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Original article

Novel 8-deaza-5,6,7,8-tetrahydroaminopterin derivatives as dihydrofolate inhibitor: Design, synthesis and antifolate activity

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Abstract

We report, for the first time, the synthesis and biological activities of 8-deaza-5,6,7,8-tetrahydroaminopterin **9**, and the 5-substituted and 5,10-disubstituted analogues **11**, **13**, **15**, and **17**. The analogues were obtained from key compound diethyl 8-deaza-5,6,7,8-tetrahydroaminopterin **8** following the catalytic reduction of the pyridine ring of diethyl 8-deaza aminopterin **5**. The five novel 8-deaza-5,6,7,8-tetrahydroaminopterin derivatives were assayed in vitro for their cytotoxicity on BGC-823, HL-60, Bel-7402 and Hela tumor cell lines, and inhibition on recombinant human dihydrofolate reductase (DHFR), among which the most potent molecule (compound **9**) was about 4- to 10-fold poorer than MTX on the four kinds of tumor cell lines, and its effect on DHFR was about 17-fold poorer than MTX. The docking studies were followed to explain the biological testing results.

Keywords: Catalytic reduction; Potential antitumor agents; Folate derivatives; Molecular modeling

1. Introduction

Folate metabolism has long been recognized as an effective target for chemotherapy of human cancers, due to its crucial role in the biosynthesis of nucleic acid precursors [1]. Methotrexate (MTX), a dihydrofolate reductase (DHFR) inhibitor, has been used clinically for more than 50 years and becomes the most commonly used anticancer antifolate drug, and remains a mainstay in the treatment of several major malignant diseases [2,3]. However, its toxic side effects and the development of resistance by tumor cells encouraged people to develop novel DHFR inhibitors as antitumor agents [4]. Numerous derivatives of folate have been synthesized to date as potential antagonists, but these have invariably encompassed structural modifications in either the pteridine ring [5–7], the bridge region [8–13], the benzene region [14–16], or the glutamate side-chain [17–21]. Only limited studies on the effect of varying the N^5-substituent of tetrahydrofolate have been reported [22]. One obstacle to the development of such compounds is the propensity of tetrahydrofolates to undergo autoxidation under relatively mild conditions, and this contrasts with their 8-deaza congeners which are significantly more stable [23]. Therefore, the most promising modified folate derivatives as effective anticancer agents are various deaza derivatives of folic acid, MTX, and aminopterin (the 10-desmethyl derivative of methotrexate, AMT) [24], for example, 8-deazafolic acid (DAF), 10-propargyl-5,8-dideazafolic acid (PDDF) [25] and 5,10-dideaza-5,6,7,8-tetrahydrofolic acid (DDATHF) [26] (Fig. 1).

It has been reported that an alkyl group substituted at modified C5-position in some analogues of AMT and MTX could increase potency against DHFR [27]. But a search of the literature indicates that there is little information on varying the substituents on N^5-position, or N^5- and N^{10}-position of tetrahydrofolate and tetrahydroaminopterin. The only example of

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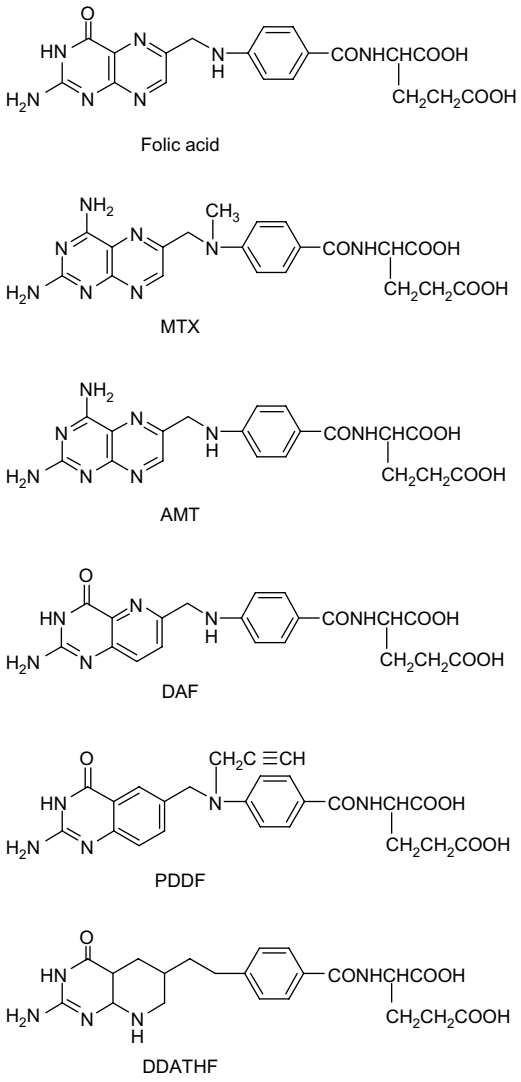


Fig. 1. Structures of folic acid, MTX, AMT, DAF, PDDF and DDATHF.

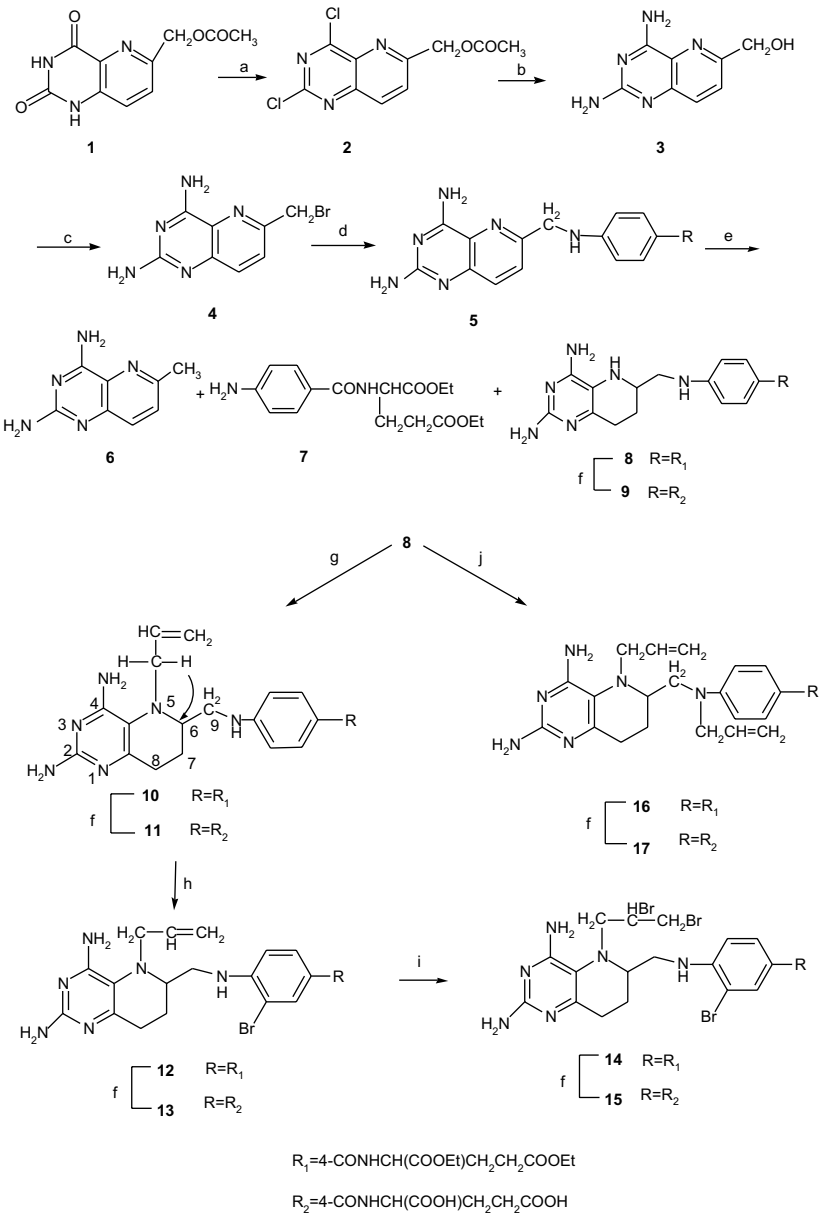
N^5-substituent of tetrahydrofolate available is the synthesis of 5,10-methenyl-8-deaza-5,6,7,8-tetrahydrofolic acid chloride derivative in 1981 [28], no or little information is available about 8-deaza-5,6,7,8-tetrahydroaminopterin and its N^5-substituted derivatives. Herein we report the synthesis and antifolate activities of 8-deaza-5,6,7,8-tetrahydroaminopterin **9**, N^5-alkyl-substituted analogues **11**, **13**, **15**, and N^5,N^{10}-disubstituted analogue **17** as part of a program involving the development of new agents as DHFR inhibitors and as antitumor agents.

2. Chemistry

Our synthesis begins with 6-acetoxymethyl-2,4-dioxopyrido[3,2-*d*]pyrimidine (**1**) [29] as shown in Scheme 1. The reaction of **1** with an excess of $POCl_3$ in the presence of catalytic amount of $(C_2H_5)_3N$ gave 2,4-dichloride derivative (**2**) in 67.2% yield. Using a modified method of Robins and Hitchings [30], **2** was reacted directly with dry saturated ammonia in absolute methanol in a sealed vessel at 150 °C for 14 h to give light yellow solid (**3**) in 81.5% yield [29,31]. Conversion of **3** to **4** was completed using PBr_3 in dry THF according to Srinivasan's procedure [32,33]. Compound **4** could not be purified because of the lability of the bromo group, and the presence of bromine atom was confirmed by mass spectra (TOF-MS, $[M+H]^+$ *m/e* 253.98, 255.98). Reaction of **4** with diethyl *N*-(*p*-aminobenzoyl)-L-glutamate in dry DMAc gave **5** in 70% overall yield from **3**.

The reduction of diethyl 8-deaza aminopterin (**5**) has not been reported, and the method mentioned in the literature was the reduction of 8-deazafolic acid [34]. Previous workers [34] have reported the catalytic hydrogenation of 8-deazafolic acid to give tetrahydro derivative, but no experimental details were given. Some studies have shown that the hydrogenation of 8-deazafolic acid in the presence of platinum was unsuccessful either in aqueous base or formic acid [28]. The approach of reducing the pyridine ring after formylation or acetylation of the N^{10}-position was then adopted [34,35]. In contrast, the hydrogenation of 8-deazafolic acid in CF_3CO_2H was rapid, but over-reduction occurred to generate a mixture that after isolation was shown to contain the predicted product 8-deazatetrahydrofolic acid in only 10% yield, carbon–nitrogen bond hydrogenolysis products 2-amino-5,6,7,8-tetrahydro-6-methylpyrido[3,2-*d*]pyrimidin-4(3*H*)-one (20%) and *N*-(4-aminobenzoyl)-L-glutamic acid diethyl ester (38%), and unidentified components [28]. Srinivasan et al. reported the reduction of diethyl 8-deazafolic acid in ethanol containing an equimolar amount of 0.1 mol/L HCl over Adams catalyst gave diethyl 5,6,7,8-tetrahydro-8-deazafolate in 35–40% yield [32]. Our attempts to reduce **5** in glacial acetic acid or aqueous HCl solution with different loading of PtO_2 were unsuccessful, in which inseparable mixtures were obtained. Fortunately, we found that in lower concentration of acetic acid (an equimolar amount of 0.5 mol/L AcOH in ethanol) and elevated temperature (40 °C), reduction of **5** over Adams catalyst finally gave diethyl 8-deaza-5,6,7,8-tetrahydroaminopterin (**8**) in 67.7% yield. The C–N bond cleavaged products 2,4-diamino-6-methylpyrido[3,2-*d*]pyrimidine (**6**) (5%) and *N*-(4-aminobenzoyl)-L-glutamic acid diethyl ester (**7**) (8%) were also isolated from the reaction mixture. Compound **8** was a mixture of diastereomers due to the appearance of another chiral center C6, and the ratio detected through chiral high performance liquid chromatography was 1:1 (Agilent 1100, Column: DIKMA AS-H, 5 μm, 4.6 × 250 mm, Mobile phase: isopropanol/*n*-hexane = 1:1).

Reaction of the compound **8** with allyl bromide gave monoallyl- or diallyl-substituted 8-deaza-5,6,7,8-tetrahydroaminopterin derivative **10** or **16** depending on the amount of allyl bromide. The use of 1.6 equiv of allyl bromide resulted in the formation of **10** and 3.2 equiv in the formation of **16**. Alkylation of **10** occurred at N^5 rather than at N^{10} was confirmed by ^1H, ^{13}C and HMBC NMR experiments. The ^1H NMR (DMSO-d_6) spectrum showed signals due to CH_2 of allyl group at δ 3.12–3.17 and 3.36–3.41. In the gHMBC spectrum, correlation signals between the long-range *J*-coupled H (3.12–3.17) of CH_2 group and C-6 (δ 51.7) could be observed clearly at cross-points (Scheme 1, compound **10**). No any of the correlation signals between the



Scheme 1. Reagents and conditions: (a) $POCl_3$, NEt_3, reflux, 8 h; (b) saturated NH_3 in dried methanol, 150 °C, 15 h; (c) PBr_3, THF, rt, 8 h; (d) DMAc, diethyl *N*-(*p*-aminobenzly)-L-glutamate, rt, 48 h; (e) PtO_2, 0.3 kPa, 0.5 mol/L AcOH, ethanol, 40 °C, 48 h; (f) 0.3 mol/L NaOH solution, THF, rt, 1–10 h; (g) allyl bromide (1.6 equiv), $NaHCO_3$, THF, rt, 12 h; (h) 1 equiv bromine, DMF, 0 °C to rt 2 h; (i) i. 1 equiv bromine, DMF, allyl bromide (3.5 equiv), $NaHCO_3$, THF, 0 °C to rt, 1 h; ii. 1 equiv bromine, DMF, 0 °C to rt, 2 h; (j) allyl bromide (3.5 equiv), $NaHCO_3$, THF, rt, 24 h.

CH_2 and 9-position or phenyl group could be seen. The bromide or tribromide compound **12** or **14** was obtained in acceptable yields from **10** when adding carefully controlled amounts of bromine. The reaction of **10** with 1 equiv of bromine resulted in the formation of **12** and 2 equiv gave compound **14**.

Finally saponification of the diesters of **8**, **10**, **12**, **14** and **16** gave the target compounds **9**, **11**, **13**, **15** and **17**, respectively.

3. Results and discussion

The compounds **9**, **11**, **13**, **15** and **17** were evaluated for cytotoxicity on cultured human promyclocytic leukemic (HL-60), human hepatocellular carcinoma (Bel-7402), stomach adenocarcinoma (BCG-823) and cervical cancer (Hela) cells (Table 1) [36,37]. And the potency of inhibiting the growth of tumor cells was measured as IC_{50} values compared to MTX. The five target compounds were also evaluated as inhibitors of recombinant human (rh) DHFR (Table 2) [38,39].

Compounds **9** were less active than MTX at concentrations 4- to 10-fold compared to MTX against the four kinds of tumor cell lines and the effect on DHFR was about 17-fold poorer than MTX. Analogues **11**, **13**, **15** and **17** in general have poor activity on the human DHFR. Only compounds **13** and **17** are more active on Bel-7402 cell line about 87- and 15-fold than MTX, respectively.

Table 1
Activity evaluation (IC_{50} μM) against tumor cell lines for compounds **9**, **11**, **13**, **15** and **17** compared with MTX

Compounds	IC_{50}, μM			
	HL-60	BGC-823	Hela	Bel-7402
9	0.90 ± 0.19	0.44 ± 0.10	0.96 ± 0.15	87.9 ± 19.7
11	0.70 ± 0.17	5.2 ± 0.92	7.85 ± 1.73	42.2 ± 8.6
13	0.57 ± 0.16	6.59 ± 1.5	0.90 ± 0.21	1.2 ± 0.23
15	48.8 ± 10.0	22.56 ± 5.05	19.4 ± 6.12	257.4 ± 47.4
17	1.42 ± 0.24	2.43 ± 0.41	21.7 ± 5.60	7.1 ± 1.8
MTX	0.21 ± 0.05	0.11 ± 0.01	0.1 ± 0.023	82.3 ± 10.2

4. Molecular modeling

Compound **9**, which is a mixture of diastereomers (racemic at C6), has certain inhibition activity on hr DHFR though lower than MTX. The **9-*R*** and **9-*S*** were subsequently flexibly docked into the binding site of DHFR complexed with MTX and NADPH (PBD entry 1U72) [40] using AutoDock 3.05 program. Default parameters were used as described in the AutoDock manual unless otherwise specified. Inhibitors of DHFR generally contain a 2,4-diamino substitution in the pyrimidine ring, as typified by MTX [39], which bound to DHFR with a conformation forming hydrogen bonds with the Ile 7, Glu 30, Asn 64, Arg 70 and Val 115 amino acid residues. The docking results revealed that the tetrahydropyrido[3,2-*d*]pyrimidine portion of **9-*R*** and **9-*S*** bound to the DHFR in a "flipped" mode via rotation of the NH_2–C2 bond by 180° [41], and the amino acid residues of DHFR forming hydrogen bonds with **9-*R*** and **9-*S*** were less than those with MTX. In this binding form, the "flipped" **9-*R*** and **9-*S*** can form hydrogen bonds with Glu 30, Asn 64, Arg 70 and Tyr 136 [Figs. 2 and 3]. To MTX complexed with DHFR, the 4-NH_2 forms hydrogen bonds with Ile 7 and Val 115. However, to compound **9** in the "flipped" mode, the 4-NH_2 which was in the opposite position in DHFR couldn't form the same hydrogen bonds as that of MTX due to the distance, and the 2-NH_2 forms hydrogen bonds with Tyr 136. In both MTX and compound **9**, the carboxyl group of the flexible glutamate chains all form hydrogen bonds with Asn 64 and Arg 70. In addition, the non-planar structure of the piperidine ring of **9** can't make π–π hydrophobic interactions with phenyl group of NADPH as the planar structure of pyrido[3,2-*d*]pyrimidine ring of the MTX do.

Table 2
Inhibition of compounds **9**, **11**, **13**, **15**, **17** and MTX on rh DHFR

Compounds	IC_{50}, μM
9	0.560 ± 0.06
11	1.408 ± 0.12
13	2.37 ± 0.11
15	9.34 ± 0.56
17	2.15 ± 0.16
MTX	0.03294 ± 0.00169

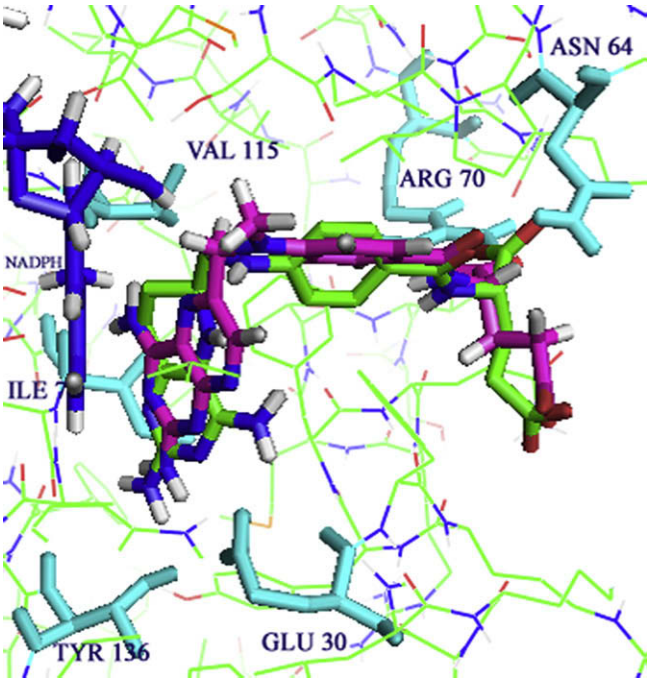


Fig. 2. Docking of **9-*S*** in the crystal structure of human DHFR (PDB code 1U72). Compound **9-*S*** is in green and MTX in red. The labeled amino acid residues Ile 7, Glu 30, Asn 64, Arg 70 and Val 115 can form hydrogen bonds with MTX. And Glu 30, Asn 64, Arg 70, Tyr 136 can form hydrogen bonds with **9-*S***. NADPH (blue) is also shown. (For interpretation of the references to colour in figure legends, the reader is referred to the web version of this article.)

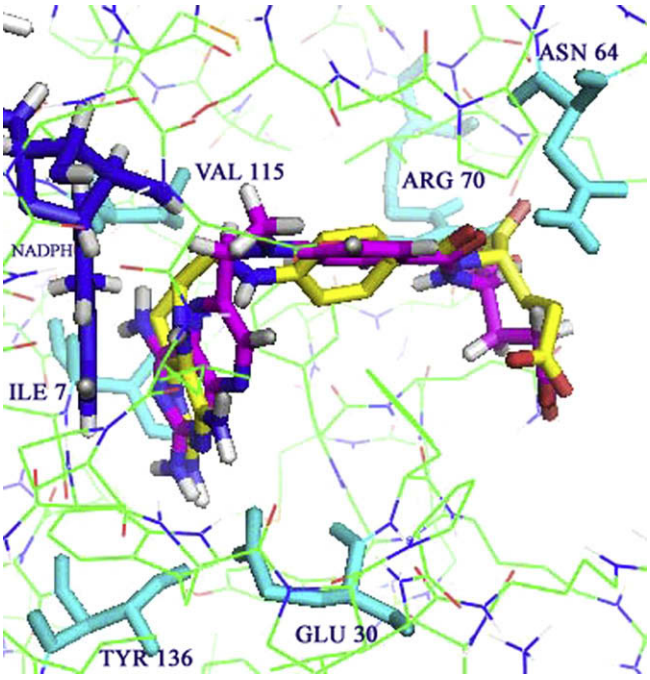


Fig. 3. Docking of **9-*R*** in the crystal structure of human DHFR (PDB code 1U72). Compound **9-*R*** is in yellow and MTX in red. The labeled amino acid residues Ile 7, Glu 30, Asn 64, Arg 70 and Val 115 can form hydrogen bonds with MTX. And Glu 30, Asn 64, Arg 70, Tyr 136 can form hydrogen bonds with **9-*R***. NADPH (blue) is also shown.

5. Conclusion

8-Deaza-5,6,7,8-tetrahydroaminopterin and N^5-substitued or N^5,N^{10}-disubstitued 8-deaza-5,6,7,8-tetrahydroaminopterin were designed and synthesized to explore the biological effect of DHFR inhibitory and antitumor activity. In the course of synthesizing target molecules **9**, **11**, **13**, **15** and **17**, we have developed a suitable condition for the reduction of diethyl 8-deaza aminopterin **5** to the tetrahydro **6** in a moderate yield. The activity of the designed compounds on DHFR was poorer than MTX. The docking results showed that the reason of poor activity of **9** may be due to the less hydrogen bonds formed with DHFR and the destruction of the pyrido[3,2-*d*]pyrimidine planar structure.

6. Experimental

6.1. Chemistry

All evaporations were carried out in vacuo with a rotary evaporator. ^{1}H and ^{13}C NMR spectra were recorded with Jeol-AL-300 and Varian INOVA-500 with DMSO-d_6 as solvent and Me_4Si as an internal standard: s = singlet, d = doublet, t = triplet, q = quartet, m = mutiplet, br s = broad singlet. *J*-values are expressed in hertz. The ESI-TOF spectra were taken on a QSTAR (ABI. SUA) mass spectrometer using methanol as the solvent. Thin layer chromatography (TLC) was performed on POLYGRAM Sil G/UV254 silica gel plates with fluorescent indicator, and the spots were visualized under 254 nm illumination.

6.1.1. Diethyl N-[4-[[2-(2,4-diamino-5,6,7, 8-tetrahydropyrido[3,2-d]pyrimidin-6-yl) methyl]amino]benzoyl]-L-glutamate (8)

To a solution of **5** (600 mg, 1.2 mmol) in ethanol (90 ml) and acetic acid (0.5 mol/L, 12 ml) was added platinum oxide catalyst (60 mg), and the suspension was hydrogenated (0.3 kPa) for 48 h at 40 °C. The reaction mixture was filtered through Celite, and the filtrate was adjusted to pH 7–8 with $NaHCO_3$ solution (5%) and evaporated to dryness under reduced pressure. The residue was added to $CHCl_3$ (10 ml) and then filtered. The filtrate was concentrated to a small volume and purified by column chromatography (silica gel). Elution with $CHCl_3$–CH_3OH (7:1) gave pure **8** (409 mg, 68%) as a white solid; mp 145 °C (decomp). ^{1}H NMR (500 MHz; DMSO-d_6) δ 1.15–1.20 (m, 6H, 2 × CH_2CH_3), 1.59–1.64 (m, 1H, CH-7), 1.97–2.01 (m, 2H, CH-7, $CH_aH_bCH_2CO$), 2.05–2.11 (m, 1H, $CH_aH_bCH_2CO$), 2.41–2.44 (t, J = 9.0 Hz, 2H, CH_2CH_2CO), 2.55–2.63 (m, 2H, CH_2-8), 3.18–3.21 (m, 2H, CH_2NH), 3.30–3.31 (m, 1H, CH-6), 4.03–4.12 (m, 4H, 2 × OCH_2), 4.37–4.41 (m, 1H, NH*CH*CO), 6.38–6.40 (t, J = 6.6 Hz, 1H, CH_2NH), 6.50 (s, 2H, NH_2-2), 6.66, 7.69 (d, each, J = 10.2 Hz, 4H, C_6H_4), 7.33 (s, 2H, NH_2-4), 8.27–8.25 (d, J = 9.0 Hz, 1H, CONH); ^{13}C NMR (125 MHz; DMSO-d_6) δ 14.1 (2 × CH_3), 23.8 (C-8), 24.2 (C-7), 25.9 (CH_2CH_2CO), 30.2 (CH_2CH_2CO), 46.8 (C-9), 49.9 (C-6), 51.8 (NH*CH*CO), 59.9 (CH_2COOCH_2), 60.4 (CHCOOCH_2), 110.8 (2 × C-Ph), 115.8 (C-4a), 120.4 (Ph-C), 129.0 (2 × C-Ph), 134.6 (C-Ph), 151.5 (C-8a), 152.2 (C-4), 157.0 (C-2), 166.5 (CONH), 172.4 (CH*C*OO), 172.6 (CH_2COO). Found $[M+H]^+$: 500.2620, $C_{24}H_{33}N_7O_5$ requires: 500.2616.

6.1.2. N-[4-[[2-(2,4-Diamino-5,6,7,8-tetrahydropyrido [3,2-d]pyrimidin-6-yl)methyl]amino]benzoyl]-L-glutamic acid (9)

A solution of **8** (85 mg, 0.2 mmol) in NaOH solution (0.3 mol/L, 3 ml) and THF (4.3 ml) was stirred at room temperature for 1 h. The organic solvent was removed by evaporation under reduced pressure, the remaining aqueous solution was acidified carefully with 0.5 mol/L HCl, and the solid was collected by filtration, washed with water, and dried in vacuo to give **9** (69.7 mg, 82%) as a white solid; mp 180 °C (decomp). ^{1}H NMR (500 MHz; DMSO-d_6) δ 1.59 (m, 1H, CH-7), 1.89–2.06 (m, 3H, H-7, CH_2CH_2CO), 2.26–2.29 (t, 2H, J = 9 Hz, CH_2CH_2CO), 2.53–2.59 (m, 2H, CH_2-8), 3.13–3.77 (m, 2H, CH_2-9), 3.29 (m, 1H, CH-6), 4.24–4.28 (m, 1H, NH*CH*CO), 6.26–6.28 (m, 1H, CH_2NH), 6.62 and 7.64 (d, each, 4H, C_6H_4, J = 10.8 Hz), 6.93 (br s, 2H, NH_2-2), 7.10 (br s, 2H, NH_2-4), 7.87–7.91 (m, 1H, CONH); ^{13}C NMR (125 MHz; DMSO-d_6) δ 23.9 (C-8), 24.3 (C-7), 27.2 (CH_2CH_2CO), 31.4 (CH_2CH_2CO), 46.9 (C-9), 49.9 (C-6), 52.7 ($CHCH_2CH_2CO$), 110.9 (C-Ph), 115.4 (C-4a), 121.1 (C-Ph), 128.7 (C-Ph), 136.1 (C-Ph), 151.3 (C-8a), 153.1 (C-4), 156.9 (C-2), 165.8 (CONH), 174.9 (COOH), 175.1 (COOH). Found $[M+H]^+$: 444.1995, $C_{20}H_{25}N_7O_5$ requires: 444.1990.

6.1.3. Diethyl N-[4-[[2-(2,4-diamino-5-allyl-5,6,7, 8-tetrahydropyrido[3,2-d]pyrimidin-6-yl)methyl]amino] benzoyl]-L-glutamate (10)

To a solution of diethyl 4-amino-8-deaza-5,6,7,8-tetrahydrofolate **8** (280 mg, 0.5 mmol) in anhydrous DMF (11 ml) were added allyl bromide (0.7 ml, 0.8 mmol) and powdered K_2CO_3 (110 mg) and the reaction mixture was stirred at room temperature for about 12 h. Solvent was removed under reduced pressure. The residue was stirred with water (10 ml) and the mixture was extracted with $CHCl_3$ (3 × 20 ml). The $CHCl_3$ layers were combined and dried over anhydrous Na_2SO_4. After evaporation of solvent to a small volume the residue was purified by column chromatography (silica gel). Elution with $CHCl_3$–CH_3OH (18:1) gave pure **10** (120 mg, 40%) as a light yellow solid; mp 74–76 °C. ^{1}H NMR (500 MHz; DMSO-d_6) δ 1.14–1.18 (m, 6H, 2 × CH_3), 1.62–1.66 (m, 1H, CH_aH_b-7), 1.94–1.99 (m, 1H, $CH_aH_bCH_2CO$), 2.03–2.09 (m, 2H, $NHCHCH_aH_bCH_2$, CH_aH_b-7), 2.82–2.44 (m, 4H, CH_2-8, CH_2CH_2COO), 2.81–2.85 (m, 1H, CH_aH_b-9), 2.95–3.01 (m, 1H, CH_aH_b-9), 3.12–3.18 (m, 2H, CH_aH_b–N^5, CH-6), 3.36–3.41 (m, 1H, CH_aH_b–N^5), 4.01–4.10 (m, 4H, 2 × OCH_2), 4.34–4.38 (m, 1H, NH$CHCH_2CH_2$), 5.09–5.11 (d, 1H, =CH_aH_b–N^5, J = 17.5 Hz), 5.23–5.27 (d, 1H, =CH_aH_b–N^5, J = 10 Hz), 5.57 (s, 2H, NH_2-4), 5.97–6.03 (m, 1H, CH_2NH), 6.03–6.11 (m, 3H, CH_2=CH–N^5, NH_2-2), 6.57 (d, 2H, J = 8.6 Hz, H-Ph), 7.64 (d, 2H, H-Ph), 8.21–8.23 (m, 1H,

CONH); ^{13}C NMR (125 MHz; DMSO-d_6) δ 14.1 (2 × CH_3), 17.8 (C-7), 24.4 (C-8), 25.8 (CH_2CH_2COO), 30.2 (CH_2COOEt), 43.3 (C-9), 51.7 (C-6), 51.8 (NH*CH*COOEt), 55.6 (CH_2CH═), 59.9 (CHCOOCH_2), 60.4 (CH_2COOCH_2), 110.9 (2 × C-Ph), 115.2 (4a), 116.9 (═CH_2), 120.2 (C-Ph), 128.9 (2 × C-Ph), 136.9 (CH═), 151.5 (C-Ph), 153.2 (8a), 158.3 (C-2), 160.0 (C-4), 166.5 (CONH), 172.2 (CH_2COO), 172.3 (CHCOO). Found $[M + H]^+$: 540.2934, $C_{27}H_{38}N_7O_5$ requires: 540.2929.

6.1.4. N-[4-[[2-(2,4-Diamino-5-allyl-5,6,7, 8-tetrahydropyrido[3,2-d]pyrimidin-6-yl)methyl]amino]benzoyl]-L-glutamic acid or 8-deaza-N^5-allyl-5,6,7,8-tetrahydroaminopterin (**11**)

A procedure similar to the preparation of **9** was followed to prepare **11** from **10**. Compound **11** was obtained in 81.5% yield as a white solid; mp 180 °C (dec). ^{1}H NMR (500 MHz; DMSO-d_6) δ 1.65–1.67 (m, 1H, CH-7), 1.89–1.94 (m, 1H, NHCHCH_aH$_b$), 2.01–2.06 (m, 2H, NHCHCH$_a$$H_b$, CH-7), 2.29–2.32 (m, 2H, CH_2CO), 2.41–2.47 (m, 2H, CH_2-8), 2.83–2.87 (m, 1H, CH_aH$_b$-9), 2.97–3.02 (m, 1H, CH$_a$$H_b$-9), 3.14–3.19 (m, 2H, C$H_aH_b$–N^5, CH-6), 3.39–3.42 (m, 1H, CH$_a$$H_b$–N^5), 4.29–4.34 (m, 1H, *CH*COOH), 5.12–5.14 (d, 1H, J = 10 Hz, ═CH_aH$_b$), 5.24–5.28 (d, 1H, J = 17 Hz, ═CH$_a$$H_b$), 5.95–5.95 (m, 1H, CH_2NH), 6.06–6.11 (m, 1H, CH_2CH═), 6.20 (br s, 2H, NH_2-4), 6.50–6.59 (m, 4H, 2 × H-Ph, NH_2-2), 7.64–7.65 (d, 2H, J = 4.4 Hz, H-Ph); ^{13}C NMR (125 MHz; DMSO-d_6) δ 17.3 (C-7), 22.4 (C-8), 26.7 (NHCHCH_2), 30.9 (CH_2CO), 43.2 (CH_2NH), 51.7 (C-6), 52.2 (*CH*COOH), 55.6 (NCH_2), 111.0 (C-Ph), 115.2 (4a), 117.4 (═CH_2), 120.8 (C-Ph), 128.8 (C-Ph), 136.5 (CH_2CH═), 151.3 (C-Ph), 156.2 (C-8a), 160.8 (C-2), 166.1 (C-4), 173.8 (CONH), 174.4 (CH_2COOH), 174.5 (CHCOOH). Found $[M + H]^+$: 484.2310, $C_{23}H_{29}N_7O_5$ requires: 484.2303.

6.1.5. Diethyl N-[4-[[2-(2,4-diamino-5-allyl-5,6,7, 8-tetrahydropyrido[3,2-d]pyrimidin-6-yl)methyl] amino]-3-bromo-benzoyl]-L-glutamate (**12**)

To a solution of **10** (10 mg, 0.02 mmol) in dried THF (0.2 ml) was added dropwise 1 μl of bromide (0.02 mmol) in an ice bath. The resulting mixture was warmed to room temperature and stirred for 2 h. Solvent was removed under reduced pressure. To the residue water (10 ml) was added and the mixture was extracted with CH_2Cl_2 (3 × 10 ml). The extracts were pooled and dried over anhydrous Na_2SO_4. After evaporation of solvent to a small volume the residue was purified by preparative thin layer chromatography (silica gel). Elution with $CHCl_3$–CH_3OH (8:1) gave pure **12** (8 mg, 69.7%) as a light yellow solid; mp 91–92 °C. ^{1}H NMR (500 MHz; DMSO-d_6) δ 1.15–1.19 (m, 6H, 2 × CH_3), 1.63–1.68 (m, 1H, CH_aH$_b$-7), 1.96–2.09 (m, 2H, CHC$H_2$$CH_2$), 2.10–2.15 (m, 1H, CH$_a$$H_b$-7), 2.37–2.44 (m, 3H, $CH_2CH_2CH_2$CO, CH_aH$_b$-8), 2.48–2.55 (m, 1H, CH_aH$_b$-8), 2.92–2.97 (m, 1H, CH_aH$_b$-9), 3.14–3.22 (m, 2H, CH$_a$$H_b$-9, C$H_aH_b$–N^5), 3.28 (m, 1H, CH-6), 3.36–3.40 (m, 1H, CH$_a$$H_b$–N^5), 4.02–4.11 (m, 4H, 2 × OCH_2), 4.36–4.40 (m, 1H, *CH*CH_2CH_2), 5.16–5.18 (d, 1H, J = 10.0 Hz, ═CH_aH$_b$), 5.30–5.33 (d, 1H, J = 17.0 Hz, ═CH$_a$$H_b$), 5.61–5.63 (m, 1H, CH_2NH), 5.74 (br s, 2H, NH_2-2), 6.00–6.06 (m, 3H, CH_2CH═, 4-NH_2), 6.63–6.64 (m, 1H, J = 9 Hz, H-Ph), 7.74 (d, 1H, J = 8.5 Hz, H-Ph), 8.03 (d, 1H, J = 1.5 Hz, H-Ph), 8.48–8.50 (d, 1H, CONH); ^{13}C NMR (125 MHz; DMSO-d_6) δ 14.1 (CH_3), 17.7 (C-7), 24.1 (C-8), 25.7 (NHCHCH_2), 30.2 (CH_2CO), 43.1 (C-9), 51.6 (C-6), 51.9 (*CH*COOH), 55.6 (C–N^5), 59.9 (OCH_2), 60.4 (OCH_2), 107.6 (C-Ph), 110.2 (C-Ph), 114.8 (C-4a), 117.5 (═CH_2), 121.7 (C-Ph), 128.6 (C-Ph), 131.5 (C-Ph), 136.4 (–CH═), 147.3 (C-Ph), 152.8 (8a), 158.1 (C-2), 159.7 (C-4), 165.2 (CONH), 172.0 (CH_2CO), 172.2 (CHCOO). Found $[M + H]^+$: 618.2044, $C_{27}H_{36}BrN_7O_5$ requires: 618.2034.

6.1.6. N-[4-[[2-(2,4-Diamino-5-allyl-5,6,7, 8-tetrahydropyrido[3,2-d]pyrimidin-6-yl)methyl] amino]-3-bromo-benzoyl]-L-glutamic acid (**13**)

A procedure similar to the preparation of **9** was followed to prepare **13** from **12**. Compound **13** was obtained in 79% yield as a white solid; mp 150 °C (dec). ^{1}H NMR (300 MHz; DMSO-d_6) δ 1.62–1.76 (m, 1H, CH_aH$_b$-7), 1.86–2.09 (m, 3H, CH$CH_2$$CH_2$, CH$_a$$H_b$-7), 2.10–2.32 (t, 2H, J = 7.2 Hz, $CH_2CH_2CH_2$CO), 2.44–2.60 (m, 2H, CH_2-8), 2.95–3.05 (m, 1H, CH_aH$_b$-9), 3.15–3.22 (m, 2H, CH$_a$$H_b$-9, C$H_aH_b$–N^5), 3.31 (m, 1H, CH-6), 3.39–3.43 (m, 1H, CH$_a$$H_b$–N^5), 4.28–4.36 (m, 1H, *CH*CH_2CH_2), 5.16–5.20 (d, 1H, J = 10.8 Hz, ═CH_aH$_b$), 5.28–5.33 (d, 1H, J = 17.1 Hz, ═CH$_a$$H_b$), 5.65–5.69 (m, 1H, CH_2NH), 6.00–6.15 (m, 1H, CH_2CH═), 6.62–6.65 (m, 1H, J = 9 Hz, H-Ph), 6.97 (s, 2H, NH_2-2), 7.72–7.75 (d, 1H, J = 8.7 Hz, H-Ph), 8.03–8.03 (d, 1H, J = 1.8 Hz, H-Ph), 8.26–8.29 (d, 1H, J = 7.8 Hz, CONH); ^{13}C NMR (75 MHz; DMSO-d_6) δ 16.8 (C-7), 21.5 (C-8), 26.4 (NHCHCH_2), 30.9 (CH_2CO), 43.1 (C-9), 51.5 (C-6), 52.3 (*CH*COOH), 55.6 (C–N^5), 107.6 (C-Ph), 110.2 (C-Ph), 115.1 (C-4a), 118.0 (═CH_2), 122.2 (C-Ph), 128.4 (C-Ph), 131.6 (C-Ph), 136.1 (–CH═), 146.6 (C-Ph), 147.2 (8a), 155.2 (C-2), 160.9 (C-4), 164.9 (CONH), 174.3 (CH_2CO), 174.4 (CHCOO). Found $[M + H]^+$: 562.1415, $C_{27}H_{36}BrN_7O_5$ requires 562.1408.

6.1.7. Diethyl N-[4-[[2-(2,4-diamino-5-(2,3-dibromopropane)-5,6,7,8-tetrahydropyrido[3,2-d]pyrimidin-6-yl)methyl]amino]3-bromo-benzoyl]-L-glutamate (**14**)

To a solution of **10** (10 mg, 0.02 mmol) in dried THF (0.2 ml) was added dropwise 1 μl of bromide (0.02 mmol) in an ice bath. The resulting mixture was warmed to room temperature and stirred for 1 h. TLC showed the disappearance of starting materials and the appearance of the major spot of **12**. Then the mixture was cooled in an ice bath and added dropwise 1 μl of bromide (0.02 mmol). After warming to room temperature the resulting solution was stirred for 2 h again. TLC showed the disappearance of the spot of **12** and the appearance of the spot of **14**. Solvent was removed under reduced pressure. To the resulting residue water (10 ml) was added and the mixture was extracted with CH_2Cl_2 (3 × 10 ml). The extracts were pooled and dried over anhydrous Na_2SO_4. After evaporation of solvent to a small volume

the residue was purified by preparative thin layer chromatography (silica gel). Elution with $CHCl_3$–CH_3OH (8:1) gave pure **14** (9 mg, 62%) as a light yellow solid; mp 110–113 °C. ^{1}H NMR (500 MHz; DMSO-d_6) δ 1.15–1.19 (m, 6H, 2 × CH_3), 1.63–1.70 (m, 1H, CH_aH_b-7), 1.91–2.11 (m, 2H, CH_2CH_2CO), 2.16–2.23 (m, 1H, CH_aH_b-7), 2.40–2.44 (m, 3H, CH_2CH_2CO, CH_aH_b-8), 2.47–2.51 (1H, CH_aH_b-8), 2.89–2.99 (m, 1H, CH_aH_b-9), 3.16–3.22 (m, 2H, CH_aH_b-9, CH-6), 3.33–3.39 (m, 2H, N^5-CH_2), 3.49–3.51 (m, 2H, CH_2Br), 3.97–4.00 (m, 1H, CHBr), 4.02–4.11 (m, 6H, 2 × OCH_2), 4.36–4.40 (m, 1H, $CHCH_2CH_2$), 4.76–4.82 (m, 1H, CH_2NH), 5.55–5.71 (br s, NH_2), 5.93 (br s, NH_2), 6.68 (d, 2H, J = 8.8 Hz, H-Ph), 7.73–7.75 (d, 1H, J = 8.5 Hz, H-Ph), 8.03–8.03 (m, 1H, H-Ph), 8.45–8.47 (d, 1H, J = 7.5 Hz, CONH). Found $[M+H]^+$: 776.0428, $C_{27}H_{36}BrN_7O_5$ requires: 776.0401.

6.1.8. N-[4-[[2-(2,4-Diamino-5-(2,3-dibromopropane)-5,6,7,8-tetrahydropyrido[3,2-d]pyrimidin-6-yl)methyl]amino]-3-bromobenzoyl]-L-glutamate (15)

A procedure similar to the preparation of **9** was followed to prepare **15** from **14**. Compound **15** was obtained in 82% yield as a white solid; mp 180 °C (dec). ^{1}H NMR (300 MHz; DMSO-d_6) δ 1.63–1.67 (m, 1H, CH_aH_b-7), 1.82–2.10 (m, 2H, CH_2CH_2CO), 2.12–2.21 (m, 1H, CH_aH_b-7), 2.26–2.35 (m, 3H, CH_2CH_2CO, CH_aH_b-8), 2.41–2.61 (m, 2H, CH_2-9), 2.95–3.06 (m, 1H, CH-6), 3.12–3.34 (m, 2H, N^5-CH_2) 4.00–4.12 (m, 1H, CHBr), 4.25–4.38 (t, 1H, CONH$CHCH_2$), 5.73 (br s, NH_2), 6.19 (br s, NH_2), 6.57 (br s, NH-10), 6.64–6.67 (d, 1H, J = 6.9 Hz, H-Ph), 7.71–7.74 (d, 1H, H-Ph), 8.03 (s, 1H, H-Ph), 8.31–8.34 (d, 1H, J = 7.8 Hz, CONHCH). Found $[M+H]^+$: 719.9765, $C_{23}H_{28}Br_3N_7O_5$ requires: 719.9775.

6.1.9. Diethyl N-[4-[[2-(2,4-diamino-5,10-diallyl-5,6,7,8-tetrahydropyrido[3,2-d]pyrimidin-6-yl)methyl]amino]benzoyl]-L-glutamate (16)

To a solution of diethyl 4-amino-8-deaza-5,6,7,8-tetrahydrofolate **8** (560 mg, 0.9 mmol) in anhydrous DMF (22 ml) were added allyl bromide (2.8 ml, 3.2 mmol) and powdered K_2CO_3 (220 mg), and the reaction mixture was stirred at room temperature for about 24 h. Solvent was removed under reduced pressure. To the resulting residue water (20 ml) was added and the mixture was extracted with $CHCl_3$ (3 × 20 ml). The extracts were pooled and dried over anhydrous Na_2SO_4. After evaporation of solvent to a small volume the residue was purified by column chromatography (silica gel). Elution with $CHCl_3$–CH_3OH (22:1) gave pure **16** (448 mg, 69%) as a yellow solid; mp 67–69 °C. ^{1}H NMR (500 MHz; DMSO-d_6) δ 1.14–1.18 (m, 6H, 2 × CH_3), 1.65–1.68 (m, 1H, CH-7), 1.95–2.01 (m, 1H, $CHCH_aH_b$), 2.05–2.09 (m, 2H, $CHCH_aH_bCH_2$, CH-7), 2.36–2.44 (m, 4H, CH_2-8, CH_2CH_2COO), 3.09–3.13 (m, 1H, CH_aH_b–N^{10}), 3.16–3.21 (m, 1H, CH-6), 3.25–3.38 (m, 3H, CH_2-9, CH_aH_b–N^{10}), 3.85–3.88 (m, 1H, CH_aH_b–N^5), 4.01–4.09 (m, 5H, OCH_2 × 2, CH_aCH_b–N^5), 4.35–4.39 (m, 1H, CONHCH), 4.93–4.97 (m, 2H, CH_aH_b=–N^{10}, CH_aCH_b=–N^5), 5.04–5.06 (d, 1H, J = 10 Hz, CH_aH_b=–N^5), 5.17–5.20 (d, 1H, J = 17.0 Hz, CH_aCH_b=–N^{10}), 5.70–5.77 (m, 1H, CH=–N^5), 5.82–5.88 (m, 1H, CH=–N^{10}), 6.59 (d, H, J = 8.5 Hz, H-Ph), 7.67 (d, 4H, H-Ph), 8.27–8.28 (m, 1H, CONH); ^{13}C NMR (125 MHz; DMSO-d_6) δ 14.1 (2 × CH_3), 17.7 (C-7), 24.0 (C-8), 25.8 (CH_2CH_2CO), 30.2 (CH_2CH_2CO), 51.3 (C-6), 51.6 (C-9), 51.8 (NHCHCO), 53.2 (CH_2–N^5), 55.5 (CH_2–N^{10}), 59.9 (OCH_2), 60.4 (OCH_2), 110.6 (2 × C-Ph), 115.3 (CH_2=–N^5), 115.8 (C-4a), 116.4 (CH_2=–N^{10}), 119.9 (C-Ph), 128. 9 (2 × C-Ph), 133.6 (CH=–N^5), 136.6 (CH=–N^{10}), 150.4 (C-Ph), 152.5 (C-8a), 157.9 (C-2), 159.7 (C-4), 166.5 (CONH), 172.2 (CH_2COO), 172.3 ($CHCOO$). Found $[M+H]^+$: 580.3246, $C_{30}H_{41}N_7O_5$ requires: 580.3242.

6.1.10. N-[4-[[2-(2,4-Diamino-5,10-diallyl-5,6,7,8-tetrahydropyrido[3,2-d]pyrimidin-6-yl)methyl]amino]benzoyl]-L-glutamic acid or 8-deaza-N^5,N^{10}-diallyl-5,6,7,8-tetrahydroaminopterin (17)

A procedure similar to the preparation of **9** was followed to prepare **17** from **16**. Compound **17** was obtained in 73% yield as a white solid; mp 140 °C (dec). ^{1}H NMR (300 MHz; DMSO-d_6) δ 1.65–1.75 (m, 1H, CH-7), 1.90–2.00 (m, 1H, $CHCH_aH_b$), 2.03–2.12 (m, 2H, $CHCH_aH_bCH_2$, CH-7), 2.23–2.36 (m, 2H, CH_2CH_2COO), 2.47–2.50 (m, 2H, CH_2-8), 3.08–3.15 (m, 1H, CH_aH_b–N^{10}), 3.20–3.40 (m, 4H, CH-6, CH_2-9, CH_aH_b–N^{10}), 3.85–3.90 (m, 1H, CH_aCH_b–N^5), 4.07–4.12 (m, 1H, CH_aCH_b–N^5), 4.28–4.38 (m, 1H, CONHCH), 4.94–5.02 (m, 2H, CH_aH_b=–N^{10}, CH_aH_b=–N^5), 5.05–5.09 (d, 1H, J = 10.5 Hz, CH_aH_b=–N^5), 5.16–5.21 (d, 1H, J = 17.1 Hz, CH_aH_b=–N^{10}), 5.71–5.82 (m, 1H, CH=–N^5), 5.84–5.90 (m, 1H, CH=–N^{10}), 6.60 (d, 2H, J = 8.4 Hz, H-Ph), 7.68 (d, 2H, H-Ph), 8.04–8.06 (m, 1H, CONH); ^{13}C NMR (75 MHz; DMSO-d_6) δ 17.3 (C-7), 22.5 (C-8), 26.7 (CH_2CH_2CO), 31.0 (CH_2CH_2CO), 51.3 (C-6), 51.5 (C-9), 52.3 (NHCHCO), 53.3 (CH_2–N^5), 55.5 (CH_2–N^{10}), 110.7 (2 × Ph-C), 115.3 (CH_2=–N^5), 115.8 (C-4a), 116.8 (CH_2=–N^{10}), 120.5 (Ph-C), 128.7 (2 × C-Ph), 133.8 (CH=–N^5), 136.4 (CH=–N^{10}), 148.9 (Ph-C), 150.2 (C-8a), 156.4 (C-2), 166.0 (C-4), 166.1 (CONH), 174.5 (CH_2COO), 174.7 ($CHCOO$). Found $[M+H]^+$: 524.2628, $C_{26}H_{33}N_7O_5$ requires: 524.2616.

6.2. Biological evaluation

Human dihydrofolate reductase and MTX were purchased from Sigma Chemical Co. RPMI-1640 medium was produced from GIBCO. Sulforhodamine B (SRB), 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT), tetrazolium salt and propidium iodide were purchased from Sigma Chemical Co. Microplate reader from FLUOsta OPTIMA, Germany and BECScan flow cytometer from Becton Sickinson FACScan, American.

6.2.1. Cell lines and culture conditions

Human promyelocytic leukemic cell line (HL-60), human hepatocellular carcinoma cell line (Bel-7402), stomach adenocarcinoma (BCG-823) and cervical cancer (Hela) were grown and maintained in RPMI-1640 medium supplemented with

10% fetal bovine serum, penicillin (100 U/ml), streptomycin concentration at 37 °C in a humidified incubators in an atmosphere of 5% CO_2. All of experiments were performed on exponentially growing cancer cells. Inhibition of cell growth was analyzed using MTT and SRB assays [35,36] after 48 h treatment of different dosages of compounds. The data are presented in Table 1 as the mean of three independent experiments.

6.2.2. Dihydrofolate reductase (DHFR) assay

All recombinant human (rh) DHFR enzymes were assayed spectrophotometrically in a solution containing 0.1 mM dihydrofolate, 0.3 mM NADPH, and 100 mM KCl at pH 7.5 at 32 °C. The change in absorbance was measured at 340 nm by microplate spectrophotometer [37,38].

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Reference

[1] A. Gangjee, Y.B. Zeng, J.J. McGuire, R.L. Kisliuk, J. Med. Chem. 48 (2005) 5329–5336.
[2] N.J. Curtin, A.N. Hughes, Lancet Oncol. 2 (2001) 298–305.
[3] A. Gangjee, H.D. Jain, J. Phan, X. Lin, X.H. Song, J.J. McGuire, R.L. Kisliuk, J. Med. Chem. 49 (2006) 1055–1065.
[4] E.C. Taylor, P.J. Harrington, S.R. Fletcher, G.P. Beardsley, R.G. Moran, J. Med. Chem. 28 (1985) 914–921.
[5] A. Gangjee, A. Vidwans, E. Elzein, J.J. McGuire, S.F. Queener, R.L. Kisliuk, J. Med. Chem. 44 (2001) 1993–2003.
[6] E.C. Taylor, J.E. Dowling, Bioorg. Med. Chem. Lett. 7 (1997) 453–456.
[7] A. Gangjee, N.P. Dubash, S.F. Queener, J. Heterocycl. Chem. 37 (2000) 935–942.
[8] C. Shih, G.B. Grindey, E.C. Taylor, P.M. Harrington, Bioorg. Med. Chem. Lett. 2 (1992) 339–342.
[9] J.I. DeGraw, P.H. Christle, W.T. Colwell, F.M. Sirotnak, J. Med. Chem. 35 (1992) 320–324.
[10] E.C. Taylor, R.P. Chaudhuri, Tetrahedron 55 (1999) 1631–1638.
[11] J.I. DeGraw, W.T. Colwell, V.H. Brown, M. Sato, R.L. Kisliuk, Y. Gaumon, J. Thorndike, F.M. Sirotnak, J. Med. Chem. 31 (1988) 150–153.
[12] J.I. DeGraw, W.T. Colwell, J.R. Piper, F.M. Sirotnak, J. Med. Chem. 36 (1993) 2228–2231.
[13] E.C. Taylor, R. Chaudhari, K. Lee, Invest. New Drugs 14 (1996) 281–285.
[14] E.C. Taylor, J.E. Dowling, J. Org. Chem. 62 (1997) 1599–1603.
[15] J.R. Piper, J.I. DeGraw, W.T. Colwell, C.A. Johnson, R.L. Smith, W.R. Waud, F.M. Sirotnak, J. Med. Chem. 40 (1997) 377–384.
[16] J.I. DeGraw, W.T. Colwell, J. Crase, R.L. Smith, J.R. Piper, W.R. Waud, F.M. Sirotnak, J. Med. Chem. 40 (1997) 370–376.
[17] V. Bavetsias, G.M.F. Bisset, R. Kimbell, F.T. Boyle, A.L. Jackman, Tetrahedron 53 (1997) 13383–13396.
[18] E.C. Taylor, L.D. Jennings, Z. Mao, B. Hu, J.G. Jun, P. Zhou, J. Org. Chem. 62 (1997) 5392–5403.
[19] E.C. Taylor, P. Zhou, L.D. Jennings, Z. Mao, B. Hu, J.G. Jun, Tetrahedron Lett. 38 (1997) 521–524.
[20] A. Gangjee, Y. Zhu, S.F. Queener, P. Francom, A.D. Broom, J. Med. Chem. 39 (1996) 1836–1845.
[21] W. Pendergast, S.H. Dickerson, I.K. Dev, R. Ferone, D.S. Duck, G.K. Smith, J. Med. Chem. 37 (1994) 838–844.
[22] A. Rosowsky, Prog. Med. Chem. 26 (1989) 3.
[23] J.A. Blair, A.J. Pearson, J. Chem. Soc. Perkin Trans. II (1974) 80.
[24] E.C. Taylor, D.C. Palmer, T.J. George, S.R. Fletcher, C.P. Tseng, P.J. Harrington, J. Org. Chem. 48 (1983) 4852–4860.
[25] J.R. Piper, N.D. Malik, M.S. Rhee, J. Galivan, F.M. Sirotnak, J. Med. Chem. 35 (1992) 332–337.
[26] E.C. Taylor, B. Hu, Heterocycles 45 (1997) 241–253.
[27] J.R. Piper, G.S. McCaleb, J.A. Montgomery, R.L. Kisliuk, Y. Gaumont, F.M. Sirotnak, J. Med. Chem. 29 (1986) 1080–1087.
[28] C. Temple, C.L. Kussner, J.D. Rose, K.L. Smithers, L.L. Bennett, J.A. Montgomery, J. Med. Chem. 24 (1981) 1254–1258.
[29] A. Srinivasan, A.D. Broom, J. Org. Chem. 46 (1981) 1777–1781.
[30] R.K. Robins, G.H. Hitchings, J. Chem. Soc. 77 (1955) 2256–2260.
[31] A. Srimivasan, V. Amarnath, A.D. Broom, F.C. Zou, Y.-C. Cheng, J. Med. Chem. 27 (1984) 1710–1717.
[32] A. Srinivasan, A.D. Broom, Tetrahedron Lett. 23 (1982) 1431–1434.
[33] A. Rosowsky, R.A. Forsch, H. Bader, J.H. Freisheim, J. Med. Chem. 34 (1991) 1447–1454.
[34] J.I. DeGraw, R.L. Kisliuk, Y. Gaumont, C.M. Baugh, J. Med. Chem. 17 (1974) 470–471.
[35] S.K. Singh, S.C. Singer, R. Ferone, K.A. Waters, R.J. Mullin, J.B. Hynes, J. Med. Chem. 35 (1992) 2002–2006.
[36] P. Skehan, R. Storeng, D. Scudiero, A. Monks, J. McMahon, D. Vistica, J.T. Warren, H. Bokesch, S. Kenney, M.R. Boyd, J. Natl. Cancer Inst. 82 (1990) 1107–1112.
[37] J. Carmichael, W.G. DeGraff, A.F. Gazdar, J.D. Minna, J.B. Mitchell, Cancer Res. 47 (1987) 936–942.
[38] K. Urakawa, M. Mihara, N. Takagi, A. Kawamura, K. Akamatsu, Y. Takeda, Eur. J. Pharmacol. 435 (2002) 237–244.
[39] R.L. Kisliuk, D. Strumpf, Y. Gaumont, R.P. Leary, L. Plante, J. Med. Chem. 20 (1977) 1531–1533.