Current Chemistry Letters 10 (2021) 427–434

Contents lists available at GrowingScience

Current Chemistry Letters

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# Amidoalkylation of heterocyclic amines by N-[5-(alkylsulfanyl)-1,3,4-thiadiazol-2-yl]- 2'-chloroacetamide and antimicrobial activity of derivatives

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**C H R O N I C L E**

*Article history*:
Received February 18, 2021
Received in revised form
April 25, 2021
Accepted May 18, 2021
Available online
May 18, 2021

*Keywords*:
*N-[5-(alkylsulfanyl)-1,3,4-thiadiazol-2-yl]-2-chloroacetamide*
*Pyperidine*
*Morpholine*
*Cytisine*
*Antimicrobial activity*

**A B S T R A C T**

Amidoalkylation of secondary heterocyclic amines by N-[5-(alkylsulfanyl)-1,3,4-thiadiazol-2-yl]-2'-chloroacetamide resulted the new compounds **5-10** that contain 1,3,4-thiadiazole-5-thione moiety alongside pyperidine, morpholine, and cytisine fragments. *In vitro* screening of antimicrobial activity of synthesized compounds showed that N-[5-(amylsulfanyl)-1,3,4-thiadiazol-2-yl]-2'-morpholinacetamide exhibited an appreciable antibacterial activity against gram-negative bacteria of *Escherichia coli* (inhibition zone diameter of 16 mm) and gram-positive bacteria of *Staphylococcus aureus* and *Bacillus subtilis* (10-13 mm).



## 1. Introduction

2-Substituted-1,3,4-thiadiazole-5-thiones have great synthetic potential due to the presence of the thiamide group NH-C=S and/or other functional groups (amino-, hydroxyl-, and etc.). Therefore, they are one of the most widely studied representatives of five-membered heterocycles. The 1,3,4-thiadiazole ring is participated as the main structural component in the molecules of many compounds that exhibit analgesic, anti-inflammatory, antimicrobial, antiviral, antitumor and other types of activity.[1-9] Despite numerous reports in the literature on the synthesis of various derivatives of 2-substituted-1,3,4-thiadiazole-5-thiones,[10-12] there is little data among them on their further chemical transformations. Therefore, our goal was to continue the study of chemical modifications of alkyl derivatives of 2-amino-1,3,4-thiadiazole-5-thione previously synthesized by us.[13]

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doi: 10.5267/j.ccl.2021.5.002

## 2. Results and Discussion

N-[5-(amyl,nonylsulfanyl)-1,3,4-thiadiazol-2-yl]-2'-chloroacetamide (**3**,**4**) were obtained at acylation of 5-amyl(nonyl)sulfanyl-1,3,4-thiadiazol-2-amine (**1,2**) with chloroacetyl chloride (CAC) in good yields (75-82%) according to literature.[14] The formation of chloroacetamide derivatives at the amino group is confirmed by the appearance of characteristic absorption bands in the IR spectra of compounds **3,4** corresponding to the C=O (1701, 1702 $cm^{-1}$) and NH (1579, 3175 and 1580, 3176 $cm^{-1}$) groups, respectively, as well as by the signals of the protons of the Cl-$CH_2$- fragment at 4.38 ppm for **3** and at 4.36 ppm for **4** in the $^1$H NMR spectra. The selection of the most suitable conditions for the interaction of chloroacetamides **3,4** with heterocyclic amines (piperidine, morpholine, succinimide, anabazine, cytisine) was carried out by varying the reaction time, solvent, temperature, and reagent ratios. The reactions were carried out in DMF at 90-95°C, in a solution of chloroform (20, 61°C), acetone (20, 56°C), benzene (20, 80°C) with and without triethylamine as acceptor of HCl. Boiling in dry benzene for 7 h at 2:1 molar ratio of reagents was found to be the most effective condition for amidomethylation of amines with chloroacetamides **3,4** (see **Fig. 1**).

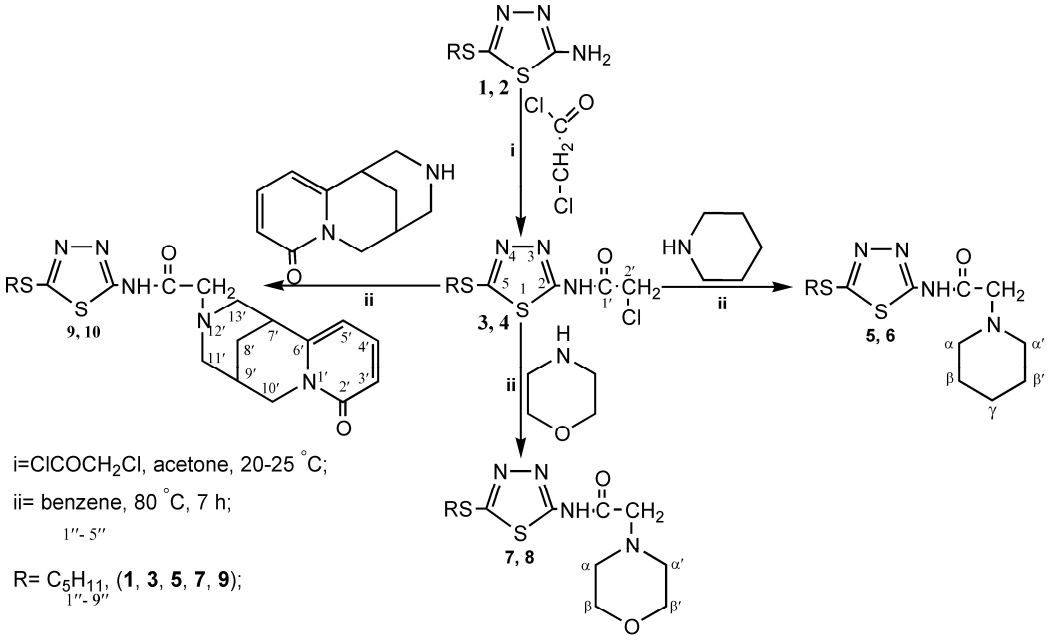


**Fig. 1.** The synthesis of compounds **3**-**10**.

Piperidine, morpholine and cytisine derivatives which are not described in literature were obtained in good yields (76-87%). They are N-[5-(amylsulfanyl)-1,3,4-thiadiazol-2-yl]-2'-piperidinacetamide (**5**), N-[5-(amylsulfanyl)-1,3,4-thiadiazol-2-yl]-2'-morpholinacetamide (**7**), and N-[5-(amylsulfanyl)-1,3,4-thiadiazol-2-yl]-2'-cytisinacetamide (**9**) and N-[5-(nonylsulfanyl)-1,3,4-thiadiazol-2-yl]-2'-piperidinacetamide (**6**), N-[5-(nonylsulfanyl)-1,3,4-thiadiazol-2-yl]-2'-morpholinacetamide (**8**), N-[5-(nonylsulfanyl)-1,3,4-thiadiazol-2-yl]-2'-cytisinacetamide (**10**). It should be noted that the yields of products **5-10** with the same amine in the case of N-[5-(nonylsulfanyl)-1,3,4-thiadiazol-2-yl]-2'-chloroacetamide **4** are comparatively higher (83-87%) than that for N-[5-(amylsulfanyl)-1,3,4-thiadiazol-2-yl]-2'-chloroacetamide **3** (76-84%). Under other conditions of interaction (DMF at 90-95°C, in chloroform solution at 20, 61°C, etc.), the target products could not be obtained, as well as with succinimide and anabazine. In some cases, the reaction mixture was highly resinous or the product yields were trace. The structure of the synthesized compounds was determined and characterized by IR-, $^1$H NMR, and $^{13}$C NMR spectral data. Thus, the characteristic absorption of amide carbonyl (-NH-CO-$CH_2$-) in the IR spectra of compounds **5,7,9** is observed at 1694-1703 $cm^{-1}$, while the absorption

band of the NH-group is appeared at 3154-3167 $cm^{-1}$, and for compounds **6,8,10** these indices were 1690-1700 $cm^{-1}$ and 3133-3157 $cm^{-1}$, respectively.

The IR spectra of N-[5-(amylsulfanyl)-1,3,4-thiadiazol-2-yl]-2'-cytisinacetamide **9** and N-[5-(nonylsulfanyl)-1,3,4-thiadiazol-2-yl]-2'-cytisinacetamide **10** contain absorption bands at 1647-1650 $cm^{-1}$ and 796-798 $cm^{-1}$, corresponding to the α-pyridone ring of the cytisine fragment,[15] which were absent in the spectra of starting compounds **3** and **4**.

All $^1H$ NMR spectra of compounds (**5-10**) contain signals of the proton of the NH group in the range 8.63-10.32 ppm. as a singlet. The protons of the corresponding alkyl (pentyl -, nonyl-) groups resonate in the form of characteristic (s, t, q, etc.), well-resolved signals in the range 0.81-3.19 ppm. The morpholine ring protons of compounds **7** and **8** were resonated at 3.34-3.94 ppm. Thus, the values of protons at carbon atoms α,α′ due to the influence of the nitrogen atom are shifted to the region of weaker fields and appear at 3.88-3.94 ppm, and the signals in a stronger field are 3.34-3.38 ppm belong to protons β,β′ of carbon atoms adjacent to the oxygen atom of the morpholine fragment of the molecule. The signals of the protons of the piperidine fragment of compounds **5** and **6** at the β,β′ atoms can be observed in the range 1.78-1.81 ppm, for α,α′ 3.39-3.41 ppm, while the signals corresponding to the protons in the γ-position are found in area 1.58-1.61 ppm.

Also, in the $^1H$ NMR spectra of compounds **9** and **10**, there are corresponding signals of the protons of the cytisine fragment and the methylene group of the N-$CH_2$-CO- fragment, which is in the form of a nitrogen-binding bridge of N-12′ cytisine. The two-proton singlet signal of this group of compounds **3** and **4**, due to the influence of neighboring N- and CO-groups, appears as a quartet and shifted from 4.36-4.38 ppm in a stronger field at 3.25-3.27 ppm. The signals of the remaining protons of the cytisine fragment of compounds **9** and **10** were manifested in the usual position and in the form characteristic of cytisine. Obtaining the target products is also confirmed by the data of $^{13}C$ NMR spectra. Thus, the signal corresponding to the carbon atom C-2' (Cl-$CH_2$-) in compounds **3** and **4** with 42.27-42.30 ppm was significantly shifted to a weak field at 57.93-63.23 ppm after the replacement of the chlorine atom by the amine fragment (RR′N-$CH_2$-) in compounds (**5-10**). It should be noted that as a result of the reactions of N-[5-(amyl, nonylsulfanyl)-1,3,4-thiadiazol-2-yl]-2'-chloroacetamide **3,4** with amines, the possible transamidation with the formation of *N*-substituted chloroacetamides of the corresponding secondary amines and the initial alkyl derivatives **1** and **2** does not observed.

**Table 1.** The antibacterial effect evaluated by diameter of inhibition zone (mm) for compounds 3-10 using agar disk diffusion assay

| Compounds | Diameters of inhibition (mm, ± SD, P≤0.05) | | | | |
|---|---|---|---|---|---|
| | Gram-positive bacteria | | Gram-negative bacteria | | Fungi |
| | *S. aureus* | *B. subtilis* | *E. coli* | *P. aeruginosa* | *C. albicans* |
| **3** | na | na* | na | na | na |
| **4** | na | na | na | na | na |
| **5** | na | na | na | na | na |
| **6** | na | na | na | na | na |
| **7** | 10.08±0.12 | 13.04±0.10 | 16.12±0.13 | 8.08±0.12 | na |
| **8** | na | na | na | na | na |
| **9** | na | na | na | na | na |
| **10** | na | na | na | na | na |
| **Ampicillin (10 µg/disc)** | 27.08±0.12 | 28.04±0.10 | nt | nt | nt |
| **Ceftriaxone (30 µg/disc)** | nt | nt* | 28.12±0.13 | 26.08±0.12 | nt |
| **Flucanazole (25 µg/disc)** | nt | nt | nt | nt | 30.04±0.10 |

*na - not active; *nt – not tested

There are reports in the literature on such cases of transamidation of heterocyclic systems, in particular, on the example of benzothiazolin-2-thiones and benzothiazolin-2-ones.[16] All synthesized compounds - N-[5-(amyl, nonylsulfanyl)-1,3,4-thiadiazol-2-yl]-2'-piperidin, morpholine, cytisine acetamide (**5-10**) and the starting compounds N-[5-(amyl- and nonylsulfanyl)-1,3,4-thiadiazol-2-yl]-2'-chloroacetamide **3,4** were tested for antibacterial and antifungal activity by a modified disk diffusion method on agar.[17,18] The test results (see **Table 1**) showed that only N-[5-(amylsulfanyl)-1,3,4-thiadiazol-2-yl]-2'-morpholinacetamide (**7**) exhibits an antibacterial effect against all tested gram-positive and gram-negative bacteria strains. *Escherichia coli* is most susceptible to the effect of compound **7** displaying inhibition zone diameter of 16.12±0.13 mm.

It should be noted that a similar compound with a longer alkyl substituent, N-[5-(nonylsulfanyl)-1,3,4-thiadiazol-2-yl]-2'-morpholinacetamide (**8**), is not active against all tested strains of bacteria. It was also found that synthesized compounds exhibit no antifungal activity against *Candida albicans*.

## 3. Conclusions

Thus, acylation with chloroacetyl chloride (CAC) at the amino group of 5-amyl(nonyl)sulfanyl-1,3,4-thiadiazol-2-amine **1,2** gave N-[5-(amyl, nonylsulfanyl)-1,3,4-thiadiazol-2-yl]-2'-chloroacetamide **3,4**. Nucleophilic substitution of the mobile chlorine atom of compounds **3,4** under the action of amines leads to the synthesis of new derivatives N-[5-(amylsulfanyl)-1,3,4-thiadiazol-2-yl]-2'-piperidine, morpholine, cytisine acetamide **5,7,9** and N-[5-(nonylsulfanyl)-1,3,4-thiadiazol-2-yl]-2'-piperidine, morpholine, cytisine acetamide **6,8,10**. At the same time, the yields of target products **6,8,10** are comparatively higher for chloroacetylamidothiadiazole with a longer alkyl chain N-[5-(nonylsulfanyl)-1,3,4-thiadiazol-2-yl]-2'-chloroacetamide (**4**). The antibacterial and antifungal properties of compounds **3-10** were evaluated against test strains of gram-positive, gram-negative bacteria and fungi. The N-[5-(amylsulfanyl)-1,3,4-thiadiazol-2-yl]-2'-morpholinacetamide (**7**) showed appreciable antibacterial activity against *Escherichia coli.*

## 4. Experimental

### *4.1. Instrumentation*

$^{1}H$ and $^{13}C$ NMR spectra were recorded on a Unity 400 spectrometer at working frequencies 400 and 100 MHz, respectively, in $CDCl_3$, $CD_3COOD$ with HMDS internal standard. IR spectra of the synthesized compounds were recorded on a Perkin Elmer-2000 Fourier spectrometer. The reactions were monitored by TLC on Silicagel 60F254 (Merck) plates using 24:1 $CHCl_3$-$C_2H_5OH$ (1), 5:1 $C_6H_6$-$CH_3OH$ (2) solvent system, developed plates were visualized under UV lamp and iodine tank. "BOETIUS" apparatus is used for determination of the melting points of compounds.

### *4.2. General procedure*

Compounds **3** and **4** were synthesized as before.[14]

#### *4.2.1. Reaction of N-[5-(alkylsulfanyl)-1,3,4-thiadiazol-2-yl]-2-chloroacetamide (3,4) with heterocyclic amines.*

A solution of the corresponding amine (0.02 mol) in benzene was dropped at room temperature into a benzene solution of compound **3** or **4** (0.01 mol). Then the reaction mixture was boiled under reflux for 7 hours. Then benzene was distilled off, the residue was washed sequentially with water, 2% NaOH solution, and again with water. The obtained products (**5-8**) in the form of white or white-milk powders were dried at room temperature, which did not require additional purification according to

TLC monitoring. Compounds **9, 10** were purified by column chromatography (KSK silica gel 100-160 μm, Reakhim, Russia, chloroform, chloroform-ethanol 50 : 1 as eluent systems).

*4.3 Physical and Spectral Data*

*4.3.1. N-[5-(amylsulfanyl)-1,3,4-thiadiazol-2-yl]-2'-chloroacetamide (3).* Yield 82%, white powder, m.p. 152-154°C, $R_f$=0.42 (1). $^1$H NMR (400 MHz, $CDCl_3$, ppm) δ: 0.85 (3H, t, J=7.2 Hz, C$\underline{H}_3$), 1.35 (4H, m, $CH_3$-C$\underline{H}_2$C$\underline{H}_2$-), 1.74 (2H, quintet, J=7.1 Hz, -C$\underline{H}_2$-), 3.19 (2H, t, J=7.3 Hz, S-C$\underline{H}_2$-), 4.38 (2H, s, C$\underline{H}_2$-CO-), 12.91 (1H, br. s., NH). $^{13}$C NMR (100 MHz, $CDCl_3$, ppm) δ: 14.14 (C-13"), 22.39 (C-12"), 29.26 (C-10"), 31.04 (C-11"), 34.49 (C-9"), 42.30 (C-2'), 159.82 (C-5), 162.20 (C-2), 165.14 (C-7'). IR, ν, cm$^{-1}$: 1579, 3175 (NH), 1702 (C=O).

*4.3.2. N-[5-(nonylsulfanyl)-1,3,4-thiadiazol-2-yl]-2'-chloroacetamide (4).* Yield 75%, white powder, m.p. 140-142°C, $R_f$=0.53 (1). $^1$H NMR (400 MHz, $CDCl_3$, ppm) δ: 0.80 (3H, t, J=5.9 Hz, C$\underline{H}_3$), 1.25 (12H, m, $CH_3$-(C$\underline{H}_2$-)$_6$), 1.73 (2H, quintet, J=7.1 Hz, -C$\underline{H}_2$-), 3.18 (2H, t, J=7.1 Hz, S-C$\underline{H}_2$-), 4.36 (2H, s, C$\underline{H}_2$-CO-), 12.62 (1H, br.s., NH). $^{13}$C NMR (100 MHz, $CDCl_3$, ppm) δ: 14.27 (C-17"), 22.85 (C-16"), 28.94 (C-11"), 29.31 (C-10"), 29.42 (C-12"), 29.64 (C-13",14"), 32.05 (C-15"), 34.65 (C-9"), 42.27 (C-2'), 159.74 (C-2), 162.23 (C-2), 165.06 (C-1'). IR, ν, cm$^{-1}$: 1580, 3176 (NH), 1701(C=O).

*4.3.3. N-[5-(amylsulfanyl)-1,3,4-thiadiazol-2-yl]-2'-piperidinacetamide (5).* Yield 84%, white-milk powder, m.p. 77-79 °C, $R_f$=0.54 (2). $^1$H NMR (400 MHz, $CD_3COOD$, ppm) δ: 0.83 (3H, t, J=7.2 Hz, C$\underline{H}_3$), 1.31 (4H, m, $CH_3$-C$\underline{H}_2$C$\underline{H}_2$-), 1.61 (2H, br.s., γ-$CH_2$-pyperidine), 1.69 (2H, quintet, J=7.2 Hz, -C$\underline{H}_2$-), 1.87 (4H, br.s., β,β′-$CH_2$- pyperidine), 3.18 (2H, t, J=7.4 Hz, S-C$\underline{H}_2$-), 3.39 (4H, br.s., α,α′-$CH_2$-pyperidine), 4.26 (2H, br.s, N-C$\underline{H}_2$-), 8.79 (1H, br.s., NH). $^{13}$C NMR (100 MHz, $CD_3COOD$, ppm) δ: 14.19 (C-13"), 22.17 (C-12"), 22.93 (γ-pyperidine), 23.61(β,β′-pyperidine), 29.75 (C-10"), 31.55 (C-11"), 34.94 (C-9"), 55.32 (α,α′- pyperidine), 57.93 (C-2'), 163.49 (C-5), 164.66 (C-2), 172.52 (C-1'). IR, ν, cm$^{-1}$: 1574, 3167 (NH), 1702 (C=O).

*4.3.4. N-[5-(nonylsulfanyl)-1,3,4-thiadiazol-2-yl]-2'-piperidinacetamide (6).* Yield 87%, white-milk powder, m.p. 94-96°C, $R_f$=0.62 (2). $^1$H NMR (400 MHz, $CD_3COOD$, ppm) δ: 0.81 (3H, t, J=6.5 Hz, C$\underline{H}_3$), 1.22 (10H, m, $CH_3$-(C$\underline{H}_2$-)$_5$), 1.38 (2H, quintet, J=5.7 Hz, -C$\underline{H}_2$-), 1.61 (2H, br.s., γ-$CH_2$-pyperidine), 1.69 (2H, quintet, J=7.1 Hz, -C$\underline{H}_2$-), 1.88 (4H, br.s., β,β′-$CH_2$-pyperidine), 3.19 (2H, t, J=7.3 Hz, S-C$\underline{H}_2$-), 3.42 (4H, br.s., α,α′-$CH_2$-pyperidine), 4.31 (2H, br.s., N-C$\underline{H}_2$-), 8.81 (1H, br.s., NH). $^{13}$C NMR (100 MHz, $CD_3COOD$, ppm) δ: 14.39 (C-17"), 22.22 (C-16"), 23.48 (γ-pyperidine), 23.59 (β,β′-pyperidine), 29.44 (C-11"), 29.95 (C-10"), 30.05 (C-12"), 30.15 (C-14"), 30.33 (C-13"), 32.75 (C-15"), 34.91 (C-9"), 55.25 (α,α′-pyperidine), 57.88 (C-2'), 163.42 (C-5), 164.63 (C-2), 172.63 (C-1'). IR, ν, cm$^{-1}$: 1563, 3154 (NH), 1690 (C=O).

*4.3.5. N-[5-(amylsulfanyl)-1,3,4-thiadiazol-2-yl]-2'-morpholinacetamide (7).* Yield 76%, white-milk powder, m.p. 100-102°C, Rf=0.58 (2). $^1$H NMR (400 MHz, $CD_3COOD$, ppm) δ: 0.83 (3H, t, J=7.2 Hz, C$\underline{H}_3$), 1.33 (4H, m, $CH_3$-C$\underline{H}_2$C$\underline{H}_2$-), 1.69 (2H, quintet, J=7.2 Hz, -C$\underline{H}_2$-), 3.18 (2H, t, J=7.2 Hz, S-C$\underline{H}_2$-), 3.34 (4H, br.s., α,α$^1$-$CH_2$-morpholine), 3.94 (4H, br.s., β,β′-$CH_2$-morpholine), 4.14 (2H, br.s., N-C$\underline{H}_2$-), 8.67 (1H, br.s., NH). $^{13}$C NMR (100 MHz, $CD_3COOD$, ppm) δ: 18.60 (C-13"), 27.40 (C-12"), 34.26 (C-10"), 36.08 (C-11"), 39.47 (C-9"), 58.36 (α,α′-morpholine), 63.23 (C-2'), 69.79 (β,β′-morpholine), 165.59 (C-5), 168.67 (C-2), 170.87 (C-1'). IR, ν, cm$^{-1}$: 1574, 3160 (NH), 1703(C=O).

*4.3.6. N-[5-(nonylsulfanyl)-1,3,4-thiadiazol-2-yl]-2'-morpholinacetamide (8).* Yield 83%, white-milk powder, m.p. 105-107°C, $R_f$=0.59 (2). $^1$H NMR (400 MHz, $CD_3COOD$, ppm) δ: 0.82 (3H, t, J=6.3 Hz, C$\underline{H}_3$), 1.23 (10H, m, -(C$\underline{H}_2$-)$_5$), 1.37 (2H, quintet, J=7.1 Hz, -C$\underline{H}_2$-), 1.68 (2H, quintet, J=7.1 Hz, -C$\underline{H}_2$-), 3.18 (2H, t, J=7.3 Hz, S-C$\underline{H}_2$-), 3.33 (4H, br.s., α,α′-$CH_2$-morpholine), 3.93 (4H, br.s., β,β′-$CH_2$-morpholine), 4.16 (2H, br.s., N-C$\underline{H}_2$-(7)), 8.63 (1H, br.s., NH). $^{13}$C NMR (100 MHz, $CD_3COOD$, ppm)

δ: 14.40 (C-17"), 23.49 (C-16"), 29.45 (C-11"), 29.96 (C-10"), 30.07 (C-12"), 30.17 (C-14"), 30.35 (C-13"), 32.77 (C-15"), 34.92 (C-9"), 53.64 (α,α′-morpholine), 58.51 (C-2'), 65.08 (β,β′-morpholine), 160.33 (C-5), 163.36 (C-2), 172.30 (C-1'). IR, ν, $cm^{-1}$: 1563, 3157 (NH), 1700 (C=O).

*4.3.7. N-[5-(amylsulfanyl)-1,3,4-thiadiazol-2-yl]-2'-cytisinacetamide (9).* Yield 81%, light-brown oil, $R_f$=0.52 (2). $^1$H NMR (400 MHz, $CDCl_3$, ppm) δ: 0.83 (3H, t, J=7.2 Hz, C$\underline{H}_3$), 1.29 (4H, m, $CH_3$-C$\underline{H}_2$C$\underline{H}_2$-), 1.66 (2H, quintet, J=7.1 Hz,-C$\underline{H}_2$-), 1.79, 1.89 (2×1H, dd, J=12.8, 12.9 Hz, H-8 cytisine), 2.48 (1H, br.s., H-9 cytisine), 2.56 (1H, d, J=10.6 Hz, H-13$_a$ cytisine), 2.58 (1H, d, J=10.9 Hz, H-11$_a$ cytisine), 2.91 (1H, d, J=11.7 Hz, H-13$_e$ cytisine), 2.97 (1H, br.s., H-7 cytisine), 3.07 (1H, d, J=11.1 Hz, H-11$_e$ cytisine), 3.12 (2H, t, J=7.4 Hz, S-C$\underline{H}_2$-), 3.25 (2H, quartet, J=16.3 Hz, N-C$\underline{H}_2$-), 3.89 (1H, dd, J=6.6, 6.7 Hz, H-10$_a$ cytisine), 4.21 (1H, d, J=15.5 Hz, H-10$_e$ cytisine), 5.58 (1H, d, J=6.9 Hz, H-5 cytisine), 6.48 (1H, d, J=9.1 Hz, H-3 cytisine), 7.25 (1H, dd, J=6.8, 6.7 Hz, H-4 cytisine), 10.32 (1H, s, NH). $^{13}$C NMR (100 MHz, $CDCl_3$, ppm) δ: 14.03 (C-13"), 22.26 (C-12"), 25.50 (C-8'), 28.08 (C-9'), 29.02 (C-10"), 30.88 (C-11"), 34.26 (C-9"), 35.27 (C-7'), 49.95 (C-10'), 60.17(C-13'), 60.72 (C-11'), 60.79 (C-2'), 105.06 (C-5′), 117.57 (C-3'), 139.21 (C-4'), 149.94 (C-6'), 157.57 (C-5), 160.85 (C-5), 163.67(C-2'), 168.18 (C-1'). IR, ν, $cm^{-1}$: 1547, 3134 (NH), 1694 (C=O), 1650 и 798 (α-pyridone ring of cytisine).

*4.3.8. N-[5-(nonylsulfanyl)-1,3,4-thiadiazol-2-yl]-2'-cytisinacetamide (10).* Yield 87%, light-brown oil, $R_f$=0.56 (2). $^1$H NMR (400 MHz, $CDCl_3$, ppm) δ: 0.81 (3H, t, J=6.7 Hz, C$\underline{H}_3$), 1.20 (10H, m, $CH_3$-(C$\underline{H}_2$-)$_5$), 1.35 (2H, quintet, J=6.6 Hz, -C$\underline{H}_2$-), 1.66 (2H, quintet, J=7.2 Hz, -C$\underline{H}_2$), 1.78, 1.91 (2x1H, dd, J=12.9, 12.2 Hz, H-8 cytisine), 2.51 (1H, br.s, H-9 cytisine), 2.59 (1H, d, J=10.6 Hz, H-13$_a$ cytisine), 2.58 (1H, д, J=10.9 Hz, H-11$_a$ cytisine), 2.93 (1H, d, J=10.7 Hz, H-13$_e$ cytisine), 2.98 (1H, br.s., H-7 cytisine), 3.07 (1H, d, H-11$_e$ cytisine), 3.13 (2H, t, J=7.4 Hz, S-C$\underline{H}_2$-), 3.27 (2H, quartet, J=17.3 Hz, N-C$\underline{H}_2$-), 3.89 (1H, dd, J=6.5, 6.6 Hz, H-10$_a$ cytisine), 4.24 (1H, d, J=15.3 Hz, H-10$_e$ cytisine), 5.94 (1H, d, J=7.2 Hz, H-5 cytisine), 6.46 (1H, d, J=9.1 Hz, H-3 cytisine), 7.26 (1H, dd, J=6.8, 6.9 Hz, H-4 cytisine), 10.28 (1H, s, NH). $^{13}$C NMR (100 MHz, $CDCl_3$, ppm) δ: 14.41 (C-17"), 22.94 (C-16"), 25.59 (C-8'), 28.23 (C-9'), 28.96 (C-11"), 29.37 (C-10"), 29.51 (C-12"), 29.71(C-13",14"), 32.12 (C-15"), 34.50 (C-9"), 35.33 (C-7'), 50.11 (C-10'), 60.29 (C-13'), 60.81 (C-2',11'), 105.49 (C-5'), 117.85 (C-3'), 139.52 (C-4'), 149.82 (C-6'), 157.62 (C-2), 161.17 (C-5), 163.86 (C-2'cytisine), 168.07 (C-1'). IR, ν, $cm^{-1}$: 1547, 3133 (NH), 1693 (C=O), 1647 and 796 (α-pyridone ring of cytisine).

*4.4. Antibacterial and antifungal activity*

The antimicrobial activity of synthesized compounds evaluated against following strains of microorganisms by modified agar disk diffusion method[17,18]: *Staphylococcus aureus* (ATCC - 25923), *Bacillus subtilis* (RKMUz - 5), *Escherichia coli* (RKMUz - 221), *Pseudomonas aeruginosa* (ATCC 27879) and *Candida albicans* (RKMUz - 247). The RKMUz bacteria and fungi strains were obtained from the microorganism cultures collection of the Institute of Microbiology, Academy of Sciences of the Republic of Uzbekistan. Compounds were dissolved in chloroform and applied on sterile paper discs (200 μg/per disc), then allowed to evaporate and were deposited on the surface of the inoculated agar plates. Ampicillin, ceftriaxone and fluconazole were used as positive controls and the chloroform as negative control. The average value of inhibition zone was calculated for the three replicates in independent assays.

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