 3.78 (s, 3H, $OCH_3$), 3.36 (t, *J* = 6.8 Hz, 2H, SC$H_2$), 2.59 (t, *J* = 6.8 Hz, 2H, $SCH_2CH_2$), 2.10 (q, *J* = 6.8 Hz, 2H, $S(CH_2)_2CH_2$); LC-MS *m/z*: 394 [M + H]$^+$. Anal. calcd. for $C_{20}H_{16}N_5O_2S$: C, 61.05; H, 4.87; N, 17.80; S, 8.15. Found: C, 61.12; H, 4.97; N, 17.74; S, 8.25.

### *Compound* ***7.5***

IR ($cm^{-1}$): 3294, 2965, 2931, 2833, 1651, 1624, 1593, 1523, 1478, 1396, 1303, 1261, 1218, 1178, 1140, 1029, 971, 902, 804, 782, 718, 646; $^1$H-NMR (500 MHz) δ: 9.49 (s, 1H, H-5), 9.27 (br.s, 1H, N*H*), 8.36 (d, *J* = 8.0 Hz, 1H, H-10), 8.1 (s, 1H, H-$3_{Ph}$), 8.04 (d, *J* = 8.0 Hz, 1H, H-7), 7.93 (t, *J* = 7.8 Hz, 1H, H-8), 7.79 (t, *J* = 7.8 Hz, 1H, H-9), 7.07 (d, *J* = 8.8 Hz, 1H, H-$3_{Ph}$), 7.00 (d, *J* = 8.8 Hz, 1H, H-$5_{Ph}$), 3.78 (s, 3H, $OCH_3$), 3.35 (t, *J* = 6.8 Hz, 2H, SC$H_2$), 2.61 (t, *J* = 6.8 Hz, 2H, $SCH_2CH_2$), 2.09 (q, *J* = 6.8 Hz, 2H, $S(CH_2)_2CH_2$); LC-MS *m/z*: 428 [M + H]$^+$, 430, 432. Anal. calcd. for $C_{20}H_{18}ClN_5O_2S$: C, 56.14; H, 4.24; N, 16.37; S, 7.49. Found: C, 56.19; H, 4.32; N, 16.43; S, 7.35.

### *Compound* ***7.6***

IR ($cm^{-1}$): 3304, 3057, 1658, 1621, 1588, 1513, 1478, 1454, 1391, 1326, 1264, 1181, 1105, 1070, 1007, 971, 901, 813, 782, 766, 714, 643; $^1$H-NMR (500 MHz) δ: 10.05 (br.s, 1H, N*H*), 9.48 (s, 1H, H-5), 8.36 (d, *J* = 7.8 Hz, 1H, H-10), 8.04 (d, *J* = 7.8 Hz, 1H, H-7), 7.93 (t, *J* = 7.8 Hz, 1H, H-8), 7.78 (t, *J* = 7.8 Hz, 1H, H-9), 7.54 (d, *J* = 8.0 Hz, 2H, H-$2_{Ph}$,$6_{Ph}$), 7.43 (d, *J* = 7.6 Hz, 2H, H-$3_{Ph}$,$5_{Ph}$), 3.36 (t, *J* = 6.8 Hz, 2H, SC$H_2$), 2.50 (m, 2H, $SCH_2CH_2$), 2.11 (q, *J* = 6.8 Hz, 2H, $S(CH_2)_2CH_2$); LC-MS *m/z*: 442 [M + H]$^+$, 444; EI-MS *m/z* (I,%): 443 (10.3), 442 (2.4) [M]$^+$, 441 (9.9), 271 (14.5), 270 (4.5), 243 (4.2), 231 (5.3), 230 (12.2), 229 (100.0), 216 (12.2), 215 (9.7), 204 (5.2), 203 (47.7), 202 (19.2), 197 (8.0), 184 96.8), 173 (55.0), 172 (18.9), 171 (59.0), 170 (18.8), 157 (5.9), 156 (5.0), 146 (5.0), 145 (14.9), 143 (6.4), 130 (9.9), 129 (44.6), 117 (3.9), 107 (6.1), 103 (6.4), 102 (33.2), 92 (13.7), 91 (27.6), 90 (10.8), 88 (5.7). Anal. calcd. for $C_{19}H_{16}BrN_5OS$: C, 51.59; H, 3.65; N, 15.83; S, 7.25. Found: C, 51.65; H, 3.69; N, 15.74; S, 7.30.

### *Compound* ***7.7***

IR ($cm^{-1}$): 3296, 3053, 2909, 2310, 1659, 1602, 1602, 1517, 1478, 1446, 1393, 1329, 1256, 3649, 1112, 960, 902, 783, 783, 769, 715, 694, 661, 643; $^1$H-NMR (500 MHz) δ: 10.26 (br.s, 1H, N*H*), 9.48 (s, 1H, H-5), 8.34 (d, *J* = 8.0 Hz, 1H, H-10), 8.07 (s, 1H, H-$2_{Ph}$), 8.03 (d, *J* = 8.0 Hz, 1H, H-7), 7.91 (t, *J* = 7.8 Hz, 1H, H-8), 7.76 (t, *J* = 7.8 Hz, 1H, H-9), 7.73 (d, *J* = 8.0 Hz, 1H, H-$6_{Ph}$), 7.50 (t, *J* = 8.0 Hz, 1H, H-$5_{Ph}$), 7.35 (d, *J* = 7.8 Hz, 1H, H-$4_{Ph}$), 3.38 (t, *J* = 6.8 Hz, 2H, SC$H_2$), 2.56 (t, *J* = 6.8 Hz, 2H, $SCH_2CH_2$), 2.13 (q, *J* = 6.8 Hz, 2H, $S(CH_2)_2CH_2$); LC-MS *m/z*: 432 [M + H]$^+$, 435. Anal. calcd. for $C_{20}H_{16}F_3N_5OS$: C, 55.68; H, 3.74; N, 16.23; S, 7.43. Found: C, 55.77; H, 3.87; N, 16.27; S, 7.41.

## Biological assays

### *Bioluminescence inhibition test*

The marine luminescent bacteria *Photobacterium leiognathi* strain Sh1, isolated from the Azov Sea Shrimp, were used for the bioluminescence analysis [13]. Bacteria were cultivated on a nutrient environment containing (g/L): pepton – 5, yeast extract – 1.5, meat extract – 1.5, sodium chloride – 30, pH = 7.4. In the acute action test (inhibiting luminescence of bacteria), bacteria were diluted with the 3% sodium chloride solution up to a concentration of $10^5$ cells/mL. 5–50 μg/mL of the studied substances suspended in DMSO were mixed with 1 mL of the diluted bacterial suspension. Vials were incubating for 10 min at 25°C, then, the intensity of bioluminescence was measured in percent (%) relative to the controls, which were prepared without the studied compounds. In the chronic action test (inhibiting growth and luminescence of bacteria), growth medium was added for potential breeding in a ratio of 1 : 50 and the mix was incubated for 16–18 h at 30°C, whereupon the intensity of bioluminescence was measured the same way as in acute action testing. Tetracycline was used as a reference. The bacterial luminescence was measured with a Bioluminometer BLM-8801 («Science», Krasnoyarsk, Russia).

### *Antimicrobial and antifungal test*

The investigation of antimicrobial and antifungal activity of amides **4**–**7** was carried out with the stiff plate agar diffusion method against *Escherichia coli*, *Staphylococcus aureus*, *Mycobacterium luteum*, *Candida tenuis*, and *Aspergillus niger*. The amount of microbial cells was 109 c.f.u./mL. Incubation period of bacteria was 24 h at 35°C, yeast – 48 to 72 h at 28–30°C. Antibiotics vancomicin, oxacillin, nystatin were used as standards. The bacterial cultures, the standards and the obtained substances were streaked across grooves in 5 mg/mL concentration, and then allowed to diffuse in the agar nutrient plate. The antimicrobial effect and degree of activity of the tested compounds were evaluated by measuring of the zone diameters (not sensitive: 11–15 mm; sensitive: 16–25 mm; highly sensitive >25 mm). The results were compared with well-known drug standards vancomicin, nystatin, and oxacillin. All experiments were repeated three times.

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