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Amides of substituted 3-(pteridin-6-yl)propanoic acids: synthesis, spectral characteristics, and cytotoxic activity

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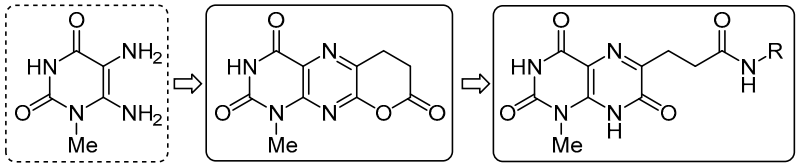


A heterocyclization reaction of 5,6-diamino-1-methyluracil and 2-oxopentanedioic acid was used in this study to synthesize 3-(1-methyl-2,4,7-trioxo-1,2,3,4,7,8-hexahydropteridin-6-yl)propanoic acid, intramolecular cyclization of which led to the formation of a tricyclic lactone, namely, 1-methyl-6,7-dihydro-2*H*-pyrano[3,2-*g*]pteridine-2,4,8-(1*H*,3*H*)-trione. Reactions of the latter with *N*-nucleophiles gave a series of amides – structural analogs of antifolates. The structure and identity of the synthesized compounds were confirmed by IR spectroscopy, ^{1}H and ^{13}C NMR spectroscopy, LC-MS analysis, and mass spectrometry. It was established that the synthesized compounds showed cytotoxic effects against human hepatocellular carcinoma (HepG2) cells and may be of interest for further studies of their antitumor activity against other cell lines.

Keywords: amides, antifolates, 5,6-diamino-1-methyluracil, 3-(1-methyl-2,4,7-trioxo-1,2,3,4,7,8-hexahydropteridin-6-yl)propanoic acid, *N*-nucleophiles, cytotoxicity, heterocyclization, structural similarity.

Folic acid is well known as a substrate and coenzyme for cellular metabolism,[1] as it participates in many biochemical processes, first of all in the biosynthesis of nitrogenous purine bases that are an integral part of nucleic acids.[2] Antifolates are structural analogs of folic acid that inhibit the metabolic pathways involving folates and their coenzyme forms. Antifolates have found applications in medicine as antibacterial, antimalarial, and antitumor drugs.[3–5] The currently used antifolates, as well as those in the pharmaceutical research and development pipeline are divided into classical and nonclassical types on the basis of their structural features and, as a consequence, the mechanisms of membrane transport. It is important to note that the strategies of targeted search for classical antifolates have been based on structural modifications of folic acid and have involved the reduction of pyridazine ring system and formylation at position 5 (Fig. 1, route 1), replacement of hydroxy group at position 4 with an amino group (route 2) followed by methylation of the exocyclic amino group (route 3) or its replacement with a hydrocarbon substituent (route 4). At the same time, few studies have been devoted to the discovery of biologically active agents among folic acid analogs by altering the sequence of structural features in the *p*-aminobenzoyl-1-glutamate moiety (position 6) and replacing the amino group with an oxo group (position 2, route 5).

The aim of this work was to synthesize 3-(1-methyl-2,4,7-trioxo-1,2,3,4,7,8-hexahydropteridin-6-yl)propanamides as promising antifolates showing cytotoxic activity, with possibilities of their further structural modification for the purpose of discovering new antitumor agents.

In order to achieve this goal, 1-methyl-5,6-diaminouracil (**1**) was selected as the starting compound and was obtained according to a published procedure from the respective nitroso derivative.[6,7] The pteridine system was formed according to the known method for heterocyclization of

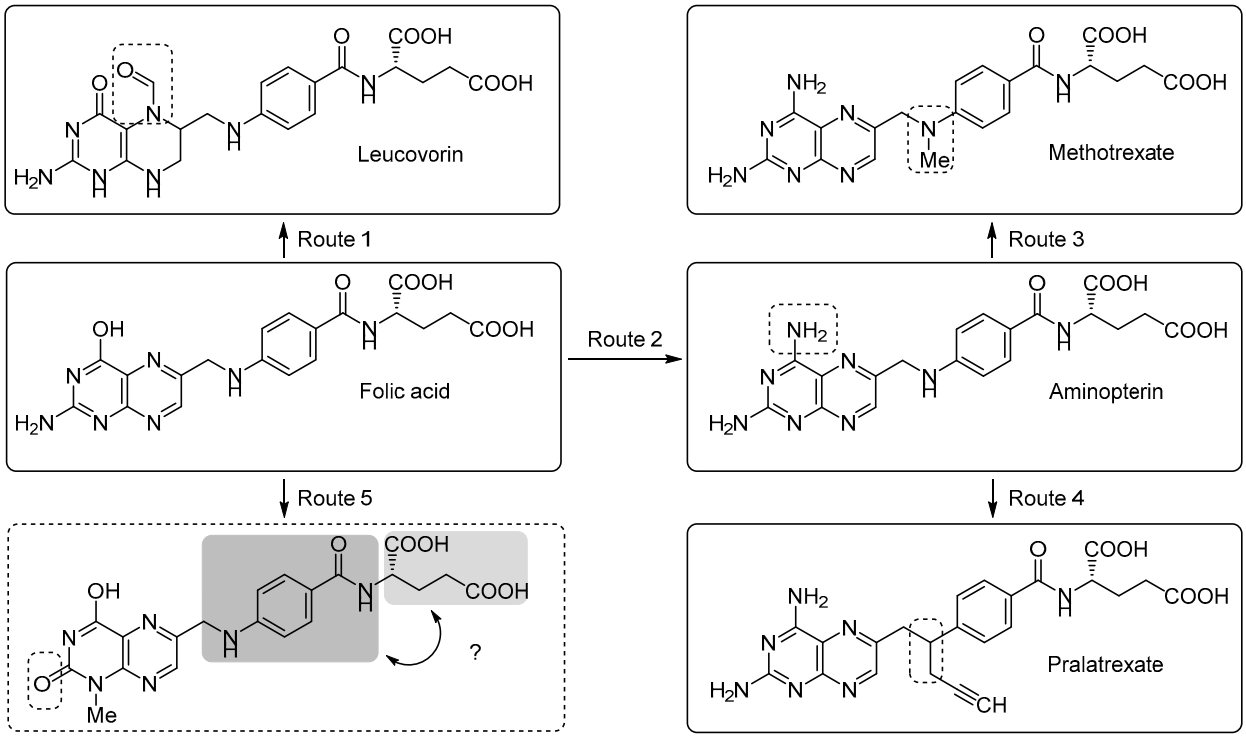


Figure 1. Structural modification of folic acid in the design of classical antifolates with therapeutic activity.

1,2-dicarbonyl compounds with substituted 5,6-diaminouracils.[8–10] Thus, the synthesis of acid **2** was performed by heating 5,6-diamino-1-methyluracil (**1**) with 2-oxopentanedioic acid in AcOH as a solvent (Scheme 1). It was established that the reaction occurred regioselectively and primarily at the more nucleophilic amino group at position 5 of the substituted uracil and the carbonyl group at the α-position of 2-oxopentanedioic acid *via* the formation of an ylidene intermediate.[11,12]

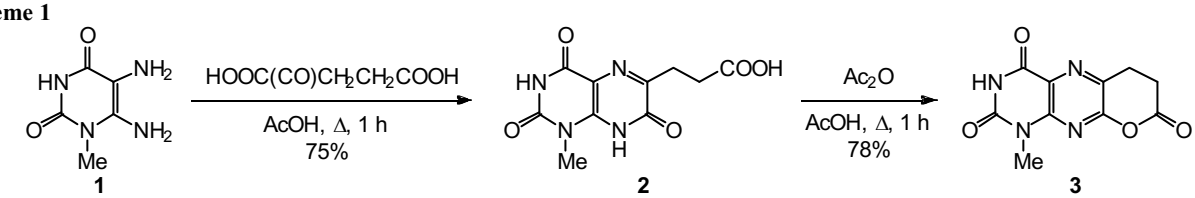
The obtained acid **2** represents a promising lead structure for further modification, and one of the most obvious approaches involves the transformation of carboxyl group into an amide group. This direction of research is additionally motivated by the fact that many of the known classical antifolates contain an amide group at position 6 (Fig. 1).

A wide range of methods are currently known for the synthesis of amides from the respective carboxylic acids, the most common of which are based on the reactions of amines with *in situ* generated activated carboxylic acid derivatives.[13] These methods often require the use of costly or toxic reagents, additional purification of solvents, and specific reaction conditions. Taking into account the aforementioned facts, as well as the structural features of acid **2**, we decided to develop a method for the synthesis of amides on the basis of a reaction between *N*-nucleophiles and lactone **3**. The latter can be obtained in high yield by heating 3-(1-methyl-2,4,7-trioxo-1,2,3,4,7,8-hexahydropteridin-6-yl)propanoic acid (**2**) in a mixture of AcOH and Ac_2O (Scheme 1).

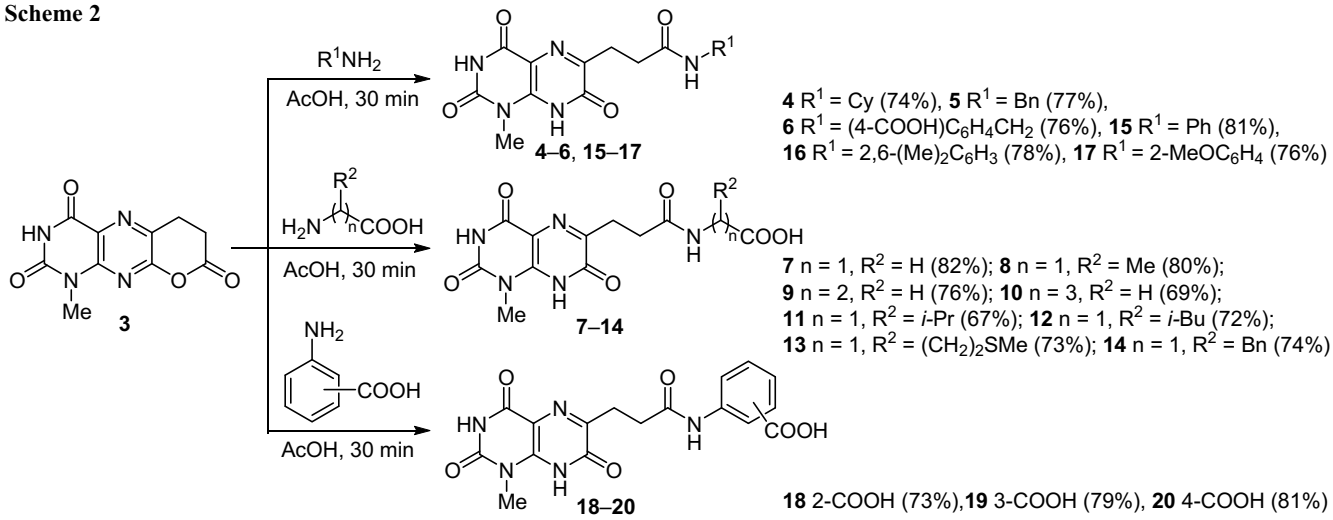
The formation of acid **2** and lactone **3** was confirmed from the obtained spectral data. Thus, ^{1}H NMR spectrum of compound **2** contained a characteristic two-proton multiplet signal at 11.57–11.98 ppm, assigned to the carboxyl group and the proton at position 8.[14] A singlet signal due to the proton at position 3 was observed in the downfield region at 11.42 ppm. The high-field region contained signals due to AB system, including two triplets at 2.93 and 2.69 ppm, corresponding to the ethylene moiety at position 6, as well as a three-proton singlet at 3.44 ppm, due to the methyl group at position 1. The formation of lactone ring (compound **3**) had a substantial effect on the spectra. Thus, ^{1}H NMR spectrum of compound **3** lacked the signals of carboxyl group protons and the protons at position 8 of the pteridine ring, while the signals of ethylene moiety showed a strong paramagnetic shift and were observed in the form of triplets at 3.06 and 3.25 ppm.

^{13}C NMR spectra of compounds **2**, **3** provided an additional confirmation of the structures. For example, ^{13}C NMR spectrum of acid **2** contained characteristic signals due to the carboxyl group at 174.1 ppm and also the carbon atoms at position 7 of the pteridine system (at

Scheme 1



Scheme 2



159.9 ppm) and the carbon atoms of the ethylene moiety (at 30.7 and 26.9 ppm). The formation of a pyran ring in compound **3** gave rise to a carbonyl carbon signal at 166.4 ppm, as well as induced a substantial diamagnetic shift of signals due to the other ring carbon atoms.

The obtained 1-methyl-6,7-dihydro-2*H*-pyrano[3,2-*g*]-pteridine-2,4,8-(1*H*,3*H*)-trione (**3**) was an effective acylating agent that readily participated in reactions with *N*-nucleophiles, giving high yields of amides **4**–**20** (Scheme 2). It should be noted that these reactions were optimally performed in anhydrous solvents, the most suitable of which was AcOH. The nucleophiles selected for these reactions were compounds containing a primary amino group (aliphatic and aromatic amines and amino acids). The selection of these reagents was influenced by the fact that the resulting amides **4**–**20** could be considered as structural analogs of antifolates.

^{1}H NMR spectra of compounds **4**–**20** contained characteristic signals due to protons of the exocyclic amide group, which, depending on their electronic environment, were observed as multiplets (compounds **4**, **5**, **9**, **10**), doublets (compounds **8**, **11**–**14**), or triplets (compounds **6**, **7**) in the range of 7.55–8.48 ppm or singlets (compounds **15**–**20**) at 8.86–11.35 ppm. We should also note the substantial paramagnetic shift of amide proton signal in the spectrum of compound **18**, which probably could be explained by the formation of a hydrogen bond. The proton signals of ethylene moiety in ^{1}H NMR spectra of amides **4**–**20** were observed as two triplets or multiplets at 2.53–3.34 and 2.74–3.20 ppm, with the integrated intensity of each corresponding to two protons. Also, ^{1}H NMR spectra of compounds **4**–**20** featured signals at 11.37–11.73 ppm due to the protons belonging to position 3 and at 3.37–3.54 ppm due to the methyl group bonded at position 1. Besides that, ^{1}H NMR spectra of compounds **4**–**20** contained signals of aliphatic and aromatic groups in the amides with the expected multiplicity and chemical shifts.[15]

^{13}C NMR spectra of amides **4**, **6**, **7**, **12**–**16**, and **18**–**20** contained the characteristic carbon signals of exocyclic amide group at 167.3–172.0 ppm, as well as carbon signals of substituents at the exocyclic nitrogen atom.

Mass spectra featured strong individual peaks of quasimolecular $[M+H]^+$ ions that unequivocally confirmed the structure and individuality of the synthesized compounds.

Mass spectrum (EI ionization) of compound **2** did not show the $[M]^{+\bullet}$ ion, with the major fragmentation pathway involving the formation of F_1 ($[M–H_2O]^{+\bullet}$ with *m*/*z* 248), F_2 ($[M–COOH]^{+\bullet}$ with *m*/*z* 221), and F_3 ($[M–CO_2]^{+\bullet}$ with *m*/*z* 220) fragment ions, which are characteristic for heterylalkylcarboxylic acids.[16] The main fragmentation pathway of $[M]^{+\bullet}$ ions in the mass spectrum of compound **20** occurred *via* α-cleavage and direct cleavage of amide bond, with the formation of F_1 ($[M–C(O)NHC_6H_4COOH]^{+\bullet}$ with *m*/*z* 220) and F_2 ($[NHC_6H_4COOH]^{+\bullet}$ with *m*/*z* 120) fragment ions. Besides that, the fragment ions characteristic for pteridine system were also observed in the mass spectrum.[17]

While continuing purposeful search for compounds with antitumor activity among antifolates and studying the effects arising from substituents at position 6 of the respective pteridine system on such activity, we selected compounds **12** and **14** for cytotoxic activity characterization on human hepatocellular carcinoma (HepG2) cells in MTT test. The experimental results showed that the ability of cell culture to reduce tetrazolium decreased in the presence of compounds **14** and **12** and depended on the dosage and time (Tables 1, 2). Thus, aqueous solutions of compound **14** at 0.125–0.5% concentrations degraded the viability of cells in the culture after incubating for 48 h (Table 1).

The sharpest decrease in the viability of cells was observed at the concentration of 0.5% (p<0.05). At the concentrations of 0.25 and 0.125% the study treatment with compound **14** increased the number of dead cells, lowering the viability index to 7.27 and 38.73% (p<0.05), respectively. The lowest of the tested concentrations (0.0625 and 0.0313%) did not produce clearly pronounced toxic effect on HepG2 cells after incubating for 48 h. Solutions of compound **14** showed reduced cytotoxicity after increasing the incubation time with HepG2 cells to 72 h. For example, incubation of the cells with solutions at the lowest concentration (0.0625

Table 1. The fraction of viable human hepatocellular carcinoma (HepG2) cells after *in vitro* incubation with compound **14** for 48, 72, and 96 h (mean value ± mean error, n = 3), %

Time, h	Concentration, %				
	0.5	0.25	0.125	0.0625	0.0313
48	4.647 ± 0.413*	7.268 ± 0.859*	38.725 ± 4.296*	64.164 ± 2.167	69.109 ± 6.553
72	5.512 ± 0.364*	13.062 ± 0.3*	17.975 ± 1.715*	98.502 ± 4.552	98.083 ± 4.552
96	34.161 ± 0.598*	30.711 ± 0.365*	37.612 ± 8.666*	113.803 ± 7.625	101.035 ± 4.662

* The difference from control is statistically significant at $p < 0.05$.

and 0.0313%) for 72 h resulted in viable cell fractions of 98.50 and 98.08%, respectively ($p > 0.05$). Increasing the concentration of the active compound (0.125, 0.25, 0.5%) during 72 h incubation led to a proportional decrease of the viable cell fraction by 17.98, 13.06, and 5.51% ($p < 0.05$), respectively. When the incubation time of the study compound solutions with HepG2 cells was increased to 96 h, the survival rate of cells at all studied concentrations was higher than after 72 h incubation, and was determined to be 34.16, 30.71, 37.61, 113.80, and 101.04% at 0.5, 0.25, 0.125, 0.0625, and 0.0313% concentrations, respectively. The observed effect can be explained by metabolism of the study compounds in the cultured hepatocytes.[18–20]

Various cytotoxic effects of compound **12** were observed after incubation for 48 h at the concentrations of 0.0313–0.5% (Table 2).

The most pronounced effects on the viability of cells were observed at 0.5% concentration – the viability of cells decreased to 9.5% ($p < 0.05$). Incubation with the study compound **12** at 0.25% concentration increased the fraction of dead cells, reducing the viable cell fraction to 48.0% ($p < 0.05$). Using 0.125, 0.0625, and 0.0313% concentrations for the given time interval did not statistically significantly reduce the viability of cells in the studied culture. The cytotoxic effect of the studied solutions of compound **12** was enhanced after longer incubation with HepG2 cells. However, it should be noted that, after incubating cells with 0.5% solution of compound **12** for 72 h, the fraction of viable cells was 15.5%, while after incubating for 96 h there was no increase of cytotoxic activity. On the contrary, the fraction of viable cells increased and reached 27.9% ($p > 0.05$). Decreasing the concentration of active substance (0.125, 0.0625, 0.0313%) during the incubation period of 72 h led to a proportional decrease of cell viability to 4.7, 38.8, 65.1% ($p < 0.05$), respectively. Prolonging the incubation of study compounds with HepG2 cells to 96 h resulted in higher survival rates of cells at all of the studied concentrations, compared to the incubation period of 72 h, with 16.7, 33.47, 90.8, and 116.1% of cells surviving at the concentrations of 0.25, 0.125, 0.0625, and 0.0313%, respectively. The observed effect can be explained by possible metabolism of study compounds in the cultured hepatocyte cells.

Thus, a heterocyclization reaction starting from 5,6-diamino-1-methyluracil and 2-oxopentanedioic acid was used to obtain 3-(1-methyl-2,4,7-trioxo-1,2,3,4,7,8-hexahydropteridin-6-yl)propanoic acid, the carboxyl group of which was activated through the lactone formation. The activated lactone was treated with *N*-nucleophiles, giving a series of amides. The structure of the synthesized compounds was established on the basis of IR spectra, ^{1}H and ^{13}C NMR spectra, LC-MS analysis, and the results of mass spectrometry. The synthesized compounds showed cytotoxic effects at 0.125–0.5% concentrations after incubating with HepG2 cells for 48 h, increasing the fraction of dead cells by 42.9 to 95.35% ($p < 0.05$). Thus, the cytotoxicity of compounds depended on the dosage and incubation time.

Experimental

IR spectra were recorded on a Bruker ALPHA FT-IR spectrometer over the wavenumber range of 7500–400 cm^{-1}, using an ATR accessory. ^{1}H and ^{13}C NMR spectra were acquired on a Varian Mercury 400 instrument (400 and 100 MHz, respectively) in DMSO-d_6 solution, using TMS as internal standard. The assignment of signals in ^{13}C NMR spectra relied on an APT experiment. LC-MS analysis was performed using an Agilent 1100 Series HPLC instrument equipped with an Agilent LC/MSD SL diode array and mass selective detector. The mobile phase consisted of a MeCN–H_2O–HCO_2H mixture, using gradient elution. The ionization type was chemical ionization at atmospheric pressure with electrospray method (APCI-ESI). Simultaneous scanning of positive and negative ions over the range of 80–1000 *m*/*z* was performed. Mass spectra were recorded on a Varian 1200L instrument, EI ionization at 70 eV, direct introduction of sample, ion source temperature 230°C, quadrupole temperature 150°C, heating rate 120°C/min over the range from 50 to 350°C, the range of recorded *m*/*z* values was 40–700. Elemental analysis (C,

Table 2. The fraction of viable human hepatocellular carcinoma (HepG2) cells after *in vitro* incubation with compound **12** for 48, 72, and 96 h (mean value ± mean error, n = 3), %

Time, h	Concentration, %				
	0.5	0.25	0.125	0.0625	0.0313
48	9.532 ± 1.374*	48.019 ± 0.878*	57.194 ± 5.16	84.540 ± 4.43	101.340 ± 4.334
72	15.518 ± 2.34*	5.752 ± 0.452*	4.733 ± 0.216*	38.766 ± 6.85*	65.129 ± 1.238*
96	27.881 ± 0.42*	16.701 ± 0.897*	33.471 ± 5.584*	90.821 ± 10.241	116.149 ± 8.703

* The difference from control is statistically significant at $p < 0.05$.

H, N) was performed on an Elementar vario EL cube instrument. Melting points were determined by capillary method on a Stuart SMP30 digital melting point apparatus.

Compound **1** was obtained according to a previously described method.[14] Commercially available reagents from Merck, Sigma-Aldrich, and Enamine were used.

3-(1-Methyl-2,4,7-trioxo-1,2,3,4,7,8-hexahydropteridin-6-yl)propanoic acid (2). A suspension of 5,6-diamino-1-methyluracil (**1**) (1.56 g, 10 mmol) in AcOH (30 ml) was treated with 2-oxopentanedioic acid (1.46 g, 10 mmol). The reaction mixture was heated, giving a clear solution from which a precipitate formed. The reaction mixture was refluxed for 1 h and cooled. The obtained precipitate was filtered off, washed with water, and dried. Yield 1.99 g (75%), light-yellow powder, mp >300°C. IR spectrum, ν, cm^{-1}: 3500, 3056, 2980, 1643, 1556, 1452, 1421, 1325, 1181, 834, 791, 750, 693, 669, 630. ^{1}H NMR spectrum, δ, ppm (*J*, Hz): 2.69 (2H, t, *J* = 7.2, $CH_2C\underline{H}_2COOH$); 2.93 (2H, t, *J* = 7.1, $C\underline{H}_2CH_2COOH$); 3.44 (3H, s, 1-NCH_3); 11.42 (1H, s, 3-NH); 11.57–11.98 (2H, m, 8-NH, COOH). ^{13}C NMR spectrum, δ, ppm: 26.9 ($\underline{C}H_2CH_2COOH$); 28.5 (NCH_3); 30.7 ($CH_2\underline{C}H_2COOH$); 118.2 (C-4a); 141.2 (C-8a); 148.1 (C-6); 150.6 (C-2); 159.9 (C-4,7); 174.1 (COOH). Mass spectrum (EI), *m*/*z* (I_{rel}, %): 248 (29), 246 (9), 221 (11), 220 (100), 167 (18), 149 (28), 121 (7), 83 (19), 68 (7), 67 (7). Mass spectrum (APCI-ESI), *m*/*z* (I_{rel}, %): 267 $[M+H]^+$ (100). Found, %: C 45.15; H 3.82; N 21.09. $C_{10}H_{10}N_4O_5$. Calculated, %: C 45.12; H 3.79; N 21.05.

1-Methyl-6,7-dihydro-2*H*-pyrano[3,2-*g*]pteridine-2,4,8(1*H*,3*H*)-trione (3). A suspension of 3-(1-methyl-2,4,7-trioxo-1,2,3,4,7,8-hexahydropteridin-6-yl)propanoic acid (**2**) (2.66 g, 10 mmol) in a mixture of AcOH (10 ml) and Ac_2O (10 ml) was refluxed for 1 h. Cooling of the solution to room temperature led to the formation of crystalline precipitate. The obtained solids were filtered off, washed with Et_2O, and dried. Yield 1.93 g (78%), white crystals, mp 294–296°C. IR spectrum, ν, cm^{-1}: 3010, 2850, 1809, 1675, 1560, 1513, 1435, 1349, 1282, 1256, 1197, 1095, 1056, 1017, 981, 883, 806, 749, 719, 673, 618. ^{1}H NMR spectrum, δ, ppm (*J*, Hz): 3.06 (2H, t, *J* = 7.4, $CH_2C\underline{H}_2CO$); 3.25 (2H, t, *J* = 7.3, $C\underline{H}_2CH_2CO$); 3.46 (3H, s, 1-NCH_3); 11.84 (1H, s, 3-NH). ^{13}C NMR spectrum, δ, ppm: 24.7 (C-7); 28.3 (C-6); 28.7 (NCH_3); 124.0 (C-4a); 135.9 (C-5a); 148.3 (C-10a); 150.4 (C-2); 155.6 (C-9a); 159.3 (C-4); 166.4 (C-8). Mass spectrum (APCI-ESI), *m*/*z* (I_{rel}, %): 249 $[M+H]^+$ (100). Found, %: C 48.43; H 3.29; N 22.61. $C_{10}H_8N_4O_4$. Calculated, %: C 48.39; H 3.25; N 22.57.

Synthesis of 3-(1-methyl-2,4,7-trioxo-1,2,3,4,7,8-hexahydropteridin-6-yl)propanamides 4–20 (General method). A solution of 1-methyl-6,7-dihydro-2*H*-pyrano-[3,2-*g*]pteridine-2,4,8(1*H*,3*H*)-trione (**3**) (1.24 g, 5 mmol) in AcOH (20 ml) was treated by adding the appropriate amine, aromatic carboxylic acid, or amino acid (5 mmol) and heated until dissolution. The reaction mixture was refluxed for 30 min, the precipitate that formed upon heating was filtered off, washed with water, and filtered.

***N*-Cyclohexyl-3-(1-methyl-2,4,7-trioxo-1,2,3,4,7,8-hexahydropteridin-6-yl)propanamide (4)**. Yield 1.28 g (74%), white powder, mp 286–288°C. IR spectrum, ν, cm^{-1}: 3020, 2910, 2860, 1694, 1526, 1434, 1322, 1192, 840, 729, 685, 632. ^{1}H NMR spectrum, δ, ppm (*J*, Hz): 2.04–1.68 (10H, m, 2,3,4,5,6,-CH_2 Cy); 2.69 (2H, t, *J* = 6.9, $CH_2C\underline{H}_2CONH$); 2.88 (2H, t, *J* = 6.9, $C\underline{H}_2CH_2CONH$); 3.37–3.54 (4H, m, 3-NCH_3, 1-CH Cy); 7.55 (1H, d, *J* = 8.9, CONH); 11.40–12.63 (2H, m, 3,8-NH). ^{13}C NMR spectrum, δ, ppm: 25.0 (C-3,5 Cy); 25.7 (C-4 Cy); 27.9 ($\underline{C}H_2CH_2CO$); 28.5 (NCH_3); 32.8 ($CH_2\underline{C}H_2CO$); 32.9 (C-2,6 Cy); 47.8 (C-1 Cy); 118.3 (C-4a); 141.9 (C-8a); 148.2 (C-6); 150.7 (C-2); 159.9 (C-7); 159.9 (C-4), 170.6 (CONH). Mass spectrum (APCI-ESI), *m*/*z* (I_{rel}, %): 348 $[M+H]^+$ (100). Found, %: C 55.36; H 6.13; N 20.19. $C_{16}H_{21}N_5O_4$. Calculated, %: C 55.32; H 6.09; N 20.16.

***N*-Benzyl-3-(1-methyl-2,4,7-trioxo-1,2,3,4,7,8-hexahydropteridin-6-yl)propanamide (5)**. Yield 1.37 g (77%), white powder, mp 289–292°C. IR spectrum, ν, cm^{-1}: 3320, 3190, 3050, 2870, 1671, 1615, 1550, 1427, 1397, 1349, 1256, 1222, 1142, 803, 750, 704. ^{1}H NMR spectrum, δ, ppm (*J*, Hz): 2.59–2.70 (2H, m, $CH_2C\underline{H}_2CONH$); 2.95–3.05 (2H, m, $C\underline{H}_2CH_2CONH$); 3.45 (3H, s, 1-NCH_3); 4.27 (2H, d, *J* = 5.8, $NHC\underline{H}_2Ph$); 6.74–7.38 (5H, m, H-2,3,4,5,6 Ph); 7.97–8.48 (1H, m, CONH); 11.40 (1H, s, 3-NH); 12.86 (1H, s, 8-NH). Mass spectrum (APCI-ESI), *m*/*z* (I_{rel}, %): 356 $[M+H]^+$ (100). Found, %: C 57.49; H 4.85; N 19.75. $C_{17}H_{17}N_5O_4$. Calculated, %: C 57.46; H 4.82; N 19.71.

4-{[3-(1-Methyl-2,4,7-trioxo-1,2,3,4,7,8-hexahydropteridin-6-yl)propanamido]methyl}benzoic acid (6). Yield 1.52 g (76%), white powder, mp >300°C. IR spectrum, ν, cm^{-1}: 3470, 3310, 3020, 2750, 1693, 1614, 1551, 1428, 1396, 1350, 1257, 1224, 1140, 763. ^{1}H NMR spectrum, δ, ppm (*J*, Hz): 2.67 (2H, t, *J* = 7.3, $CH_2C\underline{H}_2CONH$); 3.14 (2H, t, *J* = 7.3, $C\underline{H}_2CH_2CONH$); 3.45 (3H, s, 1-NCH_3); 4.33 (2H, d, *J* = 5.6, $NHC\underline{H}_2Ph$); 7.33 (2H, d, *J* = 6.8, H-3,5 Ar); 7.86 (2H, d, *J* = 7.1, H-2,6 Ar); 8.39 (1H, t, *J* = 5.7, CONH); 11.47 (1H, s, 3-NH); 11.87–12.61 (2H, m, 8-NH, COOH). ^{13}C NMR spectrum, δ, ppm: 27.5 ($\underline{C}H_2CH_2CO$); 28.5 (NCH_3); 32.3 ($CH_2\underline{C}H_2CO$); 42.3 (CH_2NH); 118.4 (C-4a); 127.4 (C-3,5 Ar); 129.5 (C-1 Ar); 129.7 (C-2,6 Ar); 141.7 (C-8a); 145.2 (C-4 Ar); 148.2 (C-6); 150.7 (C-2); 159.8–160.2 (C-4,7); 167.6 (COOH); 171.9 (CONH). Mass spectrum (APCI-ESI), *m*/*z* (I_{rel}, %): 400 $[M+H]^+$ (100). Found, %: C 54.11; H 4.26; N 17.59. $C_{18}H_{17}N_5O_6$. Calculated, %: C 54.14; H 4.29; N 17.54.

[3-(1-Methyl-2,4,7-trioxo-1,2,3,4,7,8-hexahydropteridin-6-yl)propanoyl]glycine (7). Yield 1.32 g (82%), white powder, mp 278–280°C. IR spectrum, ν, cm^{-1}: 3405, 3005, 2780, 1715, 1601, 1551, 1427, 1347, 1281, 1199, 793, 751, 632. ^{1}H NMR spectrum, δ, ppm (*J*, Hz): 2.62 (2H, t, *J* = 7.7, $CH_2C\underline{H}_2CONH$); 2.98 (2H, t, *J* = 7.5, $C\underline{H}_2CH_2CONH$); 3.44 (3H, s, 1-NCH_3); 3.73 (2H, d, *J* = 5.5, $NHC\underline{H}_2COOH$); 8.09 (1H, t, *J* = 5.6, CONH); 11.37 (1H, s, 3-NH); 12.28–12.56 (2H, m, 8-NH, COOH). ^{13}C NMR spectrum, δ, ppm: 27.7 ($\underline{C}H_2CH_2CO$); 28.5 (NCH_3); 32.4 ($CH_2\underline{C}H_2CO$); 41.0 (CH_2NH); 118.1 (C-4a); 141.7 (C-8a); 148.3 (C-6); 150.7 (C-2); 159.9 (C-7); 160.0 (C-4); 171.8 (CONH); 172.2 (COOH). Mass spectrum (APCI-ESI), *m*/*z* (I_{rel}, %): 324 $[M+H]^+$ (100). Found, %: C 44.61; H 4.09; N 21.69. $C_{12}H_{13}N_5O_6$. Calculated, %: C 44.59; H 4.05; N 21.66.

[3-(1-Methyl-2,4,7-trioxo-1,2,3,4,7,8-hexahydropteridin-6-yl)propanoyl]alanine (8). Yield 1.35 g (80%), white powder, mp 272–274°C. IR spectrum, ν, cm^{-1}: 3460, 3060, 2810, 1714, 1598, 1522, 1422, 1346, 1279, 1201, 1150, 858, 751. ^{1}H NMR spectrum, δ, ppm (*J*, Hz): 1.30 (3H, d, *J* = 7.3, NHCH(C$\underline{H}_3$)COOH); 2.60 (2H, t, *J* = 7.8, $CH_2C\underline{H}_2CONH$); 2.74–3.04 (2H, m, $C\underline{H}_2CH_2CONH$); 3.43 (3H, s, 1-NCH_3); 4.21 (1H, pent, *J* = 7.2, NHC$\underline{H}(CH_3)$COOH); 8.03 (1H, d, *J* = 7.4, CONH); 11.40 (1H, s, 3-NH); 12.19–12.78 (2H, m, 8-NH, COOH). Mass spectrum (APCI-ESI), *m*/*z* (I_{rel}, %): 338 $[M+H]^+$ (100). Found, %: C 46.31; H 4.51; N 20.79. $C_{13}H_{15}N_5O_6$. Calculated, %: C 46.29; H 4.48; N 20.76.

3-{[3-(1-Methyl-2,4,7-trioxo-1,2,3,4,7,8-hexahydropteridin-6-yl)propanoyl]amino}propanoic acid (9). Yield 1.28 g (76%), white powder, mp 254–256°C. IR spectrum, ν, cm^{-1}: 3390, 3103, 2892, 1712, 1520, 1338, 1176, 847, 688, 630. ^{1}H NMR spectrum, δ, ppm (*J*, Hz): 1.77–2.04 (2H, m, $NHCH_2C\underline{H}_2COOH$); 2.78–3.34 (6H, m, $NHC\underline{H}_2CH_2COOH$, $C\underline{H}_2C\underline{H}_2CONH$); 3.45 (3H, s, 1-$NCH_3$); 7.86 (1H, s, CONH); 11.40 (1H, s, 3-NH); 11.97–12.37 (2H, m, 8-NH, COOH). Mass spectrum (APCI-ESI), *m*/*z* (I_{rel}, %): 338 $[M+H]^+$ (100). Found, %: C 46.32; H 4.50; N 20.78. $C_{13}H_{15}N_5O_6$. Calculated, %: C 46.29; H 4.48; N 20.76.

4-{[3-(1-Methyl-2,4,7-trioxo-1,2,3,4,7,8-hexahydropteridin-6-yl)propanoyl]amino}butanoic acid (10). Yield 1.21 g (69%), white powder, mp 245–247°C. IR spectrum, ν, cm^{-1}: 3380, 3200, 2910, 1712, 1519, 1337, 1177, 846, 688, 630. ^{1}H NMR spectrum, δ, ppm (*J*, Hz): 1.66 (2H, pent, *J* = 7.1, $CONHCH_2C\underline{H}_2CH_2COOH$); 2.18 (2H, t, *J* = 7.3, $CONHCH_2CH_2C\underline{H}_2COOH$); 2.55 (2H, t, *J* = 6.5, $CH_2C\underline{H}_2CONH$); 2.90–3.02 (2H, m, $C\underline{H}_2CH_2CONH$); 3.09 (2H, q, *J* = 6.3, $NHC\underline{H}_2CH_2CH_2COOH$); 3.44 (3H, s, 1-$NCH_3$), 7.79 (1H, t, *J* = 5.4, CONH); 11.41 (1H, s, 3-NH); 12.00–12.78 (2H, m, 8-NH, COOH). Mass spectrum (APCI-ESI), *m*/*z* (I_{rel}, %): 352 $[M+H]^+$ (100). Found, %: C 47.89; H 4.90; N 19.95. $C_{14}H_{17}N_5O_6$. Calculated, %: C 47.86; H 4.88; N 19.93.

[3-(1-Methyl-2,4,7-trioxo-1,2,3,4,7,8-hexahydropteridin-6-yl)propanoyl]valine (11). Yield 1.23 g (67%), white powder, mp 269–271°C. IR spectrum, ν, cm^{-1}: 3680, 3190, 2893, 1696, 1549, 1428, 1341, 1216, 751. ^{1}H NMR spectrum, δ, ppm (*J*, Hz): 0.91 (6H, d, *J* = 6.6, NHCH(CH(C$\underline{H}_3$)$_2$)COOH); 2.07 (1H, dq, *J* = 13.3, *J* = 6.6, NHCH(C$\underline{H}$(CH$_3$)$_2$)COOH); 2.66 (2H, t, *J* = 8.1, $CH_2C\underline{H}_2CONH$); 2.97 (2H, t, *J* = 8.1, $C\underline{H}_2CH_2CONH$); 3.43 (3H, s, 1-NCH_3), 4.16 (1H, dd, *J* = 8.4, *J* = 5.6, NHC$\underline{H}$(CH(CH$_3$)$_2$)COOH); 7.87 (1H, d, *J* = 8.6, CONH); 11.40 (1H, s, 3-NH); 12.54 (2H, s, 8-NH, COOH). Mass spectrum (APCI-ESI), *m*/*z* (I_{rel}, %): 366 $[M+H]^+$ (100). Found, %: C 49.33; H 5.26; N 19.19. $C_{15}H_{19}N_5O_6$. Calculated, %: C 49.31; H 5.24; N 19.17.

[3-(1-Methyl-2,4,7-trioxo-1,2,3,4,7,8-hexahydropteridin-6-yl)propanoyl]leucine (12). Yield 1.36 g (72%), white powder, mp 216–218°C. IR spectrum, ν, cm^{-1}: 3610, 3250, 2920, 1696, 1551, 1426, 1222. ^{1}H NMR spectrum, δ, ppm (*J*, Hz): 0.90 (6H, dd, *J* = 16.3, *J* = 6.5, NHCH(CH$_2$CH(C$\underline{H}_3$)$_2$)COOH); 1.41–1.58 (1H, m, NHCH(CH$_2$C$\underline{H}$(CH$_3$)$_2$)COOH); 1.66 (2H, dt, *J* = 15.1, *J* = 6.7, NHCH(C$\underline{H}_2$CH(CH$_3$)$_2$)COOH); 2.64 (2H, t, *J* = 8.1, $CH_2C\underline{H}_2CONH$); 2.95 (2H, t, *J* = 8.1, $C\underline{H}_2CH_2CONH$); 3.44 (3H, s, 1-NCH_3); 4.16–4.29 (1H, m, NHC$\underline{H}$(CH$_2$CH(CH$_3$)$_2$)COOH); 7.98 (1H, d, *J* = 8.1, CONH); 11.41 (1H, s, 3-NH); 12.04–13.05 (2H, m, 8-NH, COOH). ^{13}C NMR spectrum, δ, ppm: 21.7, 23.3 (CHCH$_2$CH($\underline{C}$H$_3$)$_2$); 24.7 (CHCH$_2\underline{C}$H(CH$_3$)$_2$); 27.8 ($\underline{C}$H$_2$CH$_2$CO); 28.5 (NCH_3); 32.4 ($CH_2\underline{C}H_2CO$); 40.1 (CH$\underline{C}$H$_2$CH(CH$_3$)$_2$); 50.6 ($\underline{C}$HCH$_2$CH(CH$_3$)$_2$); 118.3 (C-4a); 141.6 (C-8a); 148.1 (C-6); 150.6 (C-2); 159.9 (C-7); 159.9 (C-4); 171.8 (CONH); 174.6 (COOH). Mass spectrum (APCI-ESI), *m*/*z* (I_{rel}, %): 380 $[M+H]^+$ (100). Found, %: C 50.68; H 5.61; N 18.48. $C_{16}H_{21}N_5O_6$. Calculated, %: C 50.66; H 5.58; N 18.46.

[3-(1-Methyl-2,4,7-trioxo-1,2,3,4,7,8-hexahydropteridin-6-yl)propanoyl]methionine (13). Yield 1.45 g (73%), white powder, mp 222–224°C. IR spectrum, ν, cm^{-1}: 3420, 3056, 2830, 1703, 1548, 1425, 1342, 1222, 752, 634. ^{1}H NMR spectrum, δ, ppm (*J*, Hz): 1.77–2.03 (2H, m, NHCH(CH$_2$C$\underline{H}_2$SCH$_3$)COOH); 2.05 (3H, s, NHCH(CH$_2$CH$_2$SC$\underline{H}_3$)COOH); 2.35–2.49 (2H, m, NHCH(C$\underline{H}_2$CH$_2$SCH$_3$)COOH); 2.63 (2H, t, *J* = 6.6, $CH_2C\underline{H}_2CONH$); 2.88–3.04 (2H, m, $C\underline{H}_2CH_2CONH$); 3.44 (3H, s, 1-NCH_3); 4.30 (1H, td, *J* = 8.5, *J* = 4.5, NHC$\underline{H}$(CH$_2$CH$_2$SCH$_3$)COOH); 8.07 (1H, d, *J* = 8.0, CONH); 11.41 (1H, s, 3-NH); 12.63 (2H, s, 8-NH, COOH). ^{13}C NMR spectrum, δ, ppm: 15.0 (CHCH$_2$CH$_2$S$\underline{C}$H$_3$); 27.7 ($\underline{C}$H$_2$CH$_2$CO); 28.5 (NCH_3); 30.1 (CHCH$_2\underline{C}$H$_2$SCH$_3$); 31.1 (CH$\underline{C}$H$_2$CH$_2$SCH$_3$); 32.4 ($CH_2\underline{C}H_2CO$); 51.3 ($\underline{C}$HCH$_2$CH$_2$SCH$_3$); 118.4 (C-4a); 141.6 (C-8a); 148.1 (C-6); 150.7 (C-2); 159.9 (C-4,7); 172.0 (CONH); 173.8 (COOH). Mass spectrum (APCI-ESI), *m*/*z* (I_{rel}, %): 398 $[M+H]^+$ (100). Found, %: C 45.36; H 4.85; N 17.65. $C_{15}H_{19}N_5O_6S$. Calculated, %: C 45.34; H 4.82; N 17.62.

[3-(1-Methyl-2,4,7-trioxo-1,2,3,4,7,8-hexahydropteridin-6-yl)propanoyl]phenylalanine (14). Yield 1.53 g (74%), white powder, mp 162–164°C. IR spectrum, ν, cm^{-1}: 3300, 3020, 2890, 1681, 1612, 1549, 1428, 1347, 1246, 754, 699. ^{1}H NMR spectrum, δ, ppm (*J*, Hz): 2.53–2.65 (2H, m, $CH_2C\underline{H}_2CONH$); 2.83–2.96 (2H, m, $C\underline{H}_2CH_2CONH$); 3.00–3.12 (2H, m, NHCH(C$\underline{H}_2$Ph)COOH); 3.43 (3H, s, 1-NCH_3); 4.36–4.50 (1H, m, NHC$\underline{H}$(CH$_2$Ph)COOH); 7.10–7.29 (5H, m, H-2,3,4,5,6 Ph); 8.05 (1H, d, *J* = 7.9, CONH); 11.42 (1H, s, 3-NH); 12.58 (2H, s, 8-NH, COOH). ^{13}C NMR spectrum, δ, ppm: 27.7 ($\underline{C}$H$_2$CH$_2$CO); 28.5 (NCH_3); 32.4 ($CH_2\underline{C}H_2CO$); 37.2 (CH$\underline{C}$H$_2$); 53.9 ($\underline{C}$HCH$_2$); 118.4 (C-4a); 126.7 (C-4 Ph); 128.5 (C-2,6 Ph); 129.5 (C-3,5 Ph); 138.1 (C-1 Ph); 141.6 (C-8a); 148.2 (C-6); 150.7 (C-2); 159.9 (C-7); 159.9 (C-4); 171.8 (CONH); 173.5 (COOH). Mass spectrum (APCI-ESI), *m*/*z* (I_{rel}, %): 414 $[M+H]^+$ (100). Found, %: C 55.22; H 4.65; N 16.97. $C_{19}H_{19}N_5O_6$. Calculated, %: C 55.20; H 4.63; N 16.94.

3-(1-Methyl-2,4,7-trioxo-1,2,3,4,7,8-hexahydropteridin-6-yl)-*N*-phenylpropanamide (15). Yield 1.38 g (81%), white powder, mp >295°C. IR spectrum, ν, cm^{-1}: 3410, 3190, 3010, 1679, 1595, 1563, 1498, 1438, 1310, 1192, 802, 749, 690, 626. ^{1}H NMR spectrum, δ, ppm (*J*, Hz): 2.82 (2H, t, *J* = 7.6, $CH_2C\underline{H}_2CONH$); 3.08 (2H, t, *J* = 7.7, $C\underline{H}_2CH_2CONH$); 3.46 (3H, s, 1-NCH_3); 6.90–7.03 (1H, m,

H-4 Ph); 7.12–7.27 (2H, m, H-3,5 Ph); 7.59 (2H, d, J = 6.4, H-2,6 Ph); 9.88 (1H, s, CONH); 11.44 (1H, s, 3-NH); 12.98 (1H, s, 8-NH). ^{13}C NMR spectrum, δ, ppm: 27.3 ($\underline{C}H_2CH_2CO$); 28.6 (NCH_3); 33.4 ($CH_2\underline{C}H_2CO$); 119.1–119.6 (C-4a, C-2,6 Ph); 123.4 (C-4 Ph); 129.1 (C-3,5 Ph); 139.8 (C-1 Ph); 141.6 (C-8a); 148.2 (C-6); 150.7 (C-2); 159.9 (C-4,7); 170.8 (CONH). Mass spectrum (APCI-ESI), m/z (I_{rel}, %): 342 $[M+H]^+$ (100). Found, %: C 56.33; H 4.45; N 20.55. $C_{16}H_{15}N_5O_4$. Calculated, %: C 56.30; H 4.43; N 20.52.

***N*-(2,6-Dimethylphenyl)-3-(1-methyl-2,4,7-trioxo-1,2,3,4,7,8-hexahydropteridin-6-yl)propanamide (16)**. Yield 1.44 g (78%), white powder, mp >297°C. IR spectrum, ν, cm^{-1}: 3210, 3020, 2840, 1703, 1621, 1555, 1439, 1349, 1232, 760, 643. ^{1}H NMR spectrum, δ, ppm (J, Hz): 2.14 (6H, s, 2,6-CH_3); 2.84 (2H, t, J = 7.6, $CH_2C\underline{H}_2CONH$); 3.09 (2H, t, J = 7.6, $C\underline{H}_2CH_2CONH$); 3.46 (3H, s, 1-NCH_3); 6.57–7.25 (3H, m, H-3,4,5 Ar); 9.22 (1H, s, CONH); 11.44 (1H, s, 3-NH); 12.88 (1H, s, 8-NH). ^{13}C NMR spectrum, δ, ppm: 18.5 (2,6-CH_3); 27.7 ($\underline{C}H_2CH_2CO$); 28.6 (NCH_3); 32.4 ($CH_2\underline{C}H_2CO$); 118.4 (C-4a); 126.6 (C-4 Ar); 128.0 (C-3,5 Ar); 135.7 (C-2,6 Ar); 135.7 (C-1 Ar); 141.7 (C-8a); 148.2 (C-6); 150.7 (C-2); 159.9 (C-7); 159.9 (C-4); 170.4 (CONH). Mass spectrum (APCI-ESI), m/z (I_{rel}, %): 370 $[M+H]^+$ (100). Found, %: C 58.55; H 5.20; N 18.98. $C_{18}H_{19}N_5O_4$. Calculated, %: C 58.53; H 5.18; N 18.96.

***N*-(2-Methoxyphenyl)-3-(1-methyl-2,4,7-trioxo-1,2,3,4,7,8-hexahydropteridin-6-yl)propanamide (17)**. Yield 1.41 g (76%), white powder, mp >300°C. IR spectrum, ν, cm^{-1}: 3020, 2810, 1684, 1560, 1534, 1486, 1440, 1398, 1329, 1293, 1213, 1022, 866, 801, 751, 628. ^{1}H NMR spectrum, δ, ppm (J, Hz): 2.90 (2H, t, $CH_2C\underline{H}_2CONH$); 3.08 (2H, t, J = 7.3, $C\underline{H}_2CH_2CONH$); 3.46 (3H, s, 1-NCH_3); 3.86 (3H, s, OCH_3); 6.84 (1H, t, J = 8.3, H-5 Ar); 6.91 (1H, d, J = 8.4, H-3 Ar); 6.98 (1H, t, J = 8.4, H-4 Ar); 8.03 (1H, d, J = 9.0, H-6 Ar); 8.86 (1H, s, CONH); 11.43 (1H, s, 3-NH); 12.94 (1H, s, 8-NH). Mass spectrum (APCI-ESI), m/z: 372 $[M+H]^+$ (100). Found, %: C 55.01; H 4.63; N 18.88. $C_{17}H_{17}N_5O_5$. Calculated, %: C 54.98; H 4.61; N 18.86.

2-{[3-(1-Methyl-2,4,7-trioxo-1,2,3,4,7,8-hexahydropteridin-6-yl)propanoyl]amino}benzoic acid (18). Yield 1.4 g (73%), white powder, mp 295–297°C. IR spectrum, ν, cm^{-1}: 3190, 3110, 2960, 1642, 1555, 1393, 1224, 1180, 799, 765, 718, 646. ^{1}H NMR spectrum, δ, ppm (J, Hz): 2.88 (2H, t, J = 7.4, $CH_2C\underline{H}_2CONH$); 2.99–3.20 (2H, m, $C\underline{H}_2CH_2CONH$); 3.44 (3H, s, 1-NCH_3); 7.05 (1H, t, J = 7.6, H-5 Ar); 7.49 (1H, t, J = 8.3, H-4 Ar); 7.98 (1H, d, J = 6.7, H-3 Ar); 8.57 (1H, d, J = 8.3, H-6 Ar); 11.35 (1H, s, CONH); 11.42 (1H, s, 3-NH); 12.95 (2H, s, 8-NH, COOH). ^{13}C NMR spectrum, δ, ppm: 27.1 ($\underline{C}H_2CH_2CO$); 28.5 (NCH_3); 34.4 ($CH_2\underline{C}H_2CO$); 116.8 (C-4a); 116.8 (C-1 Ar); 120.4 (Ar C-5); 122.9 (C-3 Ar); 131.4 (C-6 Ar); 134.4 (C-4 Ar); 141.1 (C-2 Ar); 141.3 (C-8a); 148.2 (C-6); 150.6 (C-2); 159.9 (C-4,7); 169.9 (CONH); 170.8 (COOH). Mass spectrum (APCI-ESI), m/z (I_{rel}, %): 386 $[M+H]^+$ (100). Found, %: C 53.02; H 3.95; N 18.20. $C_{17}H_{15}N_5O_6$. Calculated, %: C 52.99; H 3.92; N 18.18.

3-{(3-(1-Methyl-2,4,7-trioxo-1,2,3,4,7,8-hexahydropteridin-6-yl)propanoyl]amino}benzoic acid (19). Yield 1.52 g (79%), white powder, mp >300°C. IR spectrum, ν, cm^{-1}: 3330, 3160, 3080, 1711, 1632, 1541, 1429, 1346, 1275, 1241, 1190, 1136, 1022, 844, 761, 728, 686, 635. ^{1}H NMR spectrum, δ, ppm (J, Hz): 2.84 (2H, t, J = 7.4, $CH_2C\underline{H}_2CONH$); 3.09 (2H, t, J = 7.5, $C\underline{H}_2CH_2CONH$); 3.46 (3H, s, 1-NCH_3); 7.32 (1H, t, J = 8.0, H-5 Ar); 7.58 (1H, d, J = 7.8, H-4 Ar); 7.84 (1H, d, J = 8.4, H-6 Ar); 8.19 (1H, s, H-2 Ar); 10.09 (1H, s, CONH); 11.44 (1H, s, 3-NH); 12.28 (2H, s, 8-NH, COOH). ^{13}C NMR spectrum, δ, ppm: 27.2 ($\underline{C}H_2CH_2CO$); 28.6 (NCH_3); 33.4 ($CH_2\underline{C}H_2CO$); 118.4 (C-4a); 120.2 (C-2 Ar); 123.6 (C-6 Ar); 124.2 (C-4 Ar); 129.3 (C-5 Ar); 131.6 (C-1 Ar); 140.0 (C-3 Ar); 141.6 (C-8a); 148.2 (C-6); 150.7 (C-2); 159.9 (C-7); 159.9 (C-4); 167.6 (CONH); 171.1 (COOH). Mass spectrum (APCI-ESI), m/z (I_{rel}, %): 386 $[M+H]^+$ (100). Found, %: C 53.02; H 3.95; N 18.20. $C_{17}H_{15}N_5O_6$. Calculated, %: C 52.99; H 3.92; N 18.18.

4-{[3-(1-Methyl-2,4,7-trioxo-1,2,3,4,7,8-hexahydropteridin-6-yl)propanoyl]amino}benzoic acid (20). Yield 1.56 g (81%), white powder, mp >300°C. IR spectrum, ν, cm^{-1}: 3306, 3220, 1681, 1597, 1565, 1530, 1437, 1296, 1256, 1195, 860, 771, 627. ^{1}H NMR spectrum, δ, ppm (J, Hz): 2.78–2.92 (2H, m, $CH_2C\underline{H}_2CONH$); 3.05–3.16 (2H, m, $C\underline{H}_2CH_2CONH$); 3.46 (3H, s, 1-NCH_3); 7.69 (2H, d, J = 8.2, H-3,5 Ar); 7.84 (2H, d, J = 8.5, H-2,6 Ar); 10.20 (1H, s, CONH); 11.44 (1H, s, 3-NH); 11.81 (2H, s, 8-NH, COOH). ^{13}C NMR spectrum, δ, ppm: 27.0 ($\underline{C}H_2CH_2CO$); 28.6 (NCH_3); 33.5 ($CH_2\underline{C}H_2CO$); 118.6 (C-4a); 118.7 (C-3,5 Ar); 125.2 (C-1 Ar); 130.8 (C-2,6 Ar); 141.5 (C-8a); 143.8 (C-4 Ar); 148.2 (C-6); 150.7 (C-2); 159.9 (C-7); 159.9 (C-4); 167.3 (CONH); 171.4 (COOH). Mass spectrum (ESI), m/z (I_{rel}, %): 220 (15), 149 (5), 138 (8), 137 (100), 121 (9), 120 (97), 92 (36), 65 (22), 64 (4), 63 (6). Mass spectrum (APCI-ESI), m/z (I_{rel}, %): 386 $[M+H]^+$ (100). Found, %: C 53.02; H 3.95; N 18.20. $C_{17}H_{15}N_5O_6$. Calculated, %: C 52.99; H 3.92; N 18.18.

Cytotoxic activity testing of compounds 12 and 14 on human hepatocellular carcinoma (HepG2) cells *in vitro*. Human hepatocellular carcinoma (HepG2) cells were obtained from the R. E. Kavetsky Institute of Experimental Pathology, Oncology and Radiobiology, National Academy of Sciences of Ukraine. The cells were cultivated *in vitro* at 37°C in DMEM growth medium (BioWest, France) with the addition of fetal bovine serum (BioWest, France) at 10% concentration. The cell culture was incubated in 25 cm^3 culture bottles until the formation of a dense monolayer at 37 ± 0.5°C, 5% CO_2, 95% atmospheric humidity. In order to prepare solutions of the study compounds at various concentrations, the starting 10% solutions of compounds **12** and **14** (in 0.1 M NaOH solution) were titrated on immunological plate by rolling method. Saline solution was used as solvent. The following concentrations of the compounds were tested: 0.5, 0.25, 0.0125, 0.0625, and 0.0315%.

The cytotoxic effect of study compounds on human hepatrocellular carcinoma (HepG2) cells was evaluated from experiments performed on standard 96-well plates for

immunological analysis (SPL, South Korea). The cells were inoculated in the wells of plates at the concentration of $2 \cdot 10^6$ cells/ml and incubated with aqueous extracts of study compounds for 48, 72, and 96 h. Control samples of cells were incubated in parallel, by adding sterile phosphate-salt buffer instead of the solutions of study compounds. The experiments were repeated in triplicate. The optical density of solutions was measured on an Awareness Technology Stat Fax 303 Plus spectrophotometer at 492 and 630 nm wavelengths. The optical density (A) of solutions was proportional to the amount of viable cells. The number of viable cells in contact with the studied solutions was calculated according to the formula $(A_{test}/A_{control}) \cdot 100\%$.

Mathematical processing of the obtained data was performed by methods of variational statistics using Microsoft Exel and Statistica software, determining the following parameters: mean arithmetic value, error of the arithmetic mean value, statistical significance of differences between the comparison groups. The differences were considered statistically significant at $p<0.05$. The fraction of surviving cells in each well of the plate was determined after evaluating the significance in the difference of optical density in experimental wells from optical density in the control wells. As a result, each sample concentration was characterized by a set of surviving cell fraction values.[21,22]

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