Synthesis and Pharmacological Screening of Derivatives of Isoxazolo[4,3-*d*]pyrimidine and Isoxazolo[4,5-*d*]pyrimidine

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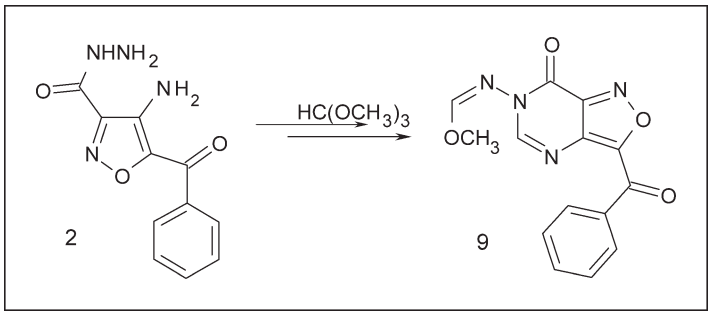

A number of derivatives of isoxazolo[4,3-*d*]pyrimidine and isoxazolo[4,5-*d*]pyrimidine were prepared with potential anticancer activity. Condensation of 4-amino-5-benzoyl-isoxazolo-3-carboxylic acid hydrazide with ethyl orthoformate and then with different amines gave a series of 3-benzoyl-7-oxo-7*H*-isoxazolo[4,3-*d*]pyrimidin-6-yl-aryliden-formamidine. A series of 5-aminomethyl-7-phenyl-isoxazolo[4,5-*d*]pyrimidine-3-carboxamide was obtained from 4-amino-5-benzoylisoxazole-3-carboxamide with acetonitrile, and chloroacetonitrile with gaseous hydrogen chloride. Some of these compounds were tested for their cytotoxic activity.

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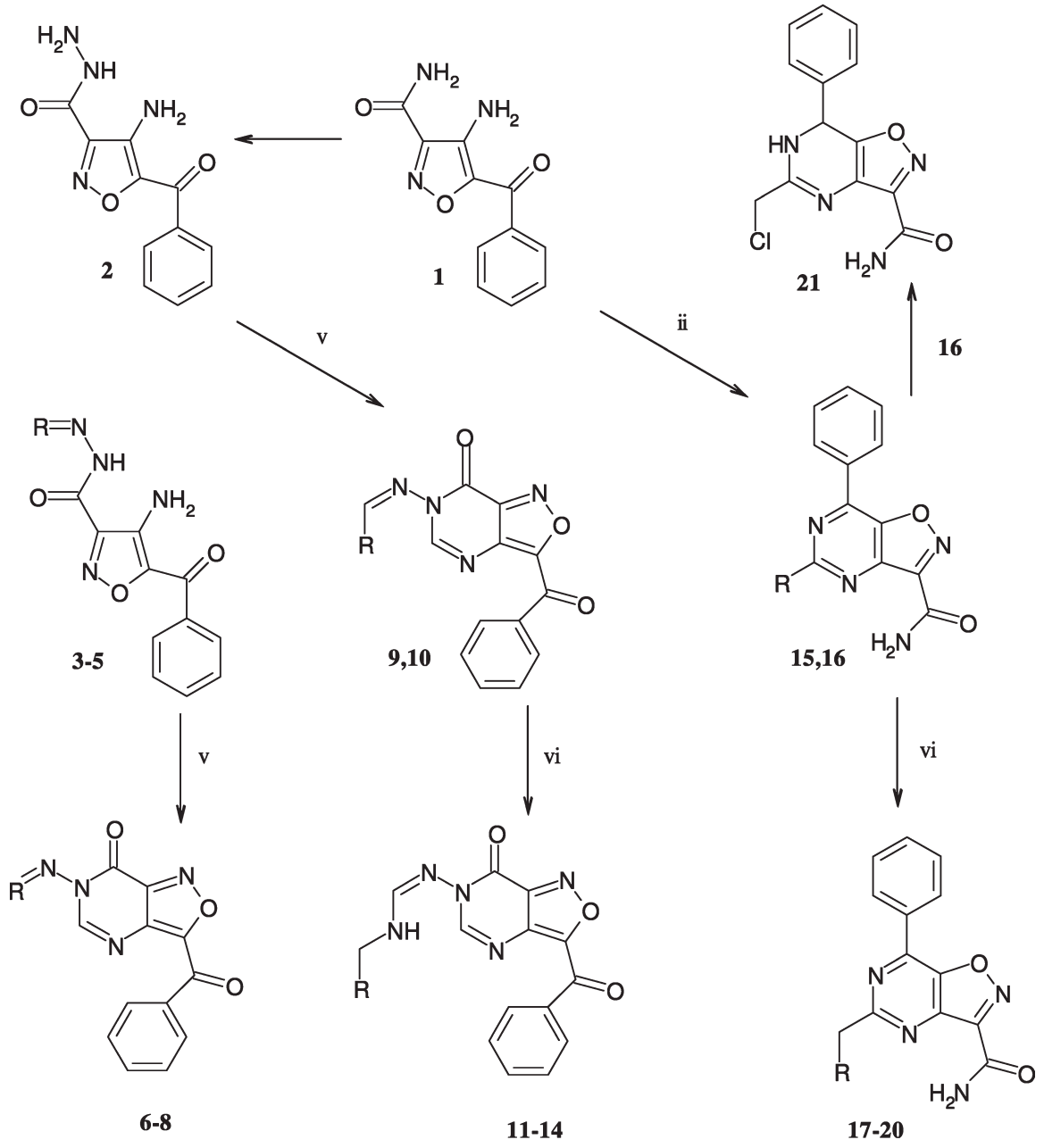
INTRODUCTION

The bases of the 9*H*-purines adenosine (adenine) and the adenosine metabolite inosine (hypoxantine) are endogenous ligands of different receptors. Adenosine is an endogenous ligand of adenosine receptors (AR). They are subdivided into four subtypes (A_1, A_{2A}, A_{2B}, and A_3) [1]. Adenosine receptors have been actively studied as potential therapeutic targets in several disorders, such as Parkinson's disease, schizophrenia, analgesia, and ischemia, and as cytostatics. Inosine is a highly selective ligand for the A_3 receptor subtype [2,3] and so is a ligand of the benzodiazepine receptor [4,5]. The nitrogen atoms at positions 3 and 7 of both purines and their derivatives take part in the formation of a hydrogen bond with these receptors [5,6]. For example, substitution of the nitrogen atom at position 7 in inosine gave 7-methylinosine, which was ineffective in binding to the benzodiazepine receptor [5]. Therefore, we have been working on synthetic derivatives of two isomers of isoxazolopyrimidine, 4,3-*d* and 4,5-*d*, which may be considered as 7,9-dideaza-7-oxa-8-aza purines, and which may have high biological and similar receptor activity. Only the isomers from four isomers of isoxazolopyrimidine have nitrogen and oxygen heteroatoms at positions analogous to the 9*H*-purines. The literature concerning derivatives of both isomers contains a few same papers in which the authors obtained isoxazolo[4,5-*d*]pyrimidine but did not assay their biological activity [7–9]. Some derivatives of isoxazolo[4,5-*d*]pyrimidine were shown to be CRF (corticotrophin releasing factor) antagonist and may be useful as cCMP phosphodiesterase inhibitors [10,11]. Another derivatives of isoxazolo[4,5-*d*]pyrimidine that are structurally similar to hypoxantine can have anxiolytic activity comparable to that of diazepam [12]. In contrast, compared with diazepam the derivatives of isoxazolo[4,3-*d*]pyrimidine are devoid of anticonvulsant and anxiolytic properties [13].

RESULTS AND DISCUSSION

Synthesis. In continuation of our program we report the synthesis of new derivatives of the title compounds. The synthetic pathways used to obtain the target compounds are depicted in Scheme 1. The starting material **1** was obtained by the Thorpe reaction according to Gewald et al. [14], by which it could be converted into hydrazide **2** [15]. Hydrazide **2** with aldehydes gave the new hydrazido-hydrazones **3**-**5**, which were condensed with ethyl orthoformate in the presence of the acetic anhydride, yielding the new derivatives of isoxazolo[4,3-

Scheme 1



Reagents: (i) H_2N-NH_2; (ii) nitrils; (v) $HC(OC_2H_5)_3$ or $HC(OCH_3)_3$; (vi) amines,

d]pyrimidines **6**-**8**. The acid hydrazide **2** was refluxed in a mixture with only equimolar amounts of triethyl ortho-formate, giving [4-amino-3-(1,3,4-oxadiazol-2-yl)isoxazol-5-yl]-(phenyl)methanone [15]. If hydrazide **2** was refluxed with an excess of triethyl orthoformate and acetic anhydride, then the derivatives of isoxazolo[4,3-*d*]pyrimidine **6**-**8** and **9**,**10** were formed, which with differ-rent amines formed new derivatives of 3-benzoyl-7-oxo-7*H*-isoxazole[4,3-*d*]pyrimidin-6-yl-aryliden-formamidine **11**–**14**. Compound **1** was heated in acetonitrile or chloroacetonitrile with gaseous hydrogen chloride to form the new derivatives of isoxazolo[4,5-*d*]pyrimidine **15**–**16**. Mechanism of the reaction was described earlier by Shishoo *et al* [16,17]. Compound **16** reacts with amines to form the new 5-(aminemethyl)-7-phenyl-isoxazolo[4,5-*d*]pyrimidine-3-carboxamide **17**–**20**. It is well

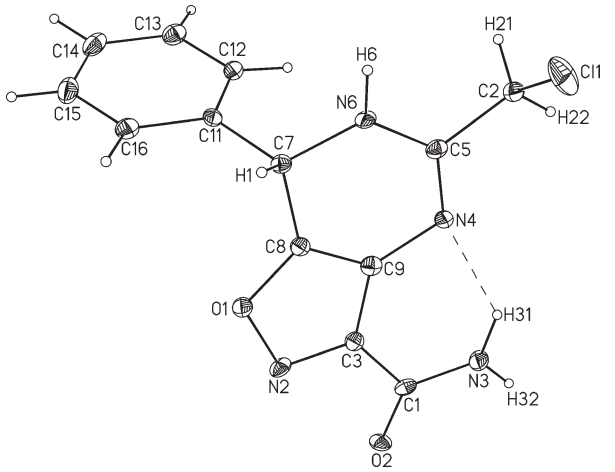


Figure 1. A view of the molecular structure of compound **21** with the atomic numbering scheme. Ellipsoids are at the 35% probability level.

known that isoxazole rings are readily cleaved by hydrogenation [18–21]. Thus the reduction of compound **16** followed by treatment with sodium borohydride in methanol at room temperature gave compound **21** with only one reduced double bond at position 6-7 from four. X-ray crystallography of compound **9** (Fig. 1) confirmed that the resulting structure was indeed compound **21** and **15**,**16**, and **17**-**20**.

However, amid **1** readily reacts with nitriles and forms compounds **15**,**16**. Of the compounds selected by the staff members of the NCI the derivatives isoxazole **3c**, isoxazole[4,3-*d*]pyrimidine **9**, and isoxazole[4,5-*d*]pyrimidine **19** were interesting.

Crystallographic part. X-ray diffraction studies: The X-ray diffraction data were collected at 100 K for a crystal of size 0.2 × 0.25 × 0.25 mm. All measurements were made on a KM4 CCD computer-controlled κ-axis diffractometer with graphite-monochromated MoK_{α}(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 SHELXS-97 and refined by full-matrix least-squares methods on F^2 using the SHELXL-97 [22] program. Nonhydrogen atoms were refined with anisotropic thermal parameters. All the H atoms were located using a differrence Fourier map and refined.

Figure 1 was drawn using the XP program [23]. Crystal data for 5-chloromethyl-7-phenyl-6,7-dihydro-isoxazlo[4,5-d]pyrimidine-3-carboxamide **21**: $C_{13}H_{11}N_4O_2Cl$, T = 100(2) K, M = 290.71, orthorhombic, space group $Pna2_1$, with a = 14.713(2) Å, b = 16.745(2) Å, c = 5.236(1) Å, V = 1290.0(3) $Å^3$, Z = 4, D_c = 1.4969 g cm^{-3}, μ = 0.303 mm^{-1}. There were 2930 unique reflections, of which 2553 were considered as observed, with final R = 0.0439 and wR = 0.079.

The molecular structure and atom numbering scheme are shown in Figure 1. Compound **21** crystallizes in a noncentrosymmetric $Pna2_1$ space group with one molecule per asymmetric unit. The determination of the structure of compound **21** confirms the S configuration of the chiral atom C7. The interatomic distances for C5-N4 [1.310(3) Å] and C3-N2 [1.320(3) Å] are typical of carbon-nitrogen double bonds [24] (1.279–1.329 Å) and the C9-N4 [1.401(3) Å] and C7-N6 [1.476(3) Å], bond lengths correspond to typical carbon-nitrogen single bonds (1.366–1.454 Å).

The isoxazole and pyrimidine rings are essentially planar, with maximum deviations from the calculated mean plane of 0.006(2) Å (C9) and 0.036(2) Å (C8), respectively. The dihedral angle between the best planes through the isoxazole ring and the pyrimidine ring is 3.0(2)°, indicating that the whole molecule is almost planar. The carboamide moiety deviates slightly from the isoxazole ring plane, the N3-C1-C3-N2 and O2-C1-C3-C9 torsion angles being −172.4(2)°, and –172.5(3)° respectively. The chloromethyl and phenyl moieties are nearly perpendicular, to the pyrimidine ring.

The dihedral angles between the pyrimidine ring plane and the Cl1-C2-C5 and C11-C16 planes are 66.6(1)° and 77.8(1)°, respectively.

In the present structure the nearly planar conformation of the title compound is stabilized by a network of intermolecular and intramolecular hydrogen bonds.

The hydrogen bond involving the amide H31 atom is bifurcated, H31 forming one intramolecular hydrogen bond with the N4 atom of the pyrimidine ring and the Cl1 atom of the chloromethyl moiety. The distances between the N(2)-H31...N(4) and N2-H31...Cl1(−x + 1, −y + 1, z − 1/2) atoms are 2.946(3) and 3.552(2) Å, respectively. Furthermore, the amide H6 forms an N6-H6...O2(x − 1/2, −y + 1/2, z + 1) hydrogen bond with the O2 atom of the carboamide group. The distances N6...O2 and H6...O2 are 2.908(3) and 2.22(3) Å, and the angle is 139(2)°. Additionally, the amide H32 atom on N2 interacts with the ring centroid Cg of the phenyl ring, forming a nonconventional hydrogen bond of the N2-H32...π (Cg) [H32... π, 2.71 Å N2... π 3.443(3) Å, N2-H32... π 149°; symmetry code: x + 1/2, −y + 1/2, z−1]. The molecules are also linked by the C7-H1...N4, C2-H21...O2, C12-H12...O2, and C15-H15...O1[3.523(3), 3.149(3), 3.550(3), and 3.437(3) Å] weak hydrogen bonds to form a chain running parallel to the axis. The distances H...O(N) [2.51(3)–2.85(3) Å] and angles [126(2)–163(2)°] suggest some kind of weak C—H...X hydrogen bond.

CCDC726160 contains the supplementary crystallographic data for this article. These data can be obtained free of charge at www.ccdc.cam.ac.uk/const/retrieving.html (or from the Cambridge Crystallographic Data

Table 1

Characterization data of compounds **3–20** and growth percent of some selected *in vitro* tumor cell lines (10 μmol).[a]

Compound	R	Ovarian Cancer OVCAR-8	Leukemia RPMI-8226	Renal Cancer UO-31	Melanoma SK-MEL-2
3	$CHCH_3$				
4	CHC_6H_5				
5	CHC_6H_4-Cl-4	45.80		**−10.37**	57.66
6	$CHCH_3$	41.53		62.54	42.59
7	CHC_6H_5				
8	C_6H_4-Cl-4				
9	OCH_3	83.79	25.74	**−12.84**	60.83
10	OC_2H_5				
11	4-Pyridine	45.76		63.61	39.94
12	C_6H_5				
13	C_6H_4Cl-4				
14	C_6H_4-F-4				
15	CH_3				
16	CH_2Cl				
17	4-Morpholine				
18	4-Methylpiperazine				
19	NH-4-morpholine	42.20	55.00	**−17.94**	
20	$N(C_4H_9)_2$				

[a] Data obtained from the NCI's *in vitro* human tumor cell screen.

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Cytotoxic screening. The cytotoxic studies were performed at the National Cancer Institute, (NCI, Bethesda, MD). The screening is a two-stage process beginning with the evaluation of the compound against 60 different human tumor cell lines representing leukemia, melanoma, and cancers of the lung, colon, brain, breast, ovary, prostate, and kidney at a single dose of 10 μmol.

Evaluation of the cytotoxic activity was performed for compounds **5**, **6**, **9**, **11**, and **19**. Only compounds **5**, **9**, and **19** showed interesting activity in the panel 62 tumor cell lines, but chiefly on the renal cancer line UO-31. Data on the active compounds are presented in Table 1.

EXPERIMENTAL

Melting points were determined with a Boethius apparatus and are uncorrected. The progress of the reaction and the purity of the compounds were monitored by TLC on analytical silica gel plates (Merck F_{254}, Darmstadt, Germany). IR spectra were recorded on a Specord M80 spectrometer (Zeiss/Analytic Jena, Germany) for KBr dises. ^{1}H NMR spectra were recorded with a Bruker Avance ARX-300 instrument (Bruker Analytic, Karlsruhe, Germany; Bruker AG, Fallanden, Switzerland). Chemical shifts are reported in ppm downfield from the internal tetramethylsilane reference and the coupling constants are in Hz. Mass spectra were recorded on a Finningan Mat 95 GC-MS (Finningan, Bremen, Germany) with an ionization energy of 70 eV. Elemental analyses were performed on a Perkin Elmer 2400 analyzer (Waltham, MA) and the results are within ± 0.4% of the theoretical values obtained for the new compounds. The chemicals and reagents for synthesis were obtained from Alfa Aesar (Karlsruhe, Germany), Chempur (Piekary Sl., Poland), and Lancaster (Frankfurt am Main).

General procedure for the synthesis of compounds 3-5. To a stirred solution of acid hydrazide **2** (2.46 g, 0.01 mol) in 20 mL of anhydrous 1,4-dioxane heated at 80°C, the appropriate aldehyde (0.012 mol) was added. The reaction mixture was heated under reflux for 5 h and the solvent was rotoevaporated. The resulting solid was recrystallized from the appropriate solvent.

4-Amino-5-benzoyl-N-(ethylideneamino)isoxazole-3-carboxamide (3). Yield: 1.78 g (65.5%), mp 191°C (1,4-dioxane); IR (potassium bromide): 3500, 3395 (NH_2), 3260 (NH), 1685, 1660 (CO), 1640 (C=N) cm^{-1}; ^{1}H NMR (DMSO-d_6): δ 1.94–2.00 (d, 3H, CH_3, J = 6.0 Hz), 6.41 (s, 2H, NH_2), 7.68 (m, 3H, arom.), 7.80–7.90 (q, 1H, CH, J = 6.0 Hz), 8.00–8.10 (d, 2H, arom.), 12.06 (s, 1H, HN-CO). Anal. Calcd. for $C_{13}H_{12}N_4O_3$: C, 57.35; H, 4.44; N, 20.58. Found: C, 57.21; H, 4.38; N, 20.37.

4-Amino-5-benzoyl-N-(phenylmethylenebenzylideneamino)isoxazole-3-carboxamide (4). Yield: 2.52 g (75.5%), mp 215C (ethanol); IR (potassium bromide): 3420, 3305 (NH_2), 3260 (NH), 1685, 1660 (CO), 1640 (C=N) cm^{-1}; ^{1}H NMR (DMSO-d_6): δ 6.44 (s, 2H, NH_2), 7.50–8.66 (m, 10H, arom.), 8.57 (s, 1H, CH), 12.42 (s, 1H, HN-CO). Anal. Calcd. for $C_{18}H_{14}N_4O_3$: C, 64.67; H, 4.22; N, 16.76. Found: C, 64.80; H, 4.23; N, 16.47.

4-Amino-5-benzoyl-isoxazole-3-carboxylic acid (4-chlorobenzylidene)-hydrazide (5). Yield: 2.54 g (69.3%), mp 249°C (THF-ethanol 1:2); IR (potassium bromide): 3480, 3385 (NH_2), 3210 (NH), 1705, 1660 (CO), 1650 (C=N) cm^{-1}; ^{1}H NMR (DMSO-d_6): δ 6.49 (s, 2H, NH_2), 7.58–8.05 (m, 9H, arom.), 8.55 (s, 1H, CH), 12.48 (s, 1H, HN-CO). Anal. Calcd. for $C_{18}H_{13}ClN_4O_3$: C, 58.63; H, 3.55; N, 15.19. Found: C, 58.81; H, 3.48; N, 14.98.

General procedure for the synthesis of compounds 6-8. To an equimolar mixture of 12.5 mL of acetic anhydride and 22 mL of triethyl orthoformate was added 0.008 mol of

the compounds **3**–**5**, or **3c** and refluxed for 5 h. After concentration *in vacuo* the residual oil was poured into 50 mL of 10% ethanolic ammonia. After 1 h of stirring at room temperature the mixture was refrigerated. The resulting solid was filtrated, washed with water, vacuum dried, and recrystallized from the appropriate solvent.

3-Benzoyl-6-ethylideneamino-6H-isoxazolo[4,3-d]pyrimidin-7-one (6). Yield: 2.23 g (79.1%), mp 180°C (1,4-dioxane); IR (potassium bromide): 1740, 1660 (CO), 1650 (C=N) cm^{-1}; ^1H NMR (DMSO-d_6): δ 2.18–2.20 (d, 3H, CH_3, J = 6.0 Hz), 7.59–7.64 (m, 3H, arom.), 7.74–7.80 (q, 1H, CH J = 6.0 Hz), 8.02–8.05 (m, 2H, arom.), 8.57 (s, 1H, pos. 5) Anal. Calcd. for $C_{14}H_{10}N_4O_3$: C, 59.57; H, 3.57; N, 19.85. Found: C, 59.38; H, 3.64; N, 20.04.

3-Benzoyl-6-(benzylidene-amino)-6H-isoxazolo[4,3-d]pyrimidin-7-one (7). Yield: 2.49 g (72.5%), mp 205°C (ethanol); ir (potassium bromide): 1720, 1700 (CO), 1650 (C=N) cm^{-1}; ^1H NMR (DMSO-d_6): δ 7.55–8.07 (m, 10H, arom.), 8.48 (s, 1H, CH), 9.15 (s, 1H, CH, pos. 5). Anal. Calcd. for $C_{19}H_{12}N_4O_3$: C, 66.28; H, 3.51; N, 16.27. Found: C, 66.29; H, 3.58; N, 16.11.

3-Benzoyl-6-[(4-chloro-benzylidene)-amino]-6H-isoxazolo[4,3-d]pyrimidin-7-one (8). Yield: 2.24 g (59.4%), mp 235°C (THF); IR (potassium bromide): 1715, 1695 (C=O), 1650 (C=N) cm^{-1}; ^1H NMR (DMSO-d_6): δ 7.61–8.11 (m, 9H, arom.), 8.47 (s, 1H, CH), 9.20 (s, 1H, CH pos. 5). Anal. Calcd. for $C_{19}H_{11}$ Cl N_4O_3: C, 60.25; H, 2.93; N, 14.79. Found: C, 60.46; H, 3.06; N, 15.03.

Methyl N-(3-Benzoyl-7-oxo-7H-isoxazolo[4,3-d]pyrimidin-6-yl)-formimidate (9). A solution of 24.6 g (0.1 mol) of the acid hydrazide **2** in an equimolar mixture of 75 mL acetic anhydride and 87 mL of trimethyl orthoformate was refluxed for 4 hours. Then the reaction mixture was concentrated *in vacuo*, filtered, and recrystallized to give compound **9**. Yield: 2.26 g (76.0%), mp 226°C (methanol); IR (potassium bromide):1760, 1680 (C=O), 1660,1650 (C=N), 1160 (C—O—C) cm^{-1}; ^1H NMR (DMSO-d_6): δ 2.09 (s, 3H, CH_3), 7.59–8.06 (m, 5H, arom.), 8.19 (s, 1H, pos. 5), 11.41 (s, 1H, pos. 6). Anal. Calcd. for $C_{14}H_{10}N_4O_4$: C, 56.38; H, 3.38; N, 18.78. Found: C, 56.12; H, 3.22; N, 18.69.

Ethyl N-(3-Benzoyl-7-oxo-7H-isoxazolo[4,3-d]pyrimidin-6-yl)formimidate (10). A solution of 2.46 g (0.01 mol) of the acid hydrazide **2** in a mixture of 12.5 mL acetic anhydride and 22 mL of trimethyl orthoformate was refluxed for 4 h. Then the reaction mixture was concentrated *in vacuo*, filtered, and recrystallized to give compound **10**. Yield: 2.12 g (68.0%), mp 222°C (1,4-dioxane); IR (potassium bromide): 1710, 1690 (C=O), 1650 (C=N), 1155 (C—O—C) cm^{-1}; ^1H NMR (DMSO-d_6): δ 1.34–1.39 (t, 3H, CH_3), 4.33–4.40 (q, 2H, CH_2), 7.59–8.05 (m, 5H, arom.), 8.23 (s, 1H, pos. 5), 8.56 (s, 1H, pos. 6). Anal. Calcd. for $C_{15}H_{12}N_4O_4$: C, 57.69; H, 3.87; N, 17.94. Found: C, 57.86; H, 3.83; N, 17.87.

General procedure for the synthesis of compounds 11-14. To a stirred solution of compound **9** (0.596g, 0.002 mol) in 15 mL of anhydrous 1,4-dioxane was added the appropriate amine (0.004 mol) and heated at 80°C for 4 h. The hot reaction mixture was treated with charcoal and filtered. Concentration of the filtrate to a small volume and addition of 5 mL ethanol resulted in the separation of a colorless solid which was collected by filtration, dried, washed with methanol, and recrystallized from ethanol.

N-(3-Benzoyl-7-oxo-7H-isoxazolo[4,3-d]pyrimidin-6-yl)-N′-pyridin-4-ylmethyl-formamidine (11). Yield: 2.08 g (55.6%), mp 195°C (ethanol); IR (potassium bromide): 3300 (NH), 1725, 1680 (C=O), 1645 (C=N) cm^{-1}; ^1H NMR (DMSO-d_6): δ 4.38 (s, 1H, NH), 4.61 (s, 2H, CH_2), 6.56 (s, 1H, CH), 7.27–7.42 (m, 3H, arom.), 7.56–7.68 (m, 3H, arom. + CH at pos. 5), 8.05–8.12 (m, 2H, arom.), 8.50–8.57 (dd, 2H, arom.). Anal. Calcd. for $C_{19}H_{14}N_6O_3$: C, 60.96; H, 3.77; N, 22.45. Found: C, 60.74; H, 3.65; N, 22.18.

N-(3-Benzoyl-7-oxo-7H-isoxazolo[4,3-d]pyrimidin-6-yl)-N′-benzyl-formamidine (12). Yield: 2.20 g (59%), mp 164°C (ethanol); IR (potassium bromide): 3300 (NH), 1715, 1680 (C=O), 1645 (C=N) cm^{-1}; ^1H NMR (DMSO-d_6): δ 4.68 (s, 1H, NH), 5.19 (s, 2H, CH_2), 6.72 (s, 1H, CH), 7.23–7.40 (m, 6H, arom.), 7.59–7.78 (m, 3H, arom. and H at pos. 5), 8.03–8.10 (m, 2H, arom.). Anal. Calcd. for $C_{20}H_{15}N_5O_3$: C, 64.34; H, 4.05; N, 18.76. Found: C, 64.56; H, 4.11; N, 18.89.

N-(3-Benzoyl-7-oxo-7H-isoxazolo[4,3-d]pyrimidin-6-yl)-N′-[(4-chloro-benzyl)-formami-dine (13). Yield: 2.03 g (49.8%), mp 169°C (ethanol); IR (potassium bromide): 3310 (NH), 1710, 1690 (C=O), 1640 (C=N) cm^{-1}; ^1H NMR (DMSO-d_6): δ 4.33 (s, 1H, NH), 4.56 (s, 2H, CH_2), 6.55 (s, 1H, CH), 7.18–7.46 (m, 5H, arom.), 7.56–7.68 (m, 3H, arom. and CH at pos. 5), 8.03–8.19 (m, 2H, arom.). Anal. Calcd. for $C_{20}H_{14}ClN_5O_3$: C, 58.90; H, 3.46; N, 17.17. Found: C, 59.08; H, 3.51; N, 17.30.

N-(3-Benzoyl-7-oxo-7H-isoxazolo[4,3-d]pyrimidin-6-yl)-N′-(4-fluoro-benzyl)-formami-dine (14). Yield: 1.76 g (45.0%), mp 2202°C (ethanol); IR (potassium bromide): 3280 (NH), 1720, 1690 (C=O), 1645 (C=N) cm^{-1}; ^1H NMR (DMSO-d_6): δ 4.32 (s, 1H, NH), 4.55 (s, 2H, CH_2), 6.54 (s, 1H, CH), 7.15–7.41 (m, 5H, arom.), 7.56–7.71 (m, 3H, arom. and CH at pos. 5), 8.06–8.08 (m, 2H, arom.). *Anal*. Calcd. for $C_{20}H_{14}FN_5O_3$: C, 61.38; H, 3.61; N, 17.89. Found: C, 61.49; H, 3.70; N, 18.08.

5-Methyl-7-phenyl-isoxazolo[4,5-d]pyrimidine-3-carboxamide (15). To a solution of amide **1** (2.31 g, 0.01 mol) in anhydrous 1,4-dioxane (40 mL) was added anhydrous acetonitrile (7 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. Then the reaction mixture was concentrated, cooled, and the resulting solid was filtered, washed with water, dried, and recrystallized from ethanol. Yield: 2.17 g (85.3%), mp 227°C (ethanol); IR (potassium bromide): 3250 ($CONH_2$), 1690 (C=O), 1600 (C=N), 760 (phenyl) cm^{-1}; ^1H NMR (DMSO-d_6): δ 3.00 (s, 3H, CH_3), 7.70–7.73 (m, 3H, arom.), 8.48–8.49 (m, 2H, arom.), 8.50–8.52 (ss, 2H, NH_2). Anal. Calcd. for $C_{13}H_{10}N_4O_2$: C, 61.41; H, 3.96; N, 22.04. Found: C, 61.62; H, 4.01; N, 21.84.

5-Chloromethyl-7-phenyl-isoxazolo[4,5-d]pyrimidine-3-carboxamide (16). Compound **16** was prepared in the same manner as described above for compound **15** except that 23.1 g (0.1 mol) of amide **1** was added to a mixture of 330 mL anhydrous 1,4-dioxane and 90 mL anhydrous chloroacetonitrile. Yield: 2.33 g (81.0%), mp 226°C (benzene); Compound **16** was obtained by another method described in our previous paper [25].

General procedure for the synthesis of compounds 17-20. A stirred mixture of compound **16** (0.576 g, 0.002 mol), 0.05 g KI, and the corresponding amine in 30 mL of anhydrous toluene was heated to ca. 80°C for 6 h. After cooling,

water (30 mL) was added and the reaction mixture was extracted with dichloromethane. The combined organic phases were washed with water, dried (Na_2SO_4), and concentrated to give a residue, which was recrystallized.

5-Morpholin-4-ylmethyl-7-phenyl-isoxazolo[4,5-d]pyrimidine-3-carboxamide (17). Yield: 2.71 g (80.1%), mp 208°C (methanol); IR (potassium bromide): 3280 (NH_2), 1715 (C=O), 1620 (C=N), 1120 (C—O—C) cm^{-1}; ^1H NMR (DMSO-d_6): δ 2.57–2.60 (m, 4H, CH_2—CH_2), 3.94 (s, 2H, CH_2), 7.58–7.68 (m, 3H, arom.), 8.41–8.45 (m, 4H, 2H, arom. and 2H, NH_2). Anal. Calcd. for $C_{17}H_{17}N_5O_3$: C, 60.17; H, 5.05; N, 20.64. Found: C, 59.79; H, 5.15; N, 20.88.

5-(4-Methyl-piperazin-1-ylmethyl)-7-phenyl-isoxazolo[4,5-d]pyrimidine-3-carboxamide (18). Yield: 2.16 g (61.5%), mp 188°C (methanol); Compound **18** was obtained by another method described in our previously paper [13], the physical and analytical data are identical.

5-(Morpholin-4-ylaminomethyl)-7-phenyl-isoxazolo[4,5-d]pyrimidine-3-carboxamide (19). Yield: 2.48 g (70.4%), mp 228°C (methanol); Yield: 2.48 g (70.4%), mp 228°C (methanol); IR (potassium bromide): 3300, 3280 (NH, NH_2), 1705 (C=O), 1630 (C=N), 1115 (C—O—C) cm^{-1}; ^1H NMR (DMSO-d_6): δ 2.21–2.23 (m, 4H, CH_2—CH_2), 3.57–3.61 (m, 4H, CH_2—CH_2), 3.94 (s, 2H, CH_2), 4.31 (s, 1H, NH), 7.65–7.67 (m, 3H, arom.), 8.36 (s, 1H, NH), 8.42–8.44 (m, 2H, arom.), 8.48 (s, 1H, NH). Anal. Calcd. for $C_{17}H_{18}N_6O_3$: C, 57.62; H, 5.12; N, 23.72. Found: C, 57.38; H, 5.18; N, 24.01.

5-Dibutylaminomethyl-7-phenyl-isoxazolo[4,5-d]pyrimidine-3-carboxamide (20). Yield: 2.84g (74.6%), mp 130°C (1,4-dioxane); IR (potassium bromide): 3300, 3200 (NH_2), 1680 (C=O), 1630 (C=N) cm^{-1}; ^1H NMR (DMSO-d_6): δ 0.82 (t, 6H, 2xCH_3), 1.20–1.32 (m, 4H, CH_2CH_2), 1.41–1.50 (m, 4H, CH_2CH_2), 2.49–2.57 (m, 4H, $N(CH_2)_2$), 4.03 (s, 2H, CH_2), 7.66–7.68 (m, 3H, arom.), 8.36 (s, 1H, NH), 8.44–8.47 (m, 2H, arom.), 8.49 (s, 1H, NH). Anal. Calcd. for $C_{21}H_{27}N_5O_2$: C, 66.12; H, 7.13; N, 18.39. Found: C, 66.36; H, 7.06; N, 18.64.

5-Chloromethyl-7-phenyl-6,7-dihydro-isoxazolo[4,5-d]pyrimidine-3-carboxamide (21). Sodium borohydride (2 g) was added portionwise to a stirred solution of compound **16** (1.44 g, 0.005 mol) in 90 mL of methanol at room temperature under nitrogen. The reaction mixture was stirred for 12 h, concentrated to ca. 30 mL *in vacuo*, and refrigerated. The solid was filtered, washed with water, vacuum dried, and recrystallized. Yield: 1.48 g (51.0%), mp 177°C (methanol); Anal. Calcd. for $C_{13}H_{11}ClN_4O_2$: C, 53.71; H, 3.81; N, 19.27. Found: C, 53.43; H, 3.82; N, 19.43.

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