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SYNTHESIS, FLUORESCENT, AND ANTIBACTERIAL PROPERTIES OF *N*-(2'-AMINOARYL)-SUBSTITUTED 1,2,4-OXADIAZOL-5(4*H*)-ONES

A.A. Shetnev[1], A.S. Konstantinova[2], A.A. Romanycheva[1], S.V. Baykov[1], A.M. Silin[2], P.N. Kalashnikov[2], S.I. Ivanovskiy[2], M.K. Korsakov[1]

Anton Andreevich Shetnev, Candidate of Chemical Sciences, Senior Researcher; **Anna Sergeevna Konstantinova,** Researcher; **Anna Alexandrovna Romanycheva**, Researcher; **Sergey Valentinovich Baykov,** Senior Researcher; **Alexander Markovich Silin,** Student; **Pavel Nikolaevich Kalashnikov,** Student; **Sergey Alexandrovich Ivanovsky,** Senior Researcher; **Mikhail Konstantinovich Korsakov,** Leading Researcher.

[1]Saint Petersburg State University, Institute of Chemistry, 26 Universitetskiy Prospekt, Peterhof, 198504, Saint Petersburg, Russia, a.shetnev@spbu.ru

[2]Yaroslavl State Pedagogical University named after K.D. Ushinsky, 108/1 Republikanskaya str., Yaroslavl, 150066, Yaroslavl Region

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Abstract. *A series of eight new 2'-aminoaryloxadiazol-5(4H)-ones has been synthesized via the reduction of the corresponding nitro derivatives with tin(II) chloride. The antibacterial and fluorescent properties of the derivatives have been studied. We obtain the compound with moderate antibacterial activity against a sensitive strain of the Gram-positive bacterium Staphylococcus aureus ATCC-25923 (3-amino-4-(3-(3,4-dichlorophenyl)-5-oxo-1,2,4-oxadiazol-4(5H)-yl)benzonitrile, MIC 64 μg/mL. It demonstrates weak fluorescent properties upon irradiation with light at λ = 355 nm.*

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Introduction

1,2,4-Oxadiazol-5(4*H*)-ones are an important class of organic compounds. Due to their structural similarity to amide and ester groups and the diversity of their biological properties, they are considered the privileged structures in medicinal chemistry [1]. The antihypertensive agent azilsartan and the antidiabetic drug aleniglipron, currently in Phase 2 clinical trials, are based on the 1,2,4-oxadiazol-5(*4H*)-one core [2].

The investigation of these compounds as antibacterial [3], antiviral [4], anti-inflammatory, and neuroactive [5] agents is of current interest.

1,2,4-Oxadiazol-5(4*H*)-one derivatives can be obtained by a variety of methods. Indeed, the cyclization reactions of amidoximes with C1-electrophilic reagents (CDI, chloroformates, carbodiimides) play a leading role [6–9]. The classical approach involving *O*-acylation followed by cyclodehydration remains the most versatile one. The modern modifications allow those efficient transformations to be made (superbasic systems, microwave irradiation) [10, 11].

In the context of the functionalization reactions of 1,2,4-oxadiazol-5(4*H*)-ones, we have previously proposed a catalytic method for the *N*-arylation of *NH*-acids of the 1,2,4-oxadiazol-5(4*H*)-one and 1,3,4-oxadiazol-2(3*H*)-one series via symmetrical and unsymmetrical diaryliodonium salts in the presence of CuI as a catalyst [12]. Furthermore, variants of non-catalytic *N*-arylation of 1,2,4-oxadiazol-5(4*H*)-ones under classical activated aromatic nucleophilic substitution conditions were also investigated [13].

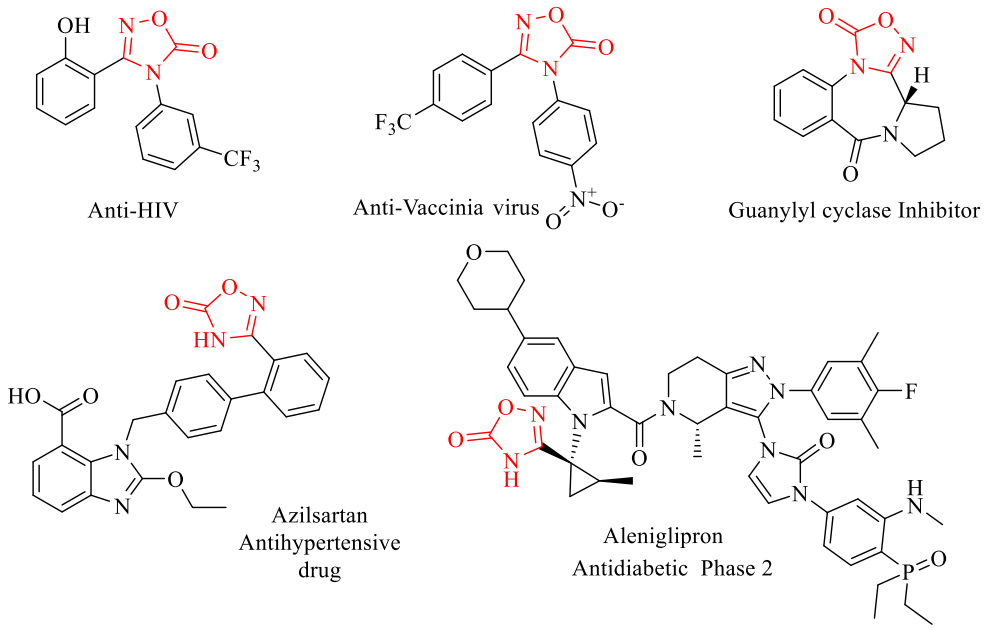


Fig. 1. Examples of biologically active compounds of the 1,2,4-oxadiazol-5(*4H*)-one class.

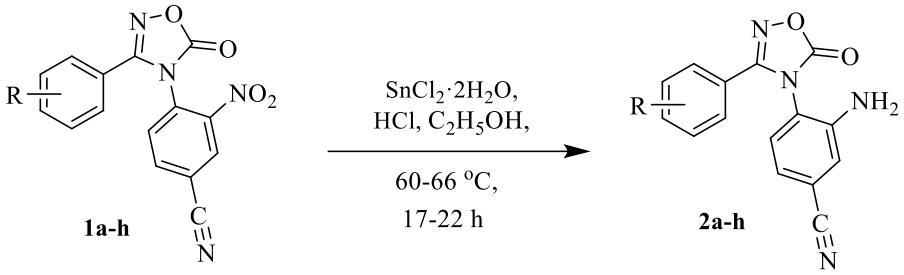
With the purpose of expanding the chemical space of available oxadiazol-5(4*H*)-ones, the present work presents the synthesis of arylamino derivatives and the investigation of their antibacterial and photophysical properties.

Main body

The reduction of the previously synthesized nitroaryl derivatives of 1,2,4-oxadiazol-5(4*H*)-ones **1** [13] was investigated (Fig. 2). The difficulty is in finding routes for the selective transformation of the nitro group into amino derivatives without destruction of the oxadiazolone ring, which is labile under reductive conditions. The use of catalytic hydrogenation methods with H_2 over 5–10% Pd/C presumably leads to ring opening and the formation of a mixture of linear amidine structures.

At the same time, the use of tin(II) chloride as a reducing agent in a hydrochloric acid–ethanol mixture, according to a method close to that reported in the literature [14, 15], leads to the selective reduction of the nitro group of nitroaryl derivatives of 1,2,4-oxadiazol-5(4*H*)-ones without affecting the heterocyclic core.

The applicability of this approach was demonstrated during the synthesis of eight amino derivatives **2** (Fig. 2).



2a: R = 4-Me,59%; **2b**:R = 4-MeO, 52%; **2c**: R = 4-Ph, 60%; **2d**: R = 4-F, 63%;
2e: R = 4-Cl, 48%; **2f:** R = 4-*i*-Pr, 54%; **2g**: R = 4-di-Cl, 63%; **2h**: R = 4-F, 3-MeO, 52%.

Fig. 2. Synthesis scheme and yields of *N*-(2'-aminoaryl)-substituted 1,2,4-oxadiazol-5(4*H*)-ones.

The proposed method provided moderate 48–63% yields of high-purity products without additional purification.

Photophysical properties. Fluorescence spectra of seven obtained compounds solutions **2b–2h** were recorded upon irradiation with light at a wavelength of λ_{ex} = 355 nm (Fig. 3).

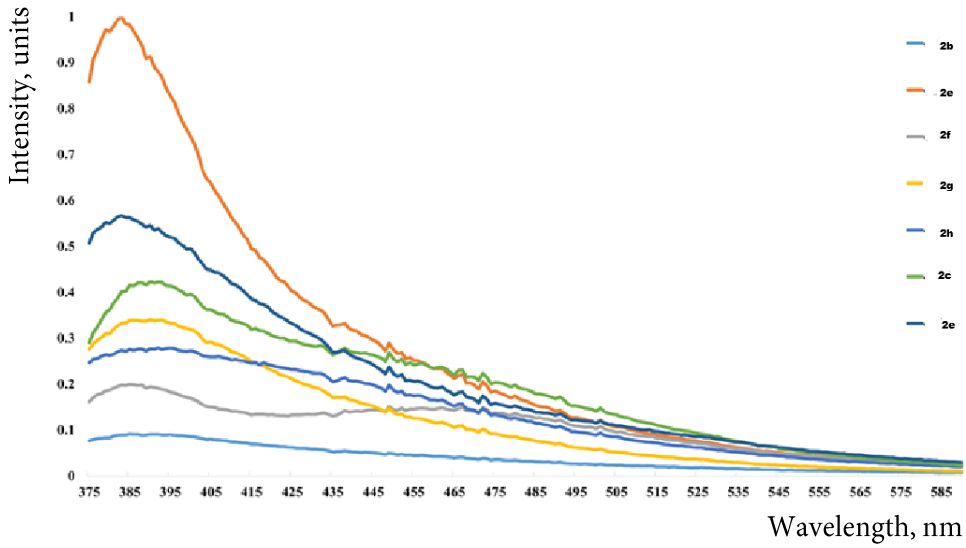


Fig. 3. Fluorescence spectra of methanol solutions of compounds **2b–2h**.

Table 1. Photophysical properties of *N*-(2'-aminoaryl)-substituted 1,2,4-oxadiazol-5*(4H)*-ones upon irradiation at λ_{ex} = 355 nm

ID	R	λ_{em}^{max}	$_{QYem}$	τ_{obs}
2b	4-MeO	395	0.88%	0.155
2c	H	394	1.43%	0.114
2d	4-F	394	0.32%	0.107
2d	4-F	460	0.32%	0.144
2e	4-Cl	395	0.11%	0.108
2f	4-*i*Pr	395	0.41%	0.118
2g	3,4-di-Cl	395	0.56%	0.13
2h	3-F, 3-MeO	393	1.02%	0.165

As a result, all tested compounds exhibited moderate fluorescent properties. The fluorescence quantum yields (**QY**$_{em}$) are in the range of 0.11–1.43%. It is typical for compounds with efficient non-radiative processes (e.g., internal conversion, intersystem crossing to the triplet state). The maximum quantum yield is observed for **2c** (R = H, 1.43%) and **2h** (R = 3-F, 3-OMe, 1.02%). The position of the emission maximum (λ_{em}^{max}) is weakly dependent on the substituent R. For all compounds, except **2d** (second peak at 460 nm), the emission lies in a narrow region of **393–395 nm**. This indicates that the emission likely originates from the same chromophore fragment (1,2,4-oxadiazol-5(4*H*)-one conjugated with the arylamino group). In the ground state and in the excited singlet state, substituents in the *para*- or *meta* positions of the aryl ring do not cause a significant solvatochromic or inductive shift. Compound **2d** exhibits two emission peaks with the same quantum yield (0.32%) and a wavelength of 394 nm characteristic of all studied compounds, as well as a bathochromically shifted band at 460 nm. The observed lifetimes (τ_{obs}) are in the nanosecond range (0.1–0.17 ns) and are characteristic of fluorescence with a small Stokes shift and a high rate of non-radiative deactivation ($k_{nr} >> k_r$). The longest τ_{obs} is observed for compound **2h** (3-F, 4-OMe 0.165 ns), which also has an increased **QY**$_{em;}$ it is explained by suppression of amino group rotation by the meta substituent. The shortest τ_{obs} is observed for **2e** (Cl, 0.108 ns) with a minimum Φ_{em}; it is associated with the presence of a heavy chlorine atom that enhances spin-orbit coupling.

Antibacterial activity. The obtained series was tested for antibacterial activity by the double serial dilution method using turbidimetric monitoring of microbial growth [16]. As test organisms, a relevant strain of Gram-positive bacteria *Staphylococcus aureus* ATCC-25923 and *Escherichia coli* C1 as a representative of Gram-negative microorganisms were selected. Based on the obtained data on the determination of the minimum inhibitory concentration (MIC) for the series of *N*-(2'-aminoaryl)-substituted 1,2,4-oxadiazol-5(4*H*)-ones, there is an absence of a broad spectrum of action. Most of the tested compounds (**2a–2f, 2h**) did not show antibacterial activity either against Gram-positive *S. aureus* ATCC-25923 or against Gram-negative *E. coli* C1 up to the maximum tested concentration of 256 μg/mL. Only one compound – **2g** (with a 3,4-dichlorophenyl substituent) – showed measurable activity against *S. aureus* (MIC = 64 μg/mL); it is classified as moderate or weak growth inhibition. Against *E. coli*, this compound was also inactive (MIC > 256 μg/mL). All the compounds in the series did not inhibited the growth of *E. coli* C1 in the tested concentration range. It may indicate low permeability of the compounds through the outer membrane of Gram-negative bacteria.

Experimental part

Commercially available reagents from Maclin, Arcos, and Khimmed were used in this work. Thin-layer chromatography was performed on Sorbfil PTSKh-P-V-UV plates, eluent: toluene : acetone : petroleum ether, 60:100:100.

Melting points were measured using a Reach Devices RD-MP melting point apparatus (manufacturer: China). ^{1}H and ^{13}C NMR spectra were recorded on a Varian 400 Unity Plus spectrometer (manufacturer: USA), solvent: DMSO-d_6. The signals of residual solvent protons were used as a reference for chemical shifts in ^{1}H NMR (δH 2.50 ppm) or ^{13}C NMR (δC 39.5 ppm);

tetramethylsilane was used as a marker. Signal multiplicities: s – singlet, d – doublet, t – triplet, dd – doublet of doublets, td – triplet of doublets, m – multiplet. High-resolution mass spectra were recorded on a Bruker Daltonics MicrOTOF-II instrument (manufacturer: USA), ionization method – electrospray ionization (ESI), ionization source temperature – 180 °C, eluent – methanol. The starting compounds **1a–h** were obtained according to the procedure described in [13].

Procedure for the preparation of *N*-(2'-aminoaryl)-substituted derivatives 2a, c–g. In a conical flask, *N*-(2'-nitroaryl) derivative **1** (300 mg, 1 equiv.) and ethanol (3 mL) were mixed at room temperature. Then, a solution of $SnCl_2 \cdot 2H_2O$ (3.5 equiv.) in concentrated HCl (0.80 mL) was added portionwise to the suspension under cooling in a water bath. After that, the reaction mixture was stirred under cooling in a water bath for 10–15 min. The reaction mixture was then heated to 60–66 °C and maintained at this temperature for 17–22 h. Further, the reaction mixture was cooled to room temperature. The reaction mixture was stirred for 2.0–2.5 h at 20–25 °C. The precipitated solid was filtered off and washed on the filter with ethanol three times with 0.5 mL each. After that, the precipitate was washed with water (2 × 2 mL), each time thoroughly stirring the precipitate on the filter. The precipitate was dried at 50 °C in a drying oven at atmospheric pressure. In the case of compound **2f**, the product was recrystallized from ethanol.

3-Amino-4-(5-oxo-3-(*p*-tolyl)-1,2,4-oxadiazol-4(5*H*)-yl)benzonitrile (2a). Yield is 0.16 g (59 %), light-beige powder, Tmelt. 225-227 °C. NMR spectrum ^{1}H, *δ*, ppm: 2.30 s (3H, CH_3), 6.30 s (2H, NH_2), 6.89 d (1H, Ar, *J* 8.1 Гц), 7.10 s (1H, Ar), 7.28 qv (4H, Ar, *J* 7.9 Hz), 7.36 d (1H, Ar, *J* 8.1 Hz). NMR spectrum ^{13}C, δ, ppm: 21.64, 114.15, 119.04, 119.30, 120.02, 120.95, 128.16, 130.22, 132.16, 142.70, 147.71, 158.20, 158.44. Mass-spectrum, *m/z*: 293.1031 $[M + H]^+$ (calculated for $C_{16}H_{13}N_4O_2^+$: 293.1033).

3-Amino-4-(3-(4-methoxyphenyl)-5-oxo-1,2,4-oxadiazol-4(5*H*)-yl)benzonitrile. Yield is 0.14 g (52 %), pale yellow powder, Tmelt 155–157 °C. NMR spectrum ^{1}H, *δ*, ppm: 3.76 s (3H, CH_3O), 6.26 s (2H, NH_2), 6.90 d (1H, Ar, *J* 7.9 Hz), 6.99 d (2H, Ar, *J* 8.2 Hz), 7.12 s (1H, Ar), 7.35 d (3H, Ar, *J* 7.9 Hz). NMR spectrum ^{13}C, δ, ppm: 56.06, 114.16, 115.18, 115.76, 119.10, 119.30, 120.12, 129.88, 132.19, 147.74, 158.12, 158.26, 162.49. Mass-spectrum, *m/z*: 309.0985 $[M + H]^+$ (calculated for $C_{16}H_{13}N_4O_3^+$: 309.0982).

3-Amino-4-(5-oxo-3-phenyl-1,2,4-oxadiazol-4(5*H*)-yl)benzonitrile (2c). Yield is 0.17 g (63 %), pale yellow powder, Tmelt. 223–225 °C. NMR spectrum ^{1}H, δ, ppm: 6.29 s (2H, NH_2), 6.89 d (1H, Ar, *J* 8.1 Hz), 7.11 s (1H, Ar), 7.37 d (1H, Ar, *J* 8.1 Hz), 7.41–7.47 m (4H, Ar), 7.52–7.55 m (1H, Ar). NMR spectrum ^{13}C, δ, ppm: 114.19, 119.05, 119.32, 119.91, 123.82, 128.26, 129.66, 132.16, 132.56, 147.71, 158.18, 158.43. Mass spectrum, *m/z*: 279.0875 $[M + H]^+$ (calculated for $C_{15}H_{11}N_4O_2^+$: 279.0877).

3-Amino-4-(3-(4-fluorophenyl)-5-oxo-1,2,4-oxadiazol-4(5*H*)-yl)benzonitrile (2d). Yield is 0.17 g (63 %), pale yellow powder, Tmelt. 236–238 °C. NMR spectrum ^{1}H, δ, ppm: 6.27 s (2H, NH_2), 6.91 d (1H, Ar, *J* 8.1 Hz), 7.11 s (1H, Ar), 7.33 t (2H, Ar, *J* 8.9 Hz), 7.39 d (1H, Ar, *J* 8.1 Hz), 7.46–7.49 m (2H, Ar). NMR spectrum ^{13}C, δ, ppm: 114.27, 116.92, 117.15, 119.08 d (*J* 5.7 Hz), 119.39, 119.67, 120.35, 130.99 d (*J* 9.2 Hz), 132.16, 147.66, 157.70, 158.11, 163.38, 165.87. Mass-spectrum, *m/z*: 297.0781 $[M + H]^+$ (calculated for $C_{15}H_{10}FN_4O_2^+$: 297.0782).

3-Amino-4-(3-(4-chlorophenyl)-5-oxo-1,2,4-oxadiazol-4(5*H*)-yl)benzonitrile (2e). Yield is 0.13 g (48 %), pale yellow powder, Tmelt. 204–206 °C. NMR spectrum ^{1}H, *δ*, ppm: 6.27 (2H, NH_2), 6.91 d (1H, Ar, *J* 8.1 Hz), 7.10 s (1H, Ar), 7.38–7.43 m (3H, Ar), 7.55 d (2H, Ar, *J* 8.2 Hz). NMR spectrum ^{13}C, δ, ppm: 114.29, 119.10 d (*J* 7.6 Hz), 119.41, 119.58, 122.67, 129.96, 130.10, 132.12, 137.57, 147.57 d (*J* 6.7 Hz), 157.65, 158.07. Mass-spectrum, *m/z*: 313.0490 [M + H]$^+$ (calculated for $C_{15}H_{10}ClN_4O_2^+$: 313.0487).

3-Amino-4-(3-(4-isopropylphenyl)-5-oxo-1,2,4-oxadiazol-4(5*H*)-yl)benzonitrile (2f). Yield is 0.12 g (54 %), pale yellow powder, Tmelt. 186–188 °C. NMR spectrum ^{1}H, *δ*, ppm: 1.15 s (3H, CH_3), 1.17 s (3H, CH_3), 2.89 p (pentet) (1H, CH, *J* 6.9 Hz), 6.28 s (2H, NH_2), 6.90 dd (1H, Ar, *J* 8.1, 1.4 Hz), 7.12 s (1H, Ar), 7.31–7.38 m (5H, Ar). NMR spectrum ^{13}C, δ, ppm: 24.06 d (*J* 4.7 Hz), 33.99, 114.22, 119.09, 119.31, 120.08, 121.30, 127.68, 128.18, 132.20, 147.73, 153.24, 158.27. Mass-spectrum, *m/z*: 321.1349 [M + H]$^+$ (calculated for $C_{18}H_{17}N_4O_2^+$: 321.1346).

3-Amino-4-(3-(4-dichlorophenyl)-5-oxo-1,2,4-oxadiazol-4(5*H*)-yl)benzonitrile (2g). Yield is 0.17 g (63 %), pale yellow powder, Tmelt. 278–280 °C (decomp.). NMR spectrum ^{1}H, *δ*, ppm: 6.29 s (2H, NH_2), 6.95 (1H, Ar, *J* 8.1 Hz), 7.12 s (1H, Ar), 7.35 d (1H, Ar, *J* 8.5 Hz), 7.44 d (1H, Ar, *J* 8.0 Hz), 7.65 s (1H, Ar), 7.77 d (1H, Ar, *J* 8.4 Hz). NMR spectrum ^{13}C, δ, ppm: 114.45, 118.99, 119.26, 119.49, 124.23, 128.40, 130.14, 132.12, 132.25, 132.54, 135.74, 147.57, 156.70, 157.94. Mass-spectrum, *m/z*: 347.0099 [M + H]$^+$ (calculated for $C_{15}H_9Cl_2N_4O_2^+$: 347.0097).

3-Amino-4-(3-(3-fluoro-4-methoxyphenyl)-5-oxo-1,2,4-oxadiazol-4(5*H*)-yl)benzonitrile (2h). Yield is 0.14 g (52 %), pale yellow powder, Tmelt. 223–225 °C. NMR spectrum ^{1}H, *δ*, ppm: 3.85 s (3H, CH_3O), 6.30 s (2H, NH_2), 6.93 d (1H, Ar, *J* 8.1 Hz), 7.13 s (1H, Ar), 7.17 d (1H, Ar, *J* 8.8 Hz), 7.24–7.28 m (2H, Ar), 7.40 d (1H, Ar, *J* 8.1 Hz). NMR-spectrum ^{13}C, δ, ppm: 56.91, 114.33, 115.01, 115.54, 115.83–115.90 м, 119.05, 119.29 d (18.3 Hz), 119.80, 125.52, 132.20, 147.72, 150.33, 150.68 d (*J* 10.0 Hz), 152.78, 157.24, 158.15. Mass-spectrum, *m/z*: 327.0886 [M + H]$^+$ (calculated for $C_{16}H_{12}FN_4O_3^+$: 327.0888).

Conclusion

A procedure has been developed for the synthesis of *N*-(2'-aminoaryl)-substituted 1,2,4-oxadiazol-5(4*H*)-ones from the corresponding nitro derivatives, providing 48–63% yields of the target products. The obtained *N*-(2'-aminoaryl)-substituted 1,2,4-oxadiazol-5(4*H*)-ones, upon excitation with light at a wavelength of 355 nm, exhibited properties of weak fluorophores (**QY**$_{em}$ < 1.5% и τ < 0.2 ns) and may be considered as potential labile fluorescent labels. Compound **2d** warrants separate theoretical calculations to explain the dual emission in its spectrum. Compound **2g** showed antimicrobial activity against a sensitive strain of *S. aureus* (MIC 64 μg/mL) and may be considered as a starting compound for the development of a new antibacterial chemotype based on *N*-aryl 1,2,4-oxadiazol-5(4*H*)-one derivatives.

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