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**Research Article**

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# Selective s-alkoxycarbonylation of isomeric 5-pyridyl-1,3,4-oxadiazoles and antimicrobial activity of the synthesized compounds

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**Abstract** Selective S-alkoxycarbonylation of isomeric - 5-(2-pyridyl)- (**1**), 5-(3-pyridyl)- (**2**), and 5-(4-pyridyl)-1,3,4-oxadiazol-2-thiones (**3**) have been studied and novel series of S-substituted derivatives (**7**-**15**) have been synthesized and characterized by physical methods of research. No formation of the amides - N-substituted derivatives (**A**) was observed. The antimicrobial tests indicated that the 2-alkyloxycarbonylthio-oxadiazole derivatives exhibited a remarkable activity against Gram-positive bacteria *Bacillus subtilis, Staphylococcus aureus* and the fungus *Candida albicans*.

**Keywords** isomeric 5-pyridyl-1,3,4-oxadiazol-2-thiones, selective S-alkoxycarbonylation, alkyl chloroformates, antibacterial and antifungal activity.

## Introduction

Among the five-membered heterocycles, containing three heteroatom such as nitrogen, oxygen and sulfur are interesting classes of heterocyclic compounds. Besides, due to a practical application of these heterocyclic compounds are of theoretical interest as models to investigate of relationship "structure – reactivity - biological activity", and etc. In this regard the 5-substituted-1,3,4-oxadiazol-2-thiones have a special attention of researchers. Many derivatives of these compounds have a wide spectrum of biological activity, such as pesticide, antibiotic, antitumor, hypotensive, antidepressant, analgesic, anti-inflammatory, and etc [1-6]. Due to presence of the ambifuncional thioamid groups (NH-C=S) in the molecules of oxadiazol-thiones, and depending on the nature of the electrophilic and nucleophilic agents and reaction conditions are possible formation of isomers - derivatives of exocyclic sulfur atoms and endocyclic nitrogen atoms or simultaneously on both reactionary centers.

Interesting representative of this class substances are 5-(2-pyridyl)- (**1**), 5-(3-pyridyl)- (**2**), and 5-(4-pyridyl)-1,3,4-oxadiazol-2-thiones (**3**), consisting of two heterocyclic fragments - pyridine and 1,3,4-oxadiazol-2-thione cycles. The combination of these in one molecule could lead to the synthesis of substances with different biological activities. Analysis of the literatures related to isomeric 5-pyridyl-1,3,4-oxadiazol-2-tiones showed that they are not widely studied. There are available literatures [7-9] related only to isomeric pyridyl thiones - 5-(4-pyridyl)-1,3,4-oxadiazol-2-thione and the information about other two isomers is very few.

On the basis of the above mentioned we have studied the effect of the location of the oxadiazole cycle in relation to the nitrogen atom of the pyridine cycle (positions 2,3,4) on the product's yield and the direction of interaction of 5-(2-pyridyl)- (**1**), 5-(3-pyridyl)- (**2**), and 5-(4-pyridyl)-1,3,4-oxadiazol-2-thiones (**3**) with alkyl chloroformates, as well as examine the antimicrobial activity of synthesized compounds.

## Materials and Methods

### Materials

All chemicals used were analytical grade, and used as purchased without further purification.

### Physical Measurements

UV spectra were recorded on a spectrometer EPS-3T Hitachi in ethanol. $^1$HNMR spectra were recorded in $CDCl_3$ on Varian 400-MR spectrometer operating accordingly at 400 MHz. Hexamethyldisiloxane (HMDSO) was used as internal standard, chemical shift of $^1$H was recorded in ppm. IR spectra were recorded on IR Fury System 2000 (Perkin-Elmer) as KBr pellets.

Mps were measured on a Boethius and MEL-TEMP apparatus manufactured by Barnstead International (USA) and were uncorrected.

The reactionary process was monitored by TLC on ALUGRAM Xtra (Germany) precoated aluminum plates using $CHCl_3$/EtOH (10:1) solvent system and developed plates were visualized under UV lamp and/or iodine tank where necessary. Solvents were purified by standard procedures.

### The antimicrobial Assay

The synthetic compounds were tested for antimicrobial activity by the agar disc diffusion method [10-11]. The antimicrobial activity was evaluated using five species of microorganism: Gram-positive bacteria *Bacillus subtilis* (RKMUz - 5) and *Staphylococcus aureus* (ATCC 25923); Gram-negative bacteria *Escherichia coli* (RKMUz - 221) and *Pseudomonas aeruginosa* (ATCC 27879); the fungus *Candida albicans* (RKMUz - 247). The RKMUz microorganism cultures were obtained from the strain collection of the Institute of Microbiology, Academy of Sciences of the Republic of Uzbekistan. Sterile nutrient agar (25g agar/l distilled water) was inoculated with bacterial cells (200 µl of bacterial cell in 2 ml 0.9% NaCl suspension and 20 ml medium) and poured into Petri dishes to give a solid medium. *Candida albicans* ($1\times10^6$ colony forming units per ml) was inoculated into sterile Mueller-Hinton-agar. Forty microliters of test material (0.2 mg/per disc of the compounds) was applied on sterile paper discs (Whatman No.1, 6 mm diameter). Ampicillin, ceftriaxone and nystatin (20 µg/disc) were used as positive controls and the solvents as negative controls. The solvents were allowed to evaporate in a stream of air. The discs were deposited on the surface of inoculated agar plates. Plates were kept for 3 h in refrigerator to enable the diffusion of the substances into the agar. Plates with bacteria were incubated for 24 h at 37°C and plates with yeasts for 48 h at 26°C. The inhibition zone (including the disc diameter) was measured and recorded after the incubation time. An average zone of inhibition was calculated for the three replicates in independent assays.

### Synthesis Method

#### Synthesis of 2-alkiloxycarbonylthio-5-(2,3,4-pyridyl)-1,3,4-oxadiazoles (7-15)

The reaction is conducted by refluxing of equimolar amounts of 5-(2-pyridyl)- (**1**), 5-(3-pyridyl)- (**2**), and 5-(4-pyridyl)-1,3,4-oxadiazol-2-thiones (**3**), alkyl chloroformates **4-6** and $K_2CO_3$ in dry acetone for 3-5 hours. Reactions were monitored by TLC (chloroform-ethanol, 10:1). After removing of acetone, solid residue was washed with water, alkali solution and water again. The resulting product was dried in the air and recrystallized from hexane or cyclohexane.

### The characterization data of the 2-alkoxycarbonylthio-5-(2-,3-,4-pyridyl)-1,3,4-oxadiazoles (7-15).

#### 2-Propoxycarbonylthio-5-(2-pyridyl)-1,3,4-oxadiazole (7)

Yield: 88%, pale brown color powder, mp 111-112°C (hexane). UV-spectra ($\lambda_{max}$): 279 nm. $^1$H NMR ($CDCl_3$, δ, ppm): 8.75 (1H, m, CH-6, pyridyl), 8.22 (1H, dt, CH-3, pyridyl), 8.01 (1H, td, CH-4, pyridyl), 7.83 (1H, m, CH-5, pyridyl), 4.32 (2H, t, $OC\underline{H}_2$), 1.64 (2H, m, $-OCH_2C\underline{H}_2$), 0.98 (3H, t, $CH_3$). IR ($\nu_{max}/cm^{-1}$): 1765 (C=O), 1600 (C=N, oxadiazole), 1210 (C-O-O, oxadiazole).

#### 2-Propoxycarbonylthio-5-(3-pyridyl)-1,3,4-oxadiazole (8)

Yield: 86%, pale yellow powder, mp 96-97°C (hexane). UV-spectra ($\lambda_{max}$): 286 nm. $^1$H NMR ($CDCl_3$, δ, ppm): 9.25 (1H, br.s , CH-2, pyridyl,), 8.78 (1H, br.s, CH-6, pyridyl), 8.22 (1H, d, CH-4, pyridyl), 7.42 (1H, dt, CH-5, pyridyl),

4.41 (2H, t, O-C$\underline{H}_2$), 1.82 (2H, m, $OCH_2C\underline{H}_2$), 1.01 (3H, t, $CH_3$). IR ($\nu_{max}$/$cm^{-1}$): 1771 (C=O), 1633 (C=N, oxadiazole), 1220 (C-O-O, oxadiazole).

**2-Propoxycarbonylthio-5-(4-pyridyl)-1,3,4-oxadiazole (9)**

Yield: 88%, pale brown color powder, mp 113-114°C (hexane). UV-spectra ($\lambda_{max}$): 282 nm. $^1$H NMR ($CDCl_3$, δ, ppm): 8.75 (2H, d, CH-2,6 pyridyl), 7.81 (2H, dd, CH-3,5 pyridyl), 4.22 (2H, t, OC$\underline{H}_2$), 1.78 (2H, m, $OCH_2C\underline{H}_2$), 1.01 (3H, t, $CH_3$). IR ($\nu_{max}$/$cm^{-1}$): 1770 (C=O), 1630 (C=N, oxadiazole), 1190 (C-O-O, oxadiazole).

**2-Isobutoxycarbonylthio-5-(2-pyridyl)-1,3,4-oxadiazole (10).**

Yield: 86%, white color powder, mp 122-123°C (hexane). UV-spectra ($\lambda_{max}$): 284 nm. $^1$H NMR ($CDCl_3$, δ, ppm): 8.75 (1H, m, CH-6, pyridyl), 8.02 (1H, dt, CH-3, pyridyl), 7.84 (1H, t.d, CH-4, pyridyl), 7.45 (1H, m, CH-5, pyridyl), 4.22 (2H, d, OC$\underline{H}_2$), 2.11 (1H, m, $CH_2C\underline{H}$), 0.99 (6H, d, $CH(C\underline{H}_3)_2$). IR ($\nu_{max}$/$cm^{-1}$): 1768 (C=O), 1610 (C=N, oxadiazole), 1200 (C-O-O, oxadiazole).

**2-Isobutoxycarbonylthio-5-(3-pyridyl)-1,3,4-oxadiazole (11)**

Yield: 84%, white color powder, mp 48-49°C (hexane). UV-spectra ($\lambda_{max}$): 285 nm.$^1$H NMR ($CDCl_3$, δ, ppm): 9.20 (1H, d, CH-2, pyridyl), 8.78 (1H, dd, CH-6, pyridyl), 8.21 (1H, ddd, CH-4, pyridyl), 7.41 (1H, dd, CH-5, pyridyl), 4.23 (2H, d, OC$\underline{H}_2$), 2.10 (1H, m, $CH_2C\underline{H}$), 1.01(6H, d, $CH(C\underline{H}_3)_2$). IR ($\nu_{max}$/$cm^{-1}$): 1770 (C=O), 1626 (C=N, oxadiazole), 1210 (C-O-O, oxadiazole).

**2-Isobutoxycarbonylthio-5-(4-pyridyl)-1,3,4-oxadiazole (12)**

Yield: 85%, yellow color powder, mp 99-100°C (hexane). UV-spectra ($\lambda_{max}$): 283 nm. $^1$H NMR ($CDCl_3$, δ, ppm): 8.83 (2H, d, CH-2,6 pyridyl), 7.86 (2H, d, CH-3,5 pyridyl), 3.15 (2H, d, OC$\underline{H}_2$), 1.60 (1H, m, $CH_2C\underline{H}$), 0.82 (6H, d, $CH(C\underline{H}_3)_2$). IR ($\nu_{max}$/$cm^{-1}$): 1772 (C=O), 1632 (C=N, oxadiazole), 1205 (C-O-O, oxadiazole).

**2-Benzoxycarbonylthio-5-(2-pyridyl)-1,3,4-oxadiazole (13)**

Yield: 90%, white color powder, mp 98-99°C (cyclohexane). UV-spectra ($\lambda_{max}$): 289 nm. $^1$H NMR ($CDCl_3$, δ, ppm): 8.69 (1H, m, CH-6, pyridyl), 8.13(1H, dt, CH-3, pyridyl), 7.80 (1H, td, CH-4, pyridyl), 7.42-7.24 (1H, m, CH-5, pyridyl; 5H, m, Ar), 4.50 (2H, s, OC$\underline{H}_2$Ar). IR ($\nu_{max}$/$cm^{-1}$): 1776 (C=O), 1615 (C=N, oxadiazole), 1182 (C-O-O, oxadiazole).

**2-Benzoxycarbonylthio-5-(3-pyridyl)-1,3,4-oxadiazole (14)**

Yield: 86%, pale brown color powder, mp 76-77°C (hexane). UV-spectra ($\lambda_{max}$): 281 nm. $^1$H NMR ($CDCl_3$, δ, ppm): 9.16 (1H, d, CH-2, pyridyl), 8.70 (1H, dd, CH-6, pyridyl), 8.22 (1H, dt, CH-4, pyridyl), 7.43-7.25 (1H, m, CH-5, pyridyl; 5H, m, Ar), 4.49 (2H, s, OC$\underline{H}_2$Ar). IR ($\nu_{max}$/$cm^{-1}$): 1768 (C=O), 1610 (C=N, oxadiazole), 1195 (C-O-O, oxadiazole).

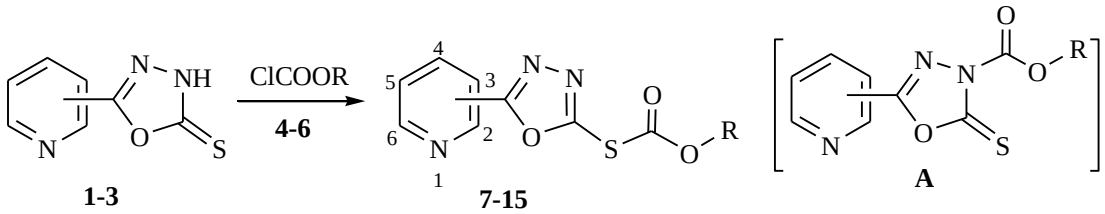
**2-Benzoxycarbonylthio-5-(4-pyridyl)-1,3,4-oxadiazole (15)**

Yield: 89%, white color powder, mp 115-116°C (hexane). UV-spectra ($\lambda_{max}$): 284 nm. $^1$H NMR ($CDCl_3$, δ, ppm): 8.78 (2H, d, CH-2,6, pyridyl), 7.79 (2H, dd, CH-3,5, pyridyl), 7.36 (5H, m, ArH), 4.49 (2H, s, OC$\underline{H}_2$Ar). IR ($\nu_{max}$/$cm^{-1}$): 1755 (C=O), 1610 (C=N, oxadiazole), 1214 (C-O-O, oxadiazole).

**Results and Discussion**

Interaction of 5-(2-pyridyl)- (**1**), 5-(3-pyridyl)- (**2**), and 5-(4-pyridyl)-1,3,4-oxadiazol-2-thiones (**3**) with alkyl chloroformates **4-6** gave only the S-alkoxycarbonyl derivatives **7-15** in high yields (85-91%). No formation of the N-substituted derivatives was observed.

The structures of the synthesized compounds **7-15** were investigated by UV-, $^1$H NMR and IR- spectra. The UV spectra of S-alkoxycarbonyl derivatives **7-15** have an absorption maxima in the range 279-285 nm, which is typical for S-substitution [12,13], whereas the absorbance of amides - N-derivatives (**A**) observed at 295-315 nm [12-15].




R=$C_3H_7$ (**7-9**), $CH_2CH(CH_3)_2$ (**10-12**), $CH_2C_6H_5$ (**13-15**)

In the $^1$H NMR spectra of the synthesized compounds (**7-15**) the signals of the pyridine ring protons have chemical shifts as singlet, doublet and multiplet with the range of 7.41-9.25 ppm. The presence of signals of protons of the alkyl (propyl, butyl) and phenyl groups corresponding to 2-alkoxycarbonylthio derivatives also confirm the structure of the obtained compounds.

In the IR spectra of compounds **7-15** are observed an absorption bands in the region of 1755-1776 $cm^{-1}$ corresponding to the C=O bond in alkoxycarbonyl, 1600-1635 $cm^{-1}$ and 1180-1220 $cm^{-1}$ of C=N, C-O-C oxadiazole ring, and also sufficiently intensive absorption near 1500 $cm^{-1}$ which can be attributed to the C=N, C=C pyridine and aromatic rings.

The antimicrobial activity of the compounds **1-3** and **7-15** against Gram-positive bacteria: *Bacillus subtilis, Staphylococcus aureus*, and Gram-negative bacteria: *Pseudomonas aeruginosa*, *Escherichia coli* as well as fungi *Candida albicans* were determined using the agar disc diffusion method. The results of the current research were displayed in Table **1** and revealed that the 2-propyl-, 2-isobutyl- and 2-benzoxycarbonylthio- derivatives of 2-alkoxycarbonylthio-5-(2-,3-,4-pyridyl)-1,3,4-oxadiazoles **(7-15)** had an antibacterial activity against Gram-positive bacteria - *B. subtilis* and *S. aureus*, and fungi *Candida albicans*, displaying inhibition zone diameters between 6-9 mm (Tab.1). The primary oxadiazolthiones **1-3** were showing activity against only *S. aureus.* The remarkable activity of derivatives **7-15** may be attributed to the presence of the 2-alkoxycarbonyl group in the oxadiazolthiones.

**Table 1:** Antimicrobial activity of compounds 1-3 and 7-15

| **Compounds** | **Gram-positive bacteria** | | **Gram-negative bacteria** | | **Fungi** |
|---|---|---|---|---|---|
| | ***B. subtilis* (RKMUz – 5)** | ***S. aureus* (ATCC 25923)** | ***P. aeruginosa* (ATCC 27879)** | ***E. coli* (RKMUz - 221)** | ***C. albicans* (RKMUz - 247);** |
| 1 | na | 6 | na | na | na |
| 2 | na | 6 | na | na | na |
| 3 | na | 6 | na | na | na |
| 7 | 6 | 7 | na | na | 6 |
| 8 | 7 | 8 | na | na | 6 |
| 9 | 7 | 7 | na | na | 6 |
| 10 | 7 | 8 | na | na | 6 |
| 11 | 6 | 6 | na | na | 6 |
| 12 | 7 | 8 | na | na | 6 |
| 13 | 6 | 6 | na | na | 6 |
| 14 | 6 | 6 | na | na | 6 |
| 15 | 8 | 9 | na | na | 6 |
| Ampicillin (20µg/disc) | 26 | 25 | nt | 26 | nt |
| Ceftriaxone (20 µg/disc) | nt | nt | 25 | nt | nt |
| Nystatin (20 µg /disc) | nt | nt | nt | nt | 20 |

na- not active; nt – not tested

## Conclusion

Thus selective S-alkoxycarbonylation of isomeric - 5-(2-pyridyl)- (**1**), 5-(3-pyridyl)- (**2**), and 5-(4-pyridyl)-1,3,4-oxadiazol-2-thiones (**3**) have been studied and due to more polarizability of sulfur atom the novel series of S-substituted derivatives (**7-15**) have been synthesized in high yields and characterized by physical methods of research. No formation of the amides - N-substituted derivatives (**A**) was observed. The nature of oxadiazolinthione and alkyl chloroformate is not significant on the reaction's direction of and the yield of the products. The 2-alkoxycarbonylthio-derivatives of isomeric 5-pyridyl-1,3,4-oxadiazol-2-thiones showed remarkable antimicrobial activity against Gram-positive bacteria *B. subtilis, S. aureus,* and fungi *Candida albicans*. Nowadays the investigations in this field are continued.

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## References

1. Weiming Xu, Jiang He, Ming He, Feifei Han, Xuehai Chen, Zhaoxi Pan, Jian Wang and Maoguo Tong. Synthesis and Antifungal Activity of Novel Sulfone Derivatives Containing 1,3,4-Oxadiazole Moieties. *Molecules*, 2011, 16: 9129-9141.
2. Jagadeesh Prasad D, B. Shivarama Holla, Nalilu Sucheta Kumari, Laxmana K., Kumara Chaluvaiah. Synthesis and Antimicrobial Evaluation of Some New Mannich Bases Bearing 1,3,4-Oxadiazoline. *Ring System International Journal of Advanced Research in Chemical Science (IJARCS)*, 2015, 2(12): 7- 14.
3. Deepak S Musmade, Nachiket S Dighe, Shashikant R Pattan, Manjusha S Sanap, Prerana A Chavan, Manisha S Kedar, Snehalata K Tambe, Pramod J Shirote. 1,3,4-Oxadiazole in medicinal chemistry: an overview. *Pharmacologyonline*, 2010, 1: 108-116.
4. Ashan M.J, Rathod VP, Singh M, Sharma R, Jadav S.S. Synthesis, Anticancer and molecular docking studies of 2-(4-chlorophenyl)-5-aryl-1,3,4-oxadiazole analogues. *Med. Chem.*, 2013, 3: 3-4.
5. Vinay KR.Sahu, Arvind K. Singh, Deepmala Yadav. Review article on 1,3,4-Oxadiazole derivaties and it's Pharmacological activities. *Int.J. Chem Tech Res*., 2011, 3(3): 1362-1369.
6. Piyush Dholaria, Kalpesh Parikh, Deepkumar Joshi. Synthetic and therapeutic journe of 1,3,4-Oxadiazoles: a review. *International Journal of Chemtech Applications.* 2015,4(3): 1-25.
7. Oza K. K., Patel H.S. Antimicrobial activity of novel 3-substituted-5-(pyridine-4-yl)-3H-1,3,4-oxadiazole-2-thione derivatives. *Bulgarian Chemical Communications*, 2010, 42(2): 103–106.
8. Zhang F., Wang XL, Shi J, Wang SF, Yin Y, et al. Synthesis, molecular modeling and biological evaluation of N-benzylidene-2-((5-(pyridin-4-yl)-1,3,4-oxadiazol-2-yl)thio)acetohydrazide derivatives as potential anticancer agents. *Bioorg. Med. Chem.* 2014, 22: 468-477.
9. Toma A., Hapău D., Vlase L., Mogoşan C., Zaharia V. Heterocycles 31: synthesis and anti-inflammatory activity of 5-(Pyridin-4-yl)-1,3,4-oxadiazole-2-thiol, 5-(Pyridin-4-yl)-1,3,4-thiadiazole-2-thiol and 5-(Pyridin-4-yl)-1,2,4-triazole-3-thiol derivatives. *Clujul Medical.* 2013, 86(1): p.35-39.
10. Wayne P. *Clinical and Laboratory Standards Institute (CLSI) performance standards for antimicrobial disk diffusion susceptibility tests* 19th ed. approved standard. CLSI document M100-S19 2009.
11. T. Lindl and J.Bauer, *Zell und Gewebekultur*. Gustav-Fischer-Verlag Jena, Berlin, 1989: 181.
12. Sandstrom J., Wennerdeck I. Tautomeric cyclic thiones. Part II. Tautomerism, acidity and electronic spectra of thioamides of the oxadiazole, thiadiazole and triazole groups. *Acta Chem.Scand.* 1966, 20(1): 57-71.
13. Rozhkova N., Sabirov K., Seytanidi K.L. About reaction of benzothiazolin-2-thione with 1-chloro-2,3-epoxypropane. *Chem. Heterocycl. Comp*. 1983, 11: 1479-1482.
14. Ziyaev A.A, Galust'yan G.G. Reaction of 5-aryl-1,3,4-oxadiazole-2(3*H*)-thiones with chloromethyl alkyl ethers. *Chemistry of Heterocyclic Compounds*. 1999, 35: 1104–1106.

15. Tozhiev I.F, Ziyaev A.A, Shakhidoyatov Kh.M. Alkylation of 5-aryl-1,3,4-oxadiazol-2(3*H*)-thions with allyl- and benzylhalogens. *Chemistry and chemical technology, Tashkent*, 2012, 4: 17-20.