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Original article

Synthesis and pharmacological screening of derivatives of isoxazolo[4,5-*d*]pyrimidine

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Abstract

A number of derivatives of isoxazolo[4,5-*d*]pyrimidine were prepared with structures similar to that of purine. Condensation of the hydrazide of 4-amino-5-benzoylisoxazolo-3-carboxylic acid **2** with ethyloxalyl chloride followed by cyclization gave 3-oxdiazolo-[1,3,4]-4-amino-5-benzoylisoxazole **7** which, upon cyclization with acetonitrile followed by reactions with different amines, gave derivatives of isoxazolo[4,5-*d*]pyrimidine **9** and **10d–g**. Compounds **8g** and **10f** were tested for their effects on the immune response in the mouse model. Both compounds significantly inhibited the humoral immune response in vivo to sheep erythrocytes at a dose of 100 μg, whereas in the delayed type hypersensitivity assay a suppressive activity was shown only by compound **10f**. In addition, compound **8g** inhibited and compound **10f** stimulated the proliferative response of mouse splenocytes to concanavalin A. The results indicated that compound **10f** was a universal inhibitor of the immune response, while compound **8g** selectively suppressed the humoral immune response.

Keywords: Isoxazoles; Isoxazole[4,5-*d*]pyrimidines; Immunosuppressant; Antitumor agents

1. Introduction

Adenosine and inosine are found naturally in DNA and RNA and play different roles in physiology. Adenosine, for example, is an endogenous nucleoside which mediates a variety of important physiological processes by interaction with specific adenosine receptors [1–3]. Inosine is an endogenous agonist of the adenosine A_3 receptor and is also an endogenous ligand of the benzodiazepine receptors [4,5]. The purine base has been utilized to obtain biologically active compounds. The idea of modifying purine bases by replacing the imidazole ring with isoxazole to obtain the corresponding structure of isoxazolopyrimidine has been the subject of numerous studies. Derivatives of isoxazolopyrimidine have significant biological activity and display analgesic [6,7], anti-inflammatory [8], bactericidal [9–11], circulatory [12], and anxiolytic activities [13]. Of the four structural isomers of isoxazolopyrimidines, only the isomer 4,5-*d* has not been thoroughly investigated. To date there have been only three [14–16] reports concerning the synthesis of derivatives of this isomer.

In this article we describe the synthesis of new isoxazoles **4**, **5**, **7**, and **8a–i** and new derivatives of isoxazolo[4,5-*d*]pyrimidine **9** and **10d–g**. Three of the compounds showed interesting biological activities.

2. Chemistry

The synthesis was carried out according to Scheme 1. The starting compound **1** was obtained by Thorpe reaction according to Gewald et al. [17]. The resulting 4-amino-5-benzoylizoxazole-3-carboxamide **1** reacted with hydrazine hydrate to produce hydrazide **2** with a good yield (81%) and hydrazone **1** of only 13%. Reaction of **2** with triethyl orthoformate in the presence of acetic anhydride underwent smooth cyclization, but did not yield derivatives of isoxazolo[4,3-*d*]pyrimidine **3,** but did yield 4-amino-5-benzoylo-3-oxdiazolo[1,3,4]isoxazole

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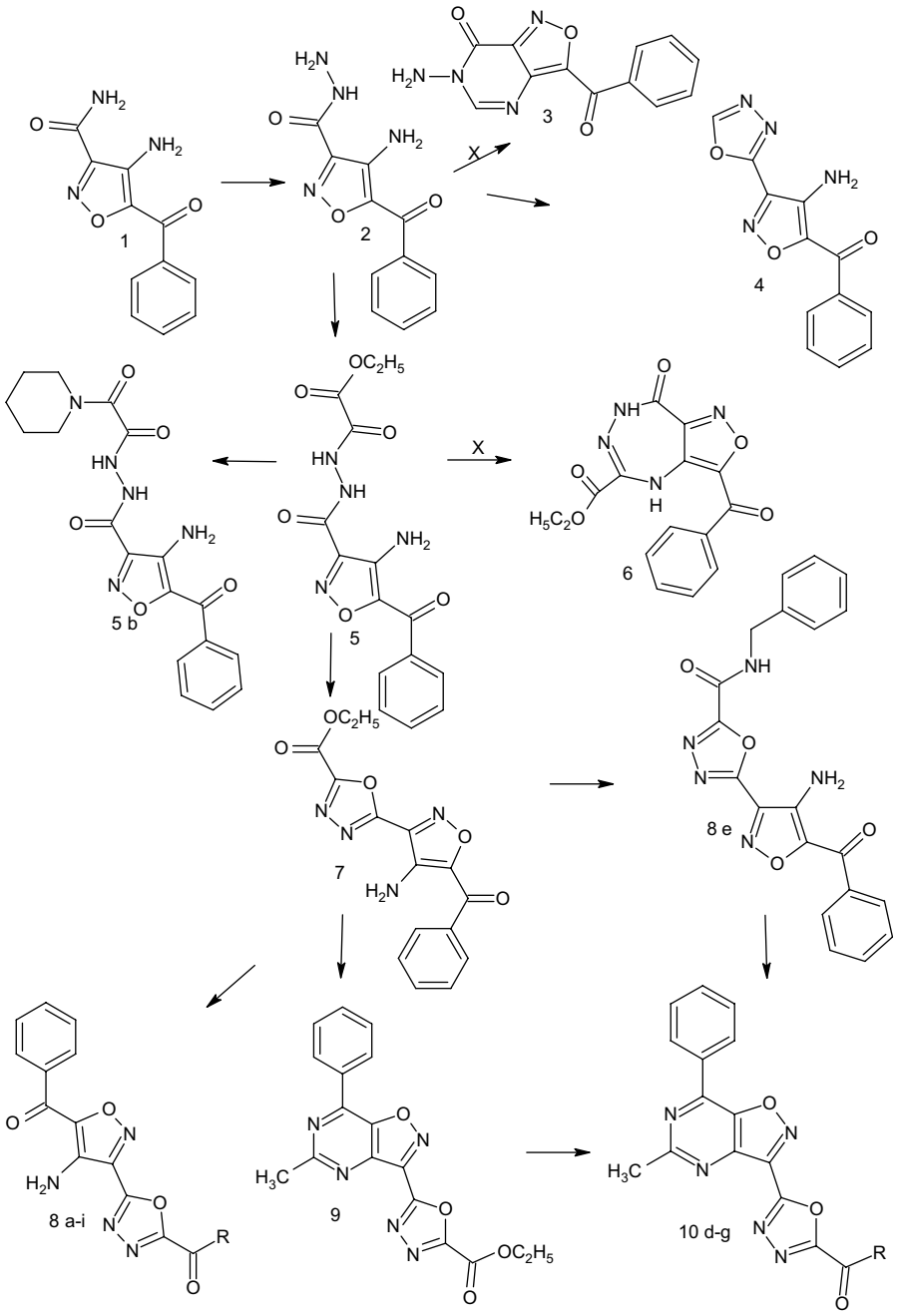

Scheme 1.

4. Treatment of the hydrazide **2** with ethyloxalyl chloride in the presence of anhydrous pyridine produced the corresponding compounds **5**, which reacted with piperidine but did not cyclize derivatives of isoxazolo[4,3-*e*]triazepine[1,2,4] **6** and only yielded the piperidine amide **5b**. Compound **5** was readily cyclized under acidic conditions with thionyl chloride to give compound **7**. The amine group at position 4 of compounds **2** and **5** was not reactive in these two cyclizations. X-ray crystallography of compound **7** confirmed that the resulting structure was indeed compound **7** (Fig. 1). Ester **7** reacts with different amines to form amides **8a–i**, whereas ester **7** and amide **8e** were heated in acetonitrile with gaseous hydrogen chloride to give the new derivatives isoxazolo[4,5-*d*]pyrimidine **9** and **10e**. Ester **9** reacts with amines to form the amides isoxazolo[4,5-*d*]pyrimidine **10d–g**. Compound **10e** was prepared by two ways from compounds **8e** and **9** and its structure also confirmed the new compounds.

3. Biological activity

Evaluation of the anticancer activity was performed for compounds **4**, **7**, **8a–i**, **9**, and **10d–g** at the National Cancer

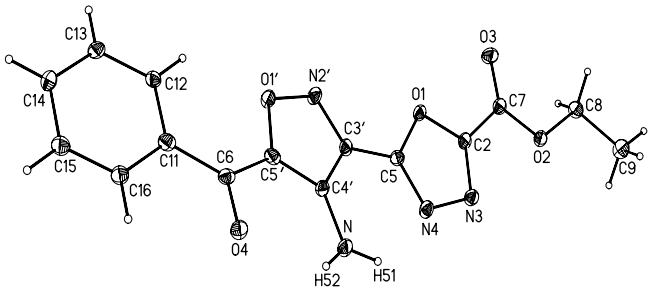

Fig. 1. A view of molecule A with the atomic numbering scheme. Ellipsoids are at the 40% probability level.

Institute (NCI) in Bethesda, MD, USA. Only compound **4** showed interesting activity on a panel of 62 tumor cell lines. Data of the active compound **4** are presented in Table 1.

4. Materials and methods

4.1. Mice

Twelve-week old male and female CBA mice were used for the experiments. The mice were kept in the Animal Facility of the Institute of Immunology and Experimental Therapy, Wroclaw, fed commercial granulated food, and had water *ad libitum*.

Table 1
Inhibition of in vitro cell lines by compound **4**[a]

Panel cell line			
MG-MID[b]	log GI_{50}[c] [M]	log TGI[d]	log LC_{50}[e] [M]
Leukemia			
CCRF-CEM	−4.74	>−4.00	>−4.00
K-562	−4.24	>−4.00	>−4.00
MOLT-4	−4.25	>−4.00	>−4.00
Non-small cell lung cancer			
HOP-92	−4.51	−4.01	>−4.00
NCI-H522	−4.47	>−4.00	>−4.00
Colon cancer			
HCC-2998	−4.57	−4.02	>−4.00
HCT-15	−4.53	>−4.00	>−4.00
CNS cancer			
SF-539	−5.56	−4.65	−4.19
U-251	−4.79	−4.16	>−4.00
Melanoma			
LOX-IMVI	−4.54	>−4.00	>−4.00
SK-MEL-2	−4.48	>−4.00	>−4.00
Renal cancer			
CAKI-1	−4.66	>−4.00	>−4.00
MG-MID	−4.25	−4.01	−4.00
Delta	1.31	0.64	0.19
Range	1.56	0.65	0.19

Compound **8d–g** and **10d–g** were also assayed for immunologic activity in selected tests.

[a] Data obtained from the NCI's in vitro disease-oriented human cells screen [21,22].

[b] MG-MID = mean graph midpoint = arithmetical mean value for all tested cell lines.

[c] The log of the molar concentration that inhibits 50% net cell growth.

[d] The log of the molar concentration leading to total growth inhibition.

[e] The log of the molar concentration leading to 50% net cell death.

4.2. Reagents

Sheep red blood cells (SRBCs) were provided by the Wroclaw Agriculture Academy. SRBCs were kept in Alsever's solution until use. Cyclosporine A (CsA) was from Sandoz (Switzerland), DMSO and concanavalin A (Con A) from Sigma RPMI 1640 medium, and fetal calf serum (FCS) was from Gibco.

4.3. Treatment of mice with the compounds

The compounds were initially dissolved in DMSO and then in 0.9% NaCl. The compounds were given to mice intraperitoneally (i.p.) at doses of 10 μg or 100 μg per mouse 2 h after immunization with SRBCs. For control, 0.2 mL of 0.9% NaCl or appropriately diluted DMSO were used.

4.4. Determination of the humoral immune response to SRBCs

Mice were immunized with 0.2 mL of a 5% SRBC suspension in 0.9% NaCl i.p. Four days later the mice were sacrificed, spleens were isolated, and the number of antibody-forming cells (AFCs) in the spleens was determined using the local hemolysis assay [18]. The results are shown as mean AFC values from 5 mice per group calculated per 10^6 viable splenocytes ± standard error (SE).

4.5. Determination of delayed type hypersensitivity (DTH) to SRBCs

The test was performed according to Lagrange et al. [19]. Mice were immunized with 0.2 mL of an SRBC suspension containing 10^5 erythrocytes intravenously. Four days later the mice were given an eliciting dose of antigen, i.e. 10^8 erythrocytes, in 0.05 mL, into the hind footpads. Twenty-four hours later the delayed type hypersensitivity reaction was measured as the footpad edema, using callipers. The background, non-specific response was elicited by administration of the eliciting dose to naive mice, which was subtracted from the response of the sensitized mice. The results are shown as mean values of DTH units from 5 mice ±SE.

4.6. The proliferative response of splenocytes to concanavalin A

A single splenocyte suspension was prepared by pressing the organs through a plastic screen into Hank's medium. The cells were centrifuged and treated with 0.83% ammonium chloride to lyse erythrocytes. Then the cells were washed twice in Hank's medium, passed through cotton wool columns to remove dead cells and debris, and finally resuspended in a culture medium consisting of RMPI 1640 supplemented with 10% FCS, L-glutamine, sodium pyruvate, and antibiotics.

The cells, 2×10^5 in 100 μL, were distributed in flat-bottom 96-well culture plates. Con A was added in a final concentration of 2.5 μg/mL and the studied compounds at the concentration range of 0.1–10 μg/mL. After three days of culture in a cell culture incubator, the proliferation rate was determined by the MTT colorimetric method [20]. The results were presented as the mean optical density (OD) at 550/630 nm from quadruplicate wells ±SE.

4.7. Statistics

The Student's *t*-test was applied for evaluation of the data. The results are presented as mean values ± SE. The results were regarded to be significant when $p < 0.05$. NS denotes not significant.

5. Results and discussion

The hydrazide **2** and compound **5** do not yield derivatives **3** and **6** because the amino groups at position **4** have decreased activity due to the electronegative group at position 5. However, if the oxdiazole ring was formed, the amine group at position 4 with acetonitrile gave compound **7**.

To evaluate the effects of the compounds on the humoral immune response in vivo, mice were immunized with SRBCs and given the compounds i.p. 2 h after immunization. Control mice were given the solvent (DMSO) or the reference suppressive drug cyclosporine A. The number of antibody-forming cells was determined 4 days later in the spleens. The results (Table 2) showed that both compounds (**8g** and **10f**) significantly suppressed the humoral immune response at the higher dose (100 μg per mouse). This inhibition was even deeper than that of CsA at the same dose.

To determine the effects of the compounds on the cellular immune response in vivo, mice were sensitized with SRBCs and given the compounds i.p. 2 h later. After 4 days the mice were challenged with the eliciting dose of antigen (SRBCs) and the delayed type hypersensitivity was measured 24 h later as footpad swelling. It appeared (Table 3) that only compound **10f** exhibited significant inhibition of DTH at both doses. This effect was even stronger than that of CsA.

The compounds were also tested (concentration range: 0.1–10 μg/mL) for their ability to alter the proliferative response of mouse splenocytes to concanavalin A, a T-cell mitogen. The results, shown in Table 4, indicate that compound **8g** inhibited, whereas compound **10f** stimulated, the proliferative response of cells to the mitogen. These effects, although small, were statistically significant.

The differential actions of the studied compounds in the humoral and cellular (delayed type hypersensitivity) responses probably resulted from the differences in their structures. The common component in the structures of both compounds, **8g** and **10f**, is the oxadiazole ring. That part of the molecule could, presumably, be responsible for the observed suppression of the humoral immune response. On the other hand, compound **10f**, which strongly suppressed the DTH response, contains isoxazolo[4,5-*d*]pyrimidine, substituted at position 7 with the phenyl radical, which may be relevant in the inhibition of the signaling pathways leading to the generation of the cellular immune response. Obviously, the isoxazole ring, substituted at position 4 with the NH_2 group and at position 5 with the benzoyl group, being the second part of the **8g** molecule, cannot affect this type of immune response. The differences in the structures of both compounds could also be the reason for the opposite effects of these compounds on the proliferative response of splenocytes to concanavalin A. However,

Table 2
Effects of the compounds on the humoral immune response in vivo to SRBCs

Compound	Dose (μg/mouse)	AFC × 10^6	±SE	*P*
Control		2153	13.58	
DMSO control	10	1553	70.5	
	100	1698	33.2	
8g	10	1361	105.1	NS
	100	574	57.5	<0.001
10f	10	1660	49.8	NS
	100	545	88.5	<0.001
CsA	10	1617	102.6	<0.02
	100	953	54.8	<0.001

Table 3
Effects of the compounds on the delayed type hypersensitivity to SRBCs

Compound	Dose (μg/mouse)	DTH units (mean)	±SE	*P*
Control		13.0	0.76	
DMSO control	10	10.9	0.18	
	100	6.4	0.37	
8g	10	7.9	0.85	NS
	100	4.9	0.60	NS
10f	10	5.9	0.79	<0.05
	100	3.5	0.42	<0.02
CsA	10	6.1	0.33	<0.01
	100	5.0	0.51	<0.01

Table 4
Effects of the compounds on the proliferative response of splenocytes to concanavalin A

Compound	Concentration (μg/mL)	OD (550/630 nm)	±SE	*P*
Control (no mitogen)		0.089	0.004	
Con A only		0.447	0.008	
DMSO control	0.1	0.438	0.007	
	1	0.451	0.003	
	10	0.429	0.003	
8g	0.1	0.442	0.018	NS
	1	0.401	0.011	<0.01
	10	0.371	0.010	<0.01
10f	0.1	0.483	0.010	<0.02
	1	0.479	0.008	<0.05
	10	0.482	0.013	<0.01
CsA	0.1	0.070	0.002	<0.001
	1	0.046	0.003	<0.001
	10	0.001	0.000	<0.001

these immunomodulatory effects in vitro were modest. In summary, both compounds exhibited distinct immunosuppressive activities; therefore further investigations applying other immunological tests are justified to reveal their potential therapeutic utility.

6. Experimental

All chemicals were obtained from Aldrich. Dry pyridine was prepared by refluxing over sodium and storage over potassium hydroxide. Melting points were determined with a Boetius apparatus and are uncorrected. The progress of the reaction and purity of compounds were monitored by TLC analytical silica gel plates (Merck F_{254}). IR spectra were recorded on a Specord M80 spectrometer for KBr discs. ^{1}H NMR spectra were recorded with a Bruker Avance DRX-300 instrument; chemical shifts are reported in ppm downfield from the internal tetramethylsilane and coupling constants in Hz. Mass spectra were recorded on a Finningan Mat 95. Elemental analyses were performed on a Carbo Erba NA 1500 analyzer.

6.1. 4-Amino-5-benzoylisoxazole-3-carbohydrazide **2**

To a solution of 100 mL of dioxane, 50 mL methanol, and 20 g of 80% hydrazine hydrate was added 23.1 g (0.1 mol) of 4-amino-5-benzoylisoxazolo-3-carboxamide **1**. The reaction mixture was stirred under reflux for 6 h. The mixture was concentrated to ca. 50 mL. After cooling, the precipitate was filtered and washed with methanol. The solids were purified by recrystallization from dioxane, giving 20 g of pure (TLC) compound **2**, yield (81.3%). Mp: 220–221 °C. IR (cm^{-1}, KBr): 3430, 3360, 3230 (NH_2, NH), 1685, 1650 (C=O), 700 (Ar). ^{1}H NMR (DMSO-d_6) δ 10.27 (s, 1H, NH), 7.56–8.07 (m, 5H, Ar), 6.35 (s, 2H, NH_2 poz.4), 4.69 (s, 2H, –NH_2). Anal. calcd. for $C_{11}H_{10}N_4O_3$ (246.22): C, 53.66; H, 4.09; N, 22.75. Found: C, 53.69; H, 4.07; N, 22.38.

6.2. [4-Amino-3-(1,3,4-oxadiazol-2-yl)isoxazol-5-yl](phenyl)methanone **4**

The acid hydrazide **2** (2.46 g, 0.01 mol) was dissolved in 30 ml of absolute dioxane. Triethyl orthoformate (3 mL) was then added while stirring and the reaction mixture was heated under reflux for 5 h. The mixture was cooled and the separated yellow product was filtered, washed with methanol, dried, and recrystallized from dioxane, giving 2.1 g (yield: 82.03%) of pure (TLC) compound **4**. Mp: 222–223 °C. IR (cm^{-1}, KBr): 3435, 3335 (NH_2), 1660, 1620, 1605 (C=O, C=N), 710 (Ar). ^{1}H NMR (DMSO-d_6) δ 7.68–8.40 (m, 6H, aromatics), 6.43 (s, 2H, NH_2). Anal. calcd. for $C_{12}H_8N_4O_3$ (256.22): C, 56.25; H, 3.15; N, 21.87. Found: C, 56.38; H, 3.41; N, 22.08.

6.3. Ethyl{2-[(4-amino-5-benzoylisoxazol-3-yl)carbonyl]hydrazine}(oxo)acetate **5**

The acid hydrazide **2** (24.6 g, 0.1 mol) was dissolved in 200 mL of absolute dioxane and 10.1 g of triethylamine was added. Ethyloxalyl chloride (15 g, 0.109 mol) was added drop wise to the mixture. The mixture was stirred at room temperature for 5 h, cooled, filtered (TEA·HCl), and the solvents were removed under reduced pressure. The residue was treated with water, and then filtered. The resulting precipitate was washed with water and methanol, dried, and recrystallized from ethyl acetate. The yield was 26.3 g (79.77%). The pure product melted at 204–205 °C. IR (cm^{-1}, KBr): 3475, 3365, 3250 (NH^2, NH), 1735, 1685, 1650 (C=O), 1605, 1545, 1497, 1427 (C=N, C=C, amide II, aromatics), 1230 (C–O–C). ^{1}H NMR (DMSO-d_6) δ 11.13 (s, 2H, CONH–NH–CO), 7.65–8.15 (m, 5H, Ar), 6.38 (s, 2H, NH_2), 4.15–4.41 (q, 2H, CH_2), 1.22–1.41 (t, 3H, CH_3). Anal. calcd. for $C_{15}H_{14}N_4O_6$ (346.30): C, 52.03; H, 4.07; N, 16.18. Found: C, 52.23; H, 4.01; N, 16.28.

6.4. Ethyl{2-[(4-amino-5-benzoylisoxazol-3-yl)carbonyl]hydrazine}(oxo)acetate **5b**

Piperidine (0.5 g, 6 mmol) was added to a solution of compound **5** (1.73 g, 5 mmol) in absolute dioxane (5 mL). The reaction mixture was refluxed for 1 h, and then allowed to attain room temperature. The separated solid was filtered, washed with cold methanol, and recrystallized from absolute ethanol. The yield of compound **5b** was 1.5 g (77.72%). Mp: 207–209 °C. IR (cm^{-1}, KBr): 3480, 3365, 3255 (NH_2, NH), 1695, 1665, 1650, 1640 (C=O, C=N). ^{1}H NMR (DMSO-d_6) 10.91–11.04 (d, 2H, CONH), 7.69–8.19 (m, 5H, Ar), 6.41 (s, 2H, NH_2), 3.38–3.53 (m, 4H, $N(CH_2)_2$), 1.38–1.82 (m, 6H, $(CH_2)_3$). Anal. calcd. for $C_{18}H_{19}N_5O_5$ (385.38): C, 56.10; H, 4.97; N, 18.17. Found: C, 56.40; H, 4.84; N, 18.10. The filtrate from this reaction contained (TLC) only compound **5**, no compound **6**.

6.5. Ethyl-5-(4-amino-5-benzoylisoxazol-3-yl)[1,3,4]oxadiazole-2-carboxylate **7**

To a solution of the ester **5** (18 g, 0.052 mol) in absolute dioxane (150 mL) was added thionyl chloride (100 mL). The reaction mixture was refluxed for 15 h, and then allowed to attain room temperature. The solvents were removed under reduced pressure. The residue was dissolved in ethyl acetate (120 mL) and treated with 4 g of Norit, boiled for a few minutes, and filtered hot. The filtrate was kept in a refrigerator for 12–16 h. The precipitated compound **7** was filtered and washed with ethanol. The product formed colorless prisms melting at 199–200 °C. The yield was 11.6 g (67.9%). IR (cm^{-1}, KBr): 3465, 3360 (NH_2), 1745, 1650, 1640 (C=O, C=N). ^{1}H NMR (DMSO-d_6) δ 7.60–8.40 (m, 5H, Ar), 6.43 (s, 2H, NH_2), 4.48–4.77 (q, 2H, CH_2), 1.43–1.61 (t, 3H, CH_3). Anal. calcd. for $C_{15}H_2N_4O_5$ (328.28): C, 54.88; H, 3.68; N, 17.07. Found: C, 54.93; H, 3.66; N, 16.89. MS, *m/z* (%): 328 (M^+, 82.0), 300.0 (18.0), 283.0 (3.5), 181.0 (5.0), 268.0 (44.0), 162.0 (95.0), 140.0 (27.0), 134.0 (78.0), 107.0 (100), 106 (82.0), 77.0 (75.0).

Atom labeling is shown in Fig. 1.

6.6. 5-(4-Amino-5-benzoylisoxazol-3-yl) [1,3,4]oxadiazole-2-carboxamide **8a–i**

To a solution of the ester **7** (1 g, 3 mmol) in absolute ethanol (5 mL) was added the appropriate amines (4 mmol). The reaction mixture was refluxed for 2–3 h, and then allowed to attain room temperature. The separated solid was filtered, washed with cold ethanol, and recrystallized. Physical and analytical data are listed in Table 1. IR (cm^{-1}): 3475, 3365, 3240 (NH_2, NHCO), 1680, 1650, 1640 (C=O, C=N), 710 (Ar).

Compound ***8a***: ^{1}H NMR (DMSO-d_6) δ 7.55–8.29 (m, 5H, Ar), 7.12–7.20 (t, 1H, CONH), 6.48 (s, 2H, NH_2), 3.44–3.54 (m, 2H, N–CH_2–), 1.66–1.78 (m, 2H, CH_2), 1.01–1.06 (t, 3H, CH_3). MS, *m/z* (%): 341 (M^+, 34.0), 313 (11.0), 255 (7.0), 228 (9.0), 181 (35.0), 161 (17.0), 134 (20.0), 106 (44.0), 105 (100), 96.0 (43), 86.0 (13.0), 77.0 (6.0).

Compound ***8b***: ^{1}H NMR (DMSO-d_6) δ 7.50–8.28 (m, 5H, Ar), 6.39 (s, 2H, NH_2) 3.73–4.16 (m, 4H, $N(CH_2)_2$), 1.70–1.76 (m, 6H, $(CH_2)_3$).

Compound ***8i***: ^{1}H NMR (DMSO-d_6) δ 7.49–8.13 (m, 5H, Ar), 6.54 (s, 2H, NH_2), 3.96–4.00 (m, 4H, CH_2–O–CH_2), 3.61–3.71 (m, 4H, $N(CH_2)_2$).

6.7. Ethyl-5-(5-methyl-7-phenylisoxazolo[4,5-d]pyrimidin-3-yl)[1,3,4]oxadiazole-2-carboxy-late **9**

To a solution of the ester **7** (9.84 g, 0.03 mol) in anhydrous dioxane (100 mL) was added anhydrous acetonitrile (25 mL) and dry gaseous hydrogen chloride was bubbled into the solution for 6 h at room temperature. The reaction mixture was stirred at room temperature for 6 days. After the reaction the mixture was concentrated, cooled, and the separated solid was filtered, washed with water, dried, and recrystallized from ethyl acetate. The yield was 8.86 g (84.11%). The pure product melted at 191–192 °C. IR (cm^{-1}, KBr): 1745, 1640 (C=O, C=N), 1230 (C–O–C). ^{1}H NMR (DMSO-d_6) δ 8.16–8.24 (m, 2H, *o*,*o*′Ar), 7.65–7.73 (m, 3H, *m*,*p*,*m*′ Ar), 4.51–4.79 (q, 2H, CH_2), 3.05 (s, 3H, CH_3) <1.45–1.63 (t, 3H, CH_3). Anal. calcd. for $C_{17}H_{13}N_5O_4$ (351.32): C, 58.12; H, 3.73; N, 19.94. Found: C, 57.95; H, 3.57; N, 20.01.

6.8. Amidoderivatives of 3-(1,3,4-oxadiazol-2-yl)-5-methyl-7-phenylisoxazolo[4,5-d]-pyrimidine **10d–g**

6.8.1. Method A

The amide **8e** (0.79 g, 2 mmol) was dissolved in (10 mL) of anhydrous dioxane and (3 mL) anhydrous acetonitrile. Dry gaseous hydrogen chloride was bubbled into the solution for 6 h at room temperature. Stirring was maintained at room temperature for 6 days. The solvents were removed under reduced pressure, and the residue was treated with water, and then filtered. The resulting precipitate was washed with water, dried, and recrystallized.

6.8.2. Method B

To a solution of ester **9** (0.70 g, 2 mmol) in anhydrous tetrahydrofurane, appropriate amines (3 mmol) were added. The resulting suspension was heated under reflux for 2 h, and then allowed to attain room temperature. The precipitate formed was filtered off, washed with ethanol and water, dried, and recrystallized. Physical and analytical data are listed in Table 5.

Compound ***10d***: IR (cm^{-1}, KBr) 3330 (NH), 1690 (C=O), 1635, 1605, 1552 (C=N, C=C, aromatics), 1215 (C–O–C). ^{1}H NMR (DMSO-d_6) δ 9.75 (s, 1H, CONH), 7.75–8.21 (m, 5H, Ar), 3.40–3.64 (m, 6H, CH_2–O–CH_2 + CO–N–CH_2), 2.98 (s, 3H, CH_3), 2.47–2.64 (m, 6H, N=$(CH_2)_3$).

Compound ***10e***: IR (cm^{-1} KBr) 3335 (NH), 1690, 1640 (C=O, C=N), 710 (Ar). ^{1}H NMR (DMSO-d_6) δ 10.13–10.28 (t, 1H, CONH), 7.43–8.63 (m, 10H, Ar_2), 4.53–4.63 (d, 2H, CH_2), 3.00 (s, 3H, CH_3).

Compound ***10f***: IR (cm^{-1}, KBr) 3300 (NH), 1687, 1640, 1605 (C=O, C=N), 705 (Ar). ^{1}H NMR (DMSO-d_6) δ 10.18–10.22 (t, 1H, CONH), 7.40–8.69 (m, 9H, $C_6H_5 + C_5H_4N$), 4.57–4.63 (d, 2H, N–CH_2), 3.02 (s, 3H, CH_3).

Compound ***10g***: IR (cm^{-1}, KBr) 3305 (NH), 1680, 1640, 1603 (C=O, C=N). ^{1}H NMR (DMSO-d_6) δ 10.20–10.25

Table 5
Physical and analytical data of compounds **8a–i**, **10d–g**

Cpd. No.	Crystallized from	Mp	Yield	Formula	Molecular weight	Calc. (%)			Found (%)		
						C	H	N	C	H	N
8a	Ethyl acetate	206–207	76.92	$C_{16}H_{15}N_5O_4$	341.32	56.30	4.43	20.52	56.49	4.40	20.65
8b	Ethanol	168–169	67.86	$C_{18}H_{17}N_5O_4$	367.36	58.85	4.66	19.06	58.70	4.59	19.02
8c	Ethanol	257–258	60.24	$C_{19}H_{19}N_5O_4$	381.39	59.84	5.02	18.36	60.15	4.96	18.22
8d	Ethanol	186–187.5	72.93	$C_{19}H_{20}N_6O_5$	412.40	55.34	4.89	20.38	55.13	4.90	20.53
8e	Propanol	200–201	86.00	$C_{20}H_{15}N_5O_4$	389.37	61.69	3.88	17.99	62.01	3.75	18.24
8f	Ethanol	190–181	75.63	$C_{19}H_{14}N_6O_4$	390.36	58.46	3.61	21.53	58.70	3.54	22.05
8g	Ethanol	213–214	83.19	$C_{19}H_{14}N_6O_4$	390.36	58.46	3.61	21.53	58.86	3.59	21.82
8h	Ethanol	160–161.5	77.32	$C_{18}H_{18}N_6O_4$	382.37	56.54	4.74	21.98	56.48	4.76	22.16
8i	Acethyl acetate	246–248	62.17	$C_{17}H_{15}N_5O_5$	369.33	55.29	4.09	18.96	54.99	4.03	18.72
10d	Ethanol	209–210	69.36	$C_{21}H_{21}N_7O_4$	435.44	57.93	4.86	22.52	58.12	4.84	22.76
10e	Acethyl acetate	227–228	A. 70.47 B. 77.76	$C_{22}H_{16}N_6O_3$	412.41	64.07	3.91	20.38	64.38	4.00	20.62
10f	Ethanol	251–252	68.03	$C_{21}H_{15}N_7O_3$	413.41	61.01	3.66	23.72	60.86	3.70	23.49
10g	Ethanol	224–225	73.13	$C_{21}H_{15}N_7O_3$	413.39	61.01	3.66	23.72	61.17	3.61	23.91

(t, 1H, CONH), 7.44–8.69 (m, 9H, $C_6H_5 + C_5H_4N$), 4.59–4.66 (d, 2H, N–CH_2), 2.96 (s, 3H, CH_3).

6.9. Crystallographic part

6.9.1. X-ray diffraction studies

The X-ray diffraction data were collected at 100 K for a crystal of size $0.15 \times 0.25 \times 0.25$ mm. All measurements were made on a KM4 CCD computer-controlled κ-axis diffractometer with graphite-monochromated Mo Kα (0.71073 Å) radiation. The intensities were corrected for Lorentz and polarization effects, but no corrections were made for absorption. The structure was solved by direct methods with SHELXS97 and refined on F^2 by full-matrix least-square methods using the SHELXL97 [23] program. Non-hydrogen atoms were refined with anisotropic thermal parameters. The carbon-bonded H-atoms were included in calculating the positions and refined using a riding model with isotropic displacement parameters equal to 1.2 Ueq or 1.5 Ueq of the attached C atoms. The H-atoms of the amino groups were located from a difference Fourier map and refined.

Fig. 1 is a view of one of the two independent molecules prepared using the XP program [24].

Crystal data for **7**: $C_{15}H_{12}N_4O_5$, $T = 100(2)$ K, $M = 328.29$, triclinic, space group P-1, with $a = 8.242(2)$ Å, $b = 12.435(3)$ Å, $c = 14.041(3)$ Å, $\alpha = 88.49(3)$, $\beta = 75.61(3)^\circ$, $\gamma = 88.89(3)^\circ$, $V = 1393.2(5)$ Å^3, $Z = 4$, $D_c = 1.565$ g cm^{-3}, $\mu = 0.121$ mm^{-1}, $R = 0.0490$, $wR = 0.112$ (5420 observed reflections) for 448 variables.

There are two independent molecules A and B in the asymmetric unit, one is shown in Fig. 1. All the bond distances and angles are very close and do not deviate from those observed in similar compounds. The isoxazole and oxadiazole rings are planar within the limits of experimental error. The amino groups are situated in the planes of the isoxazole rings and the values of the torsion angles N(5)–C(4′)–C(5′)–O(1′) and N(51)–C(41′)–C(51′)–O(11′) are $-179.3(3)^\circ$ and $179.5(4)^\circ$, respectively. The main differences between the molecular structures could be attributed to the different planarity of the molecules.

The angle between the plane defined by the isoxazole ring atoms and the plane defined by the oxadiazole atoms is $11.7(2)^\circ$ in molecule A. In molecule B these groups have different positions and therefore the angle formed between them is $5.6(3)^\circ$.

Furthermore, the conformational difference between the two molecules is due to the 2-$COOCH_2CH_3$ group orientation. In both cases, this group is situated in the plane of the oxadiazole ring. The angles between the mean planes of the $COOCH_2CH_3$ portion of the oxadiazole ring are $2.5(4)^\circ$ (molecule A) and $11.3(4)^\circ$ (molecule B) and show slight deviations from co-planarity in both molecules.

The molecules A and B are connected to each other by two hydrogen bonds between the donor atoms (N(5), N(51)) and acceptor atoms (N(3), N(31)). The isoxazole rings of molecules A and B are connected to each other by two hydrogen bonds, in which N(3) (molecule A) and N(31) molecule (B) atoms are the acceptors for the amino groups (N(5), N(51)). The distances between the N(5)···N(3) and N(51)···N(31) atoms are 3.116(2) and 3.100(2) Å, respectively.

In the structure there are two intramolecular hydrogen bonds between amino N(5), N51 and O(4), O(41) oxygen atoms. The distances between the N(5)···(O4) and N(51)···O(41) atoms are 2.827(2) and 2.814(2) Å, respectively.

Furthermore, hydrogen bonding involving the amino H(5) and H(51) atoms are bifurcated and forms two intramolecular H-bonds with N(4) and N(41) atoms of the oxadiazole rings. The distances between the N(5)···N(4) and N(51)···N(41) are 2.970(2) and 2.938(2) Å, respectively.

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