Synthesis and Biological Evaluation of 5-Arylfuro[2,3-*d*]pyrimidines as Novel Dihydrofolate Reductase Inhibitors

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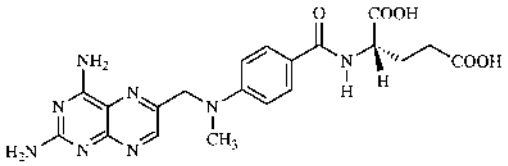
A series of about fifty novel 5-arylfuro[2,3-*d*]pyrimidine derivatives were synthesized as potential inhibitors of dihydrofolate reductase (DHFR) arising from different species. Weak enzyme inhibition was observed for most of the compounds, with only a few reaching IC_{50} values less than 30 μM. With regards to antibacterial and antimalarial potency, only seven compounds showed a modest *in vitro* activity against some bacteria strains and only three products proved significantly active against *P. falciparum*.

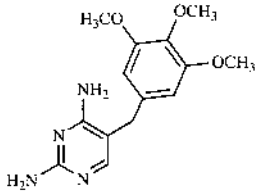
Key words furo[2,3-*d*]pyrimidine; antifolate; trimethoprim analogue; dihydrofolate reductase inhibition; antibacterial; antimalarial

Several enzymes involved in the biosynthesis of nucleic acid precursors have become attractive biochemical target sites over the past twenty years. In particular, dihydrofolate reductase (DHFR), a crucial NADPH-linked dehydrogenase that catalyses the reduction of dihydrofolate (and folate with a lower efficiency) to tetrahydrofolate has attracted considerable attention.[1] The inhibition of this ubiquitous enzyme, which plays an essential role in maintaining cellular pools of tetrahydrofolate derivatives, has led to major drugs for use in cancer chemotherapy and as antiprotozoan and antibacterial agents.[2] These compounds are conventionally divided into two main classes : "classical" antifolates bearing a glutamate moiety (also present in the natural folic substrate) and "non-classical" antifolates devoid of this side-chain, which confer a rather lipophilic character to such molecules. The prominent example of "classical" DHFR inhibitors is methotrexate (MTX, **1**) which is a potent and still widely employed antineoplastic agent,[3] whereas "non-classical" antimetabolites, such as trimethoprim (TMP, **2**), trimetrexate (TMQ, **3**), piritrexim (PTX, **4**) and pyrimethamine (PM, **5**) are more particularly used against pathogenic microorganisms. In this context, during the last decade, a renewed interest in this area appeared because of the persistent demand for novel drugs for treatment and prophylaxis of opportunistic infections in immunosuppressed AIDS victims.[4]

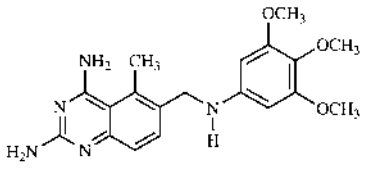
As an application of the synthetic strategy to the 6-arylfuro[2,3-*d*]pyrimidine system that we developed a few years ago,[5] we describe herein the preparation and biological evaluation of several conformationally constrained analogues of **2**. The new potential folate antagonists prepared in the present work are related to several other 6/5-fused heterocyclic systems including cyclopenta[*d*]pyrimidines,[6] pyrrolo[2,3-*d*]pyrimidines,[7] pyrrolo[3,2-*d*]pyrimidines,[6b,8] thieno[2,3-*d*]-pyrimidines,[9] purines[10] and furo[2,3-*d*]pyrimidines;[11] but



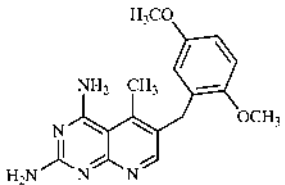
MTX 1



TMP 2



TMQ 3



PTX 4

PM 5

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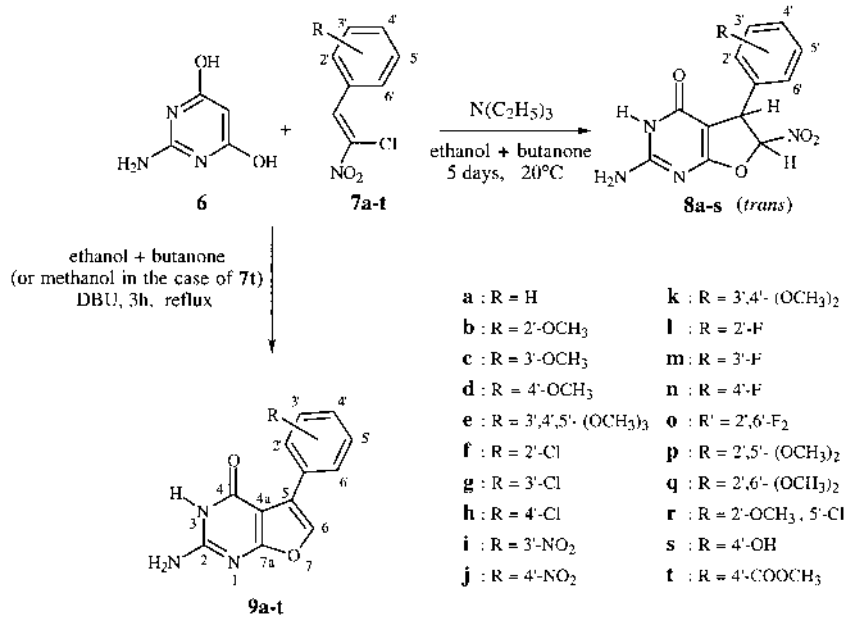

Chart 1

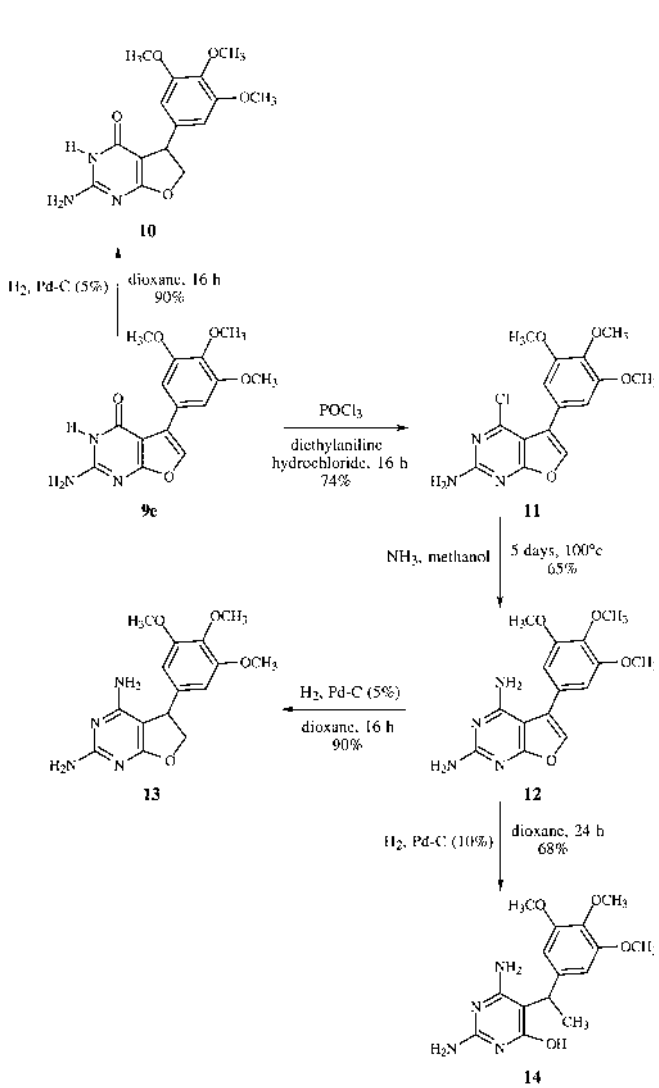

Chart 2

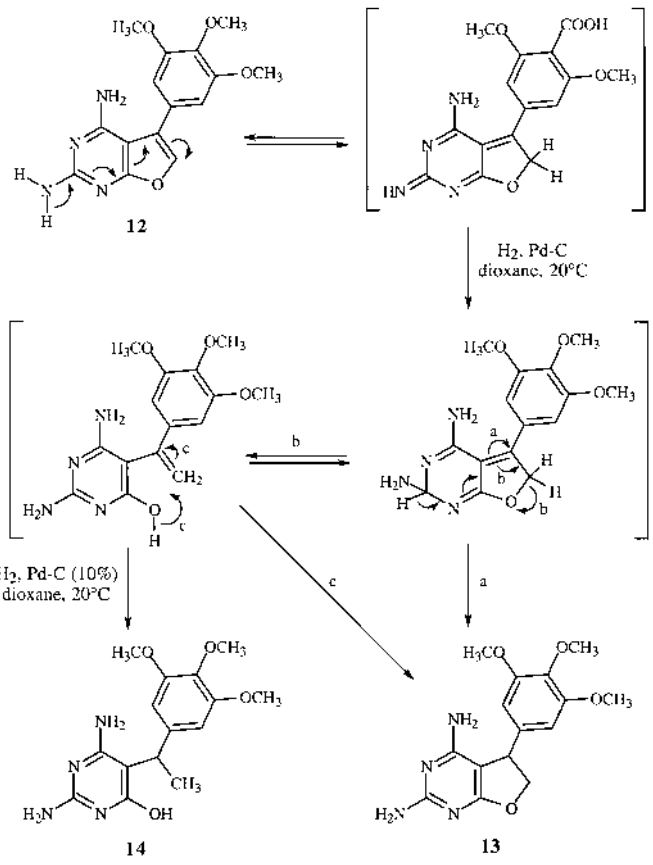

Chart 3

differ from these previously described antifolate-designed molecules in that, as in the model TMP itself, only two C–C bonds separate the side-aromatic ring from the 5-position of the pyrimidine nucleus.

Chemistry

As depicted in Chart 1, the 5-aryl-2-aminofuro[2,3-*d*]pyri-

midin-4-(3*H*)-ones (**8a**—**s** and **9a**—**t**) were generally synthesized from 2-amino-4,6-dihydroxypyrimidine (**6**) and the corresponding 2-chloro-2-nitroethenylbenzenes (**7**). Compounds **8a**—**s** were prepared, at room temperature, in a mixture of ethanol–butanone in the presence of triethylamine, whereas furo[2,3-*d*]pyrimidines **9a**—**t** were obtained in a refluxing mixture of ethanol and butanone in the presence of 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU).

The novel 3,4,5-trimethoxyphenyl derivatives (**10**—**14**), related to the TMP model (**2**), were prepared, as shown in Chart 2, starting from 2-amino-5-(3,4,5-trimethoxyphenyl)-furo[2,3-*d*]pyrimidin-4-(3*H*)-one (**9e**). Direct catalytic hydrogenation of **9e** gave 2-amino-5,6-dihydro-5-(3,4,5-trimethoxyphenyl)furo[2,3-*d*]pyrimidin-4-(3*H*)-one (**10**). Chlorination of **9e** with phosphorous oxychloride in the presence of diethylaniline hydrochloride provided 2-amino-4-chloro-5-(3,4,5-trimethoxyphenyl)furo[2,3-*d*]pyrimidin-4-(3*H*)-one (**11**) which was further converted into 2,4-diamino-5-(3,4,5-trimethoxyphenyl)furo[2,3-*d*]pyrimidine (**12**) by heating for five days in an autoclave with ammonia in methanol. Catalytic reduction of **12** in dioxane gave either 2,4-diamino-5,6-dihydro-5-(3,4,5-trimethoxyphenyl)furo[2,3-*d*]pyrimidine [**13** (by using 5% Pd–C)] or 6-hydroxy-5-[1-(3,4,5-trimethoxyphenyl)-1-ethyl]-2,4-pyrimidinediamine [**14** (by using 10% Pd–C)]. A plausible mechanism to explain the *a priori* unexpected opening of the furan ring leading to the formation of **14** is detailed in Chart 3. In the same context, because of the similar substitution of the side-aromatic ring with that of the "non classical" antifolate PTX (**4**), 2-amino-5,6-dihydro-5-(2,5-dimethoxyphenyl)furo[2,3-*d*]pyrimidin-4-(3*H*)-one (**15**) was also prepared by catalytic hydrogenation of 2-amino-5-(2,5-dimethoxyphenyl)furo[2,3-*d*]pyrimidin-4-(3*H*)-one (**9p**), as depicted in Chart 4.

Diethyl *N*-[4-(2-amino-4(3*H*)-oxofuro[2,3-*d*]pyrimidin-5-yl)benzoyl]-L-glutamate (**17**) was synthesized in good yield by condensation of **16** with diethyl L-glutamate in the presence of *N*-methylmorpholine (NMM) and triazine in anhydrous dimethylformamide (DMF) at room temperature. 4-(2-Amino-4(3*H*)-oxofuro[2,3-*d*]pyrimidin-5-yl)benzoic acid (**16**) was obtained by hydrolysis of the corresponding methyl

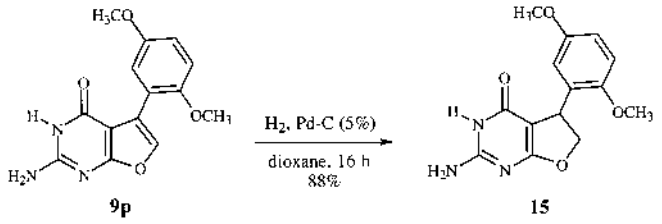

Chart 4

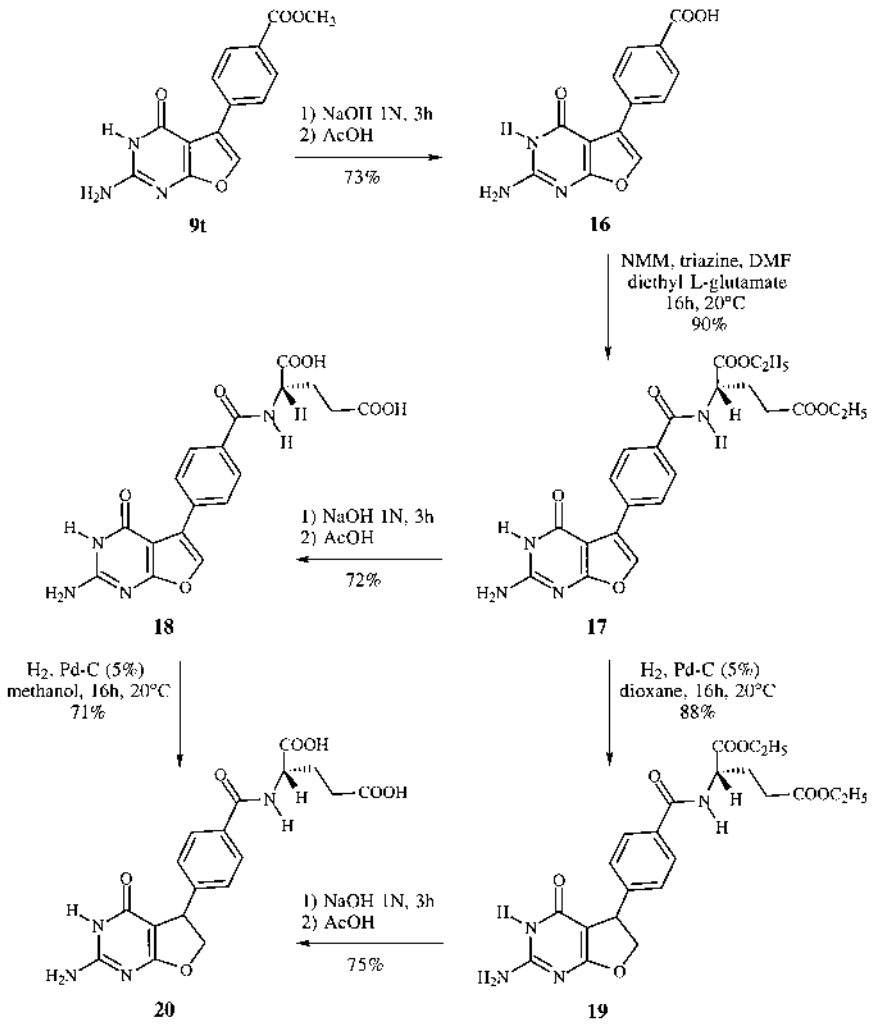

Chart 5

ester (**9t**). Further transformations of this ester **17** gave *N*-[4-(2-amino-4(3*H*)-oxofuro[2,3-*d*]pyrimidin-5-yl)benzoyl]-L-glutamic acid (**18**) [by alkaline hydrolysis followed by acidification] or diethyl *N*-[4-(2-amino-5,6-dihydro-4(3*H*)-oxofuro[2,3-*d*]pyrimidin-5-yl)benzoyl]-L-glutamate (**19**) [by palladium-catalysed reduction]. *N*-[4-(2-Amino-5,6-dihydro-4(3*H*)-oxofuro[2,3-*d*]pyrimidin-5-yl)benzoyl]-L-glutamic acid (**20**) was then prepared either by hydrogenation of **18** or by hydrolysis of **19** in comparable overall yields based on **17** (Chart 5).

Biological Results

Along with TMP (**2**), the compounds synthesized in the present study were evaluated as inhibitors of DHFR from different species (*Escherichia coli*, *Staphylococcus aureus*, *Staphylococcus aureus* TMP-resistant, *Pneumocystis carinii* and human), for *in vitro* antibacterial activity (several strains, TMP-resistant and susceptible, of *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Enterococcus faecium*, *Enterococcus faecalis*, *Pseudomonas aeruginosa* and *Escherichia coli*) and for *in vitro* antimalarial activity (*Plasmodium falciparum* NF 54 and the pyrimethamine resistant *Plasmodium falciparum* K1) . The test results are shown in Tables 1—3, respectively. In order to summarize these Tables, we have chosen to mention only those products exhibiting a significant activity (*i.e.* $IC_{50}<300\ \mu M$ for at least one of the tested DHFR, minimum inhibitory concentrations value (MIC)$\leq$ 64 μg/ml for at least one of the tested strains in the antibacterial assays, and $IC_{50}<5\ \mu$g/ml for the antimalarial evaluation).

The results reported in Table 1 deal with enzyme inhibition and show that several, of the evaluated compounds exhibited a moderate IC_{50} ($<30\ \mu M$), but only a few have a strong IC_{50} ($<10\ \mu M$, which is the threshold usually required to expect activity in the corresponding species). However, it is worth pointing out that, in several cases, for a given compound (*e.g.* **8d**, **8h**, **8l**, **8r**, **9b**, **9c**, **9p**, **9r**, **10**, **11**, **12**, **13** or **15**), significant differences in inhibition were observed between the examined enzymes. The selectivity for the human enzyme was generally poor but, in many cases, inhibition of the human enzyme was more pronounced than that of bacterial or *P. carinii* enzymes. This was especially observed with derivatives **17**, **18**, **19** and **20** bearing a L-glutamic side chain. Nevertheless, activities in this series remain much lower than those determined for MTX (**1**) which exhibits an IC_{50} of about 0.004 μM against recombinant human DHFR.[11b,c]

From the MICs gathered in Table 2, it appears that only seven compounds (**8a**, **9a**, **9d, 9l**, **9o**, **9s** and **15**) showed slight activity against some strains (MICs between 16 and 64 μg/ml). Most active compounds contained mono-substituted phenyl substituents, whereas compounds with multiple-substituted phenyls were inactive. Substances with *in vitro* activity had relatively low IC_{50} values against the corresponding enzyme, although the converse was not true. Factors such as permeability and intracellular accumulation must also play a role. The marginal activity observed both in the enzyme and *in vitro* tests is too weak for any certain correlation between DHFR inhibition and antibacterial activity. The fact that antibacterial activity was not always observed against the strains most sensitive to TMP (**2**) suggests some differences in the mode of binding to DHFR.

Table 1. Inhibition of Dihydrofolate Reductases

Compounds	IC_{50} (μM)[a]				
	E. coli	*S. aureus*	*S. aureus* TMP-resistant	*P. carinii*	Human
8a	24	25	240	34	110
8d	>300	240	22	100	50
8e	>300	>100	>300	130	>100
8h	>300	>300	>300	21	>100
8k	>300	>300	>300	100	>300
8l	>300	90	>300	50	>300
8q	>300	>300	>300	100	>300
8r	>300	>300	300	20	>300
9a	38	30	30	32	27
9b	80	20	38	>300	>300
9c	>300	100	30	50	5
9d	38	30	18	50	60
9e	>300	300	>300	>300	>300
9g	42	30	34	24	6
9h	19	17	11	14	6
9i	>300	>300	210	140	30
9j	100	120	170	75	>300
9l	100	>300	48	65	4
9n	100	160	32	100	10
9o	70	60	60	100	4
9r	15	13	230	9	85
9s	300	11	90	140	120
10	>300	10	>300	60	>300
11	17	21	300	>300	>300
12	15	19	>300	>300	>300
13	300	11	>300	>300	>300
15	10	2	>300	30	>300
17	>300	>300	>300	>300	15
18	>300	110	>100	110	17
19	>300	>300	>300	>300	19
20	300	>300	25	190	9
TMP (**2**)	0.0078	0.0034	16	43	900

a) The symbol (>) indicates that the IC_{50} was not reached at the highest concentration tested.

Table 2. *In Vitro* Antibacterial Activity

Strains	MIC (μg/ml)[a]							
	TMP (**2**)	**8a**	**9a**	**9d**	**9l**	**9o**	**9s**	**15**
S. aureus Smith	0.5	>64	>64	>64	>64	>64	64	>64
S. aureus ATCC25923	0.5	>64	>64	>64	>64	>64	>64	>64
S. aureus MR 8736 (norA)	0.5	>64	>64	>64	>64	>64	>64	>64
S. aureus QR 56	0.5	>64	>64	>64	>64	>64	>64	>64
S. aureus H 19982	1	>64	≤16	>64	>64	>64	32	64
S. aureus Sa NYC 1310	8	64	>64	>64	>64	>64	64	>64
S. aureus 101	32	>64	>64	32	>64	>64	64	>64
S. aureus 744	>32	>64	>64	>64	>64	>64	>64	>64
S. aureus 157/4696	>32	>64	>64	>64	>64	>64	>64	>64
S. epidemidis ATCC 14990	0.125	64	≤16	>64	>64	>64	≤16	>64
S. epidemidis H 13688	4	64	≤16	>64	>64	>64	32	>64
S. epidemidis SP 15	>32	>64	>64	>64	>64	>64	64	>64
S. epidemidis 3-7	>32	>64	>64	>64	>64	>64	64	>64
E. faecium van A E 25-1	>32	>64	>64	>64	>64	>64	64	>64
E. faecalis J 21	0.125	>64	>64	64	>64	>64	64	>64
E. faecalis ATCC 29212	0.25	>64	>64	>64	>64	>64	>64	>64
E. faecium ATCC 19434	0.125	>64	>64	64	>64	>64	64	>64
P. aeruginosa B A	8	>64	>64	>64	>64	>64	>64	>64
E. coli D C 2	≤0.06	≤16	≤16	>64	≤16	≤16	32	≤16
E. coli ATCC 25922	0.5	>64	>64	>64	>64	>64	>64	>64
E. coli S 6133	32	>64	>64	>64	> 64	>64	>64	>64

a) The symbol (>) indicates that the MIC was not reached at the highest concentration tested. The symbol (≤) indicates that the MIC was lower or equal to the reported concentrations.

Table 3. *In Vitro* Antimalarial Activity

Strains	IC_{50} (μg/ml)			
	PM (**5**)	**8c**	**9b**	**15**
P. falciparum K 1	4	4.33	3.75	0.88
P. falciparum NF 54	0.0054	5	3.12	0.855

The IC_{50} summarized in Table 3 indicate that three compounds, **8c**, **9b** and especially **15**, exhibited slight activity in the *in vitro* test against *P. falciparum*. The common structural feature of these compounds is a methoxy-substituted phenyl substituent. The compounds were equally active against pyrimethamine-resistant (K1) and sensitive (NF 54) strains , suggesting a different mode of binding to DHFR, or another mode of action.

Experimental

Melting points were measured on a Köfler hot stage apparatus and are uncorrected. Mass spectra were obtained with a Nermag-Ribermag R10-10C spectrometer applying a desorption chemical ionization (CI) technique using ammonia as the reagent gas or an electrospray ionization (ESI) method. Infrared spectra were obtained with a Perkin-Elmer 1710 spectrophotometer for chloroform solutions or KBr discs. The ^{1}H-NMR (90 and 300 MHz) spectra were recorded on Varian EM 390 and Bruker AC 300 spectrometers, respectively. Chemical shifts are expressed as parts per million downfield from tetramethylsilane. Splitting patterns are designated as follows: s (singlet), d (doublet), dd (doublet of doublet), t (triplet), q (quadruplet), m (multiplet), br (broad signal) and sh (shoulder). Coupling constants (*J* values) are listed in hertz (Hz). Reactions were monitored by analytical thin layer chromatography and products were visualized by exposure to UV light. Merck silica gel (230—400 mesh ASTM) was used for column chromatography. Methanol and CH_2Cl_2 employed as eluents were distilled on a rotary evaporator prior to use. Anhydrous triethylamine was obtained by distillation from calcium hydride. Dry tetrahydrofuran (THF) was prepared by distillation from benzophenone/sodium. Commercial anhydrous ethanol and butanone were used without further purification. Starting 2-amino-4,6-dihydroxypyrimidine (**6**), reagents, and palladium 10% catalyst were purchased from Aldrich Chemical Co. Palladium 5% on activated carbon was obtained from Strem Chemicals Inc. Large quantities of the required *Z*-(2-chloro-2-nitroethenyl)benzenes **7a**—**t** were prepared according to our previously reported procedure.[12,13] Compounds **7a**—**j**[12] and **7k**—**o**[13] have already been described. The new β-chloro-β-nitrostyrenes **7p**—**t** were especially synthesized on a 0.2 mole scale for the present work.

***Z*-(2-Chloro-2-nitroethenyl)-2,5-dimethoxybenzene (7p)** Yield 79% (38.5 g), mp 89—90 °C (recrystallized from heptane). ^{1}H-NMR ($CDCl_3$) δ: 3.60 (s, 3H), 3.93 (s, 3H), 6.73 (d, 1H, *J*=8.2), 7.06 (dd, 1H, *J*=8.2, 2.3), 7.66 (d, 1H *J*=2.3), 8.83 (s, 1H); IR ν: 1620 , 1540, 1310 cm^{-1}; *Anal*. Calcd for $C_{10}H_{10}ClNO_4$: C, 49.29; H, 4.13; N, 5.75. Found: C, 49.43; H, 4.23; N, 5.51.

***Z*-(2-Chloro-2-nitroethenyl)-2,6-dimethoxybenzene (7q)** Yield 82% (40.0 g), mp 112—114 °C (recrystallized from heptane). ^{1}H-NMR ($CDCl_3$) δ: 3.87 (s, 6H), 6.60 (d, 2H, *J*=8.2), 7.39 (t, 1H, *J*=8.2), 8.35 (s, 1H). IR ν: 1621 , 1539, 1322 cm^{-1}. *Anal*. Calcd for $C_{10}H_{10}ClNO_4$: C, 49.30; H, 4.14; N, 5.75. Found: C, 49.46; H, 4.18; N, 5.74.

***Z*-(2-Chloro-2-nitroethenyl)-5-chloro-2-methoxybenzene (7r)** Yield 91% (45.1 g), mp 90—91 °C (recrystallized from hexane). ^{1}H-NMR ($CDCl_3$) δ: 3.75 (s, 3H), 6.39 (d, 1H, *J*=8.3 Hz), 6.73 (dd, 1H, *J*=8.3, 2.7), 7.15 (d, 1H, *J*=2.7), 8.41 (s, 1H). IR ν: 1622 , 1541, 1312 cm^{-1}. *Anal*. Calcd for $C_9H_7C_{l2}NO_3$: C, 43.57; H, 2.84; N, 5.64. Found: C, 43.81; H, 2.75; N, 5.49.

***Z*-4-(2-Chloro-2-nitroethenyl)phenol (7s)** Yield 73% (29.1 g), mp 132—134 °C (recrystallized from a benzene/heptane mixture). ^{1}H-NMR ($CDCl_3$) δ: 7.25—7.31 (br d, 2H, AA′BB′ system, *J*=8.3), 7.46—7.52 (br d, 2H, AA′BB′ system, *J*=8.3), 8.27 (s, 1H), 9.33 (br s, 1H exchangeable with D_2O). IR ν: 1618 , 1544, 1313 cm^{-1}. *Anal*. Calcd for $C_8H_6C_lNO_3$: C, 48.14; H, 3.03; N, 7.02. Found: C, 48.35; H, 3.11; N, 6.92.

Methyl *Z*-4-(2-Chloro-2-nitroethenyl)benzoate (7t) Yield 92% (44.3 g), mp 177—179 °C (recrystallized from heptane). ^{1}H-NMR ($CDCl_3$) δ: 4.00 (s, 3H), 7.86—7.96 (br d, 2H, AA′BB′ system, *J*=7.8), 8.13—8.23 (br d, 2H, AA′BB′, *J*=7.8), 8.42 (s, 1H). IR ν: 1690, 1620 , 1540, 1311 cm^{-1}. *Anal*. Calcd for $C_{10}H_8C_lNO_4$: C, 49.70; H, 3.33; N, 5.79. Found: C, 49.53; H, 3.37; N, 5.76. The starting methyl 4-formylbenzoate (mp 60—62 °C recrystallized from pentane) was easily prepared in 95% yield by adding thionyl chloride dropwise, at room temperature, to a mixture of 4-formylbenzoic acid and methanol.

2-Amino-5,6-dihydro-6-nitro-5-phenylfuro[2,3-*d*]pyrimidin-4(3*H*)-one (8a) A solution of *Z*-(2-chloro-2-nitroethenyl)benzene (**7a**) (918 mg, 5 mmol) in a mixture of anhydrous ethanol (33 ml) and butanone (17 ml) was placed in a dried, two-necked, 100 ml round-bottomed flask fitted with a septum inlet. 2-Amino-4,6-dihydroxypyrimidine (**6**) (690 mg, 5.5 mmol) was then added in one portion under argon atmosphere. Dry triethylamine (2.53 g, 3.5 ml, 25 mmol) was introduced *via* a syringe and the mixture was efficiently stirred for five days. The reaction mixture was suction-filtered through a short pad of Celite. The Celite was thoroughly rinsed with a CH_2Cl_2/methanol 9/1 mixture and the combined volatile materials were evaporated under reduced pressure. The crude product was chromatographed over a silica gel column (300 g, eluent CH_2Cl_2/methanol: 9/1). Removal of the solvent followed by subsequent recrystallisation in a mixture water/ethanol afforded pure **8a** in 60% yield (822 mg), mp >260 °C. ^{1}H-NMR (DMSO-d_6) δ: 4.70 (d, 1H, *J*=2.1), 6.51 (d, 1H, *J*=2.1), 7.06 (br s, 2H exchangeable with D_2O), 7.23—7.40 (m, 5H), 10.93 (br s, 1H exchangeable with D_2O). IR ν: 1692 cm^{-1}. MS *m/z* (CI): 196 $(M-C_6H_5)^+$, 228 $(M-NO_2)^+$, 275 $(M+H)^+$, 292 $(M+NH_4)^+$. *Anal*. Calcd for $C_{12}H_{10}N_4O_4$: C, 52.56; H, 3.68; N, 20.43. Found: C, 52.29; H, 3.72; N, 20.52.

2-Amino-5-aryl-5,6-dihydro-6-nitrofuro[2,3-*d*]pyrimidin-4(3*H*)-ones **8b**—**s** were synthesized from **7b**—**s** in a manner similar to that described above for the preparation of **8a**.

2-Amino-5,6-dihydro-5-(2-methoxyphenyl)-6-nitrofuro[2,3-*d*]pyrimidin-4(3*H*)-one (8b) Yield 40% (609 mg), mp >260 °C. ^{1}H-NMR (DMSO-d_6) δ: 3.86 (s, 3H), 4.80 (d, 1H, *J*=2.3), 6.18 (d, 1H, *J*=2.3), 6.76 (br s, 2H exchangeable with D_2O), 6.86—7.40 (m, 3H), 7.45—7.51 (m, 1H), 10.82 (br s, 1H exchangeable with D_2O). IR ν: 1672 cm^{-1}. MS *m/z* (CI): 258 $(M-NO_2)^+$, 305 $(M+H)^+$, 322 $(M+NH_4)^+$. *Anal*. Calcd for $C_{13}H_{12}N_4O_5$: C, 51.32; H, 3.98; N, 18.41. Found: C, 51.63; H, 4.09; N, 18.13.

2-Amino-5,6-dihydro-5-(3-methoxyphenyl)-6-nitrofuro[2,3-*d*]pyrimidin-4(3*H*)-one (8c) Yield 46% (700 mg), mp >260 °C. ^{1}H-NMR (DMSO-d_6) δ: 3.88 (s, 3H), 4.66 (d, 1H, *J*=2.6), 6.53 (d, 1H, *J*=2.6), 6.83—7.16 (m, 3H), 7.26—7.56 (m, 1H), 7.73 (br s, 2H exchangeable with D_2O), 10.13 (br s, 1H exchangeable with D_2O). IR ν: 1690 cm^{-1}. MS *m/z* (CI): 258 $(M-NO_2)^+$, 305 $(M+H)^+$, 322 $(M+NH_4)^+$. *Anal*. Calcd for $C_{13}H_{12}N_4O_5$: C, 51.32; H, 3.98; N, 18.41. Found: C, 51.11; H, 3.81; N, 18.58.

2-Amino-5,6-dihydro-5-(4-methoxyphenyl)-6-nitrofuro[2,3-*d*]pyrimidin-4(3*H*)-one (8d) Yield 58% (882 mg), mp >260 °C. ^{1}H-NMR (DMSO-d_6) δ: 3.73 (s, 3H), 4.63 (d, 1H, *J*=2.1), 6.33 (d, 1H, *J*=2.1), 6.96 (br s, 2H exchangeable with D_2O), 6.82—6.91 (br d, 2H, AA′BB′ system, *J*=8.5), 7.08—7.20 (br d, 2H, AA′BB′ system, *J*=8.5), 10.75 (br s, 1H exchangeable with D_2O). IR ν: 1687 cm^{-1}. MS *m/z* (CI): 258 $(M-NO_2)^+$, 305 $(M+H)^+$, 322 $(M+NH_4)^+$. *Anal*. Calcd for $C_{13}H_{12}N_4O_5$: C, 51.32; H, 3.98; N, 18.41. Found: C, 51.21; H, 3.83; N, 18.63.

2-Amino-5,6-dihydro-6-nitro-5-(3,4,5-trimethoxyphenyl)furo[2,3-*d*]pyrimidin-4(3*H*)-one (8e) Yield 65% (1.18 g), mp >260 °C. ^{1}H-NMR (DMSO-d_6) δ: 3.77 (s, 3H), 3.83 (s, 6H), 4.64 (d, 1H, *J*=2.4), 6.46 (d, 1H, *J*=2.4), 6.89 (br s, 2H exchangeable with D_2O), 7.00 (s, 2H), 10.80 (br s, 1H exchangeable with D_2O); IR ν: 1693 cm^{-1}; MS *m/z* (CI): 318 $(M-NO_2)^+$, 365 $(M+H)^+$, 382 $(M+NH_4)^+$. *Anal*. Calcd for $C_{15}H_{16}N_4O_7$: C, 49.45; H, 4.43; N, 15.38. Found: C, 49.29; H, 4.60; N, 15.27.

2-Amino-5-(2-chlorophenyl)-5,6-dihydro-6-nitrofuro[2,3-*d*]pyrimidin-4(3*H*)-one (8f) Yield 61% (941 mg), mp >260 °C. ^{1}H-NMR (DMSO-d_6) δ: 4.89 (d, 1H, *J*=2.1), 6.55 (d, 1H, *J*=2.1), 6.70 (br s, 2H exchangeable with D_2O), 7.10—7.20 (m, 1H), 7.26—7.66 (m, 3H), 10.93 (br s, 1H exchangeable with D_2O). IR ν: 1687 cm^{-1}. MS *m/z* (CI): 262, 264 $(M-NO_2)^+$, 309, 311 $(M+H)^+$, 326, 328 $(M+NH_4)^+$. *Anal*. Calcd for $C_{12}H_9ClN_4O_4$: C, 46.69; H, 2.94; N, 18.15. Found: C, 46.49; H, 3.10; N, 18.31.

2-Amino-5-(3-chlorophenyl)-5,6-dihydro-6-nitrofuro[2,3-*d*]pyrimidin-4(3*H*)-one (8g) Yield 59% (911 mg), mp >260 °C. ^{1}H-NMR (DMSO-d_6) δ: 4.79 (d, 1H, *J*=2.3), 6.46 (d, 1H, *J*=2.3), 6.95—7.29 (m, 4H), 7.10 (br s, 2H exchangeable with D_2O), 10.24 (br s, 1H exchangeable with D_2O). IR ν: 1688 cm^{-1}. MS *m/z* (CI): 262, 264 $(M-NO_2)^+$, 309, 311 $(M+H)^+$, 326, 328 $(M+NH_4)^+$. *Anal*. Calcd for $C_{12}H_9C_lN_4O_4$: C, 46.69; H, 2.94; N, 18.15. Found: C, 46.53; H, 3.07; N, 18.21.

2-Amino-5-(4-chlorophenyl)-5,6-dihydro-6-nitrofuro[2,3-*d*]pyrimidin-4(3*H*)-one (8h) Yield 64% (988 mg), mp >260 °C. ^{1}H-NMR (DMSO-d_6)

δ: 4.73 (d, 1H, J=2.1), 6.46 (d, 1H, J=2.1), 7.10 (br s, 2H exchangeable with D_2O), 7.20—7.30 (br d, 2H, AA′BB′ system, J=7.6), 7.43—7.53 (br d, 2H, AA′BB′ system, J=7.6), 10.80 (br s, 1H exchangeable with D_2O). IR ν: 1683 cm^{-1}. MS *m/z* (CI): 262, 264 $(M-NO_2)^+$, 309, 311 $(M+H)^+$, 326, 328 $(M+NH_4)^+$. *Anal*. Calcd for $C_{12}H_9C_lN_4O_4$: C, 46.69; H, 2.94; N, 18.15. Found: C, 46.71; H, 3.04; N, 18.22.

2-Amino-5,6-dihydro-6-nitro-5-(3-nitrophenyl)furo[2,3-*d*]pyrimidin-4(3*H*)-one (8i) Yield 49% (782 mg), mp >260 °C. ^{1}H-NMR (DMSO-d_6) δ: 4.36 (d, 1H, J=2.6), 6.23 (d, 1H, J=2.6), 6.95 (br s, 2H exchangeable with D_2O), 7.13—7.58 (m, 4H), 10.47 (br s, 1H exchangeable with D_2O). IR ν: 1693 cm^{-1}. MS *m/z* (CI): 273 $(M-NO_2)^+$, 320 $(M+H)^+$, 337 $(M+NH_4)^+$. *Anal*. Calcd for $C_{12}H_9N_5O_6$: C, 45.15; H, 2.84; N, 21.94. Found: C, 45.41; H, 2.81; N, 21.79.

2-Amino-5,6-dihydro-6-nitro-5-(4-nitrophenyl)furo[2,3-*d*]pyrimidin-4(3*H*)-one (8j) Yield 51% (814 mg), mp >260 °C. ^{1}H-NMR (DMSO-d_6) δ: 4.27 (d, 1H, J=2.3), 6.19 (d, 1H, J=2.3), 6.86—7.04 (br d, 2H, AA′BB′ system, J=7.8), 7.08—7.26 (br d, 2H, AA′BB′ system, J=7.8), 7.15 (br s, 2H exchangeable with D_2O), 10.79 (br s, 1H exchangeable with D_2O). IR ν: 1679 cm^{-1}. MS *m/z* (CI): 273 $(M-NO_2)^+$, 320 $(M+H)^+$, 337 $(M+NH_4)^+$. *Anal*. Calcd for $C_{12}H_9N_5O_6$: C, 45.15; H, 2.84; N, 21.94. Found: C, 45.31; H, 2.77; N, 21.87.

2-Amino-5,6-dihydro-5-(3,4-dimethoxyphenyl)-6-nitrofuro[2,3-*d*]pyrimidin-4(3*H*)-one (8k) Yield 54% (903 mg), mp >260 °C. ^{1}H-NMR (DMSO-d_6) δ: 3.73 (s, 3H), 3.83 (s, 3H), 4.63 (d, 1H, J=2.7), 6.46 (d, 1H, J=2.7), 6.66 (d, 1H, J=8.6), 6.87 (d, 1H, J=8.6), 6.85—7.30 (m, 1H), 6.89 (br s, 2H exchangeable with D_2O), 10.76 (br s, 1H exchangeable with D_2O). IR ν: 1673 cm^{-1}. MS *m/z* (CI): 288 $(M-NO_2)^+$, 335 $(M+H)^+$, 352 $(M+NH_4)^+$. *Anal*. Calcd for $C_{14}H_{14}N_4O_6$: C, 50.30; H, 4.22; N, 16.76. Found: C, 50.37; H, 4.39; N, 16.63.

2-Amino-5,6-dihydro-5-(2-fluorophenyl)-6-nitrofuro[2,3-*d*]pyrimidin-4(3*H*)-one (8l) Yield 60% (877 mg), mp >260 °C. ^{1}H-NMR (DMSO-d_6) δ: 4.70 (d, 1H, J=2.2), 6.37 (d, 1H, J=2.2), 7.15 (br s, 2H exchangeable with D_2O), 6.97—7.28 (m, 1H), 7.32—7.59 (m, 3H), 10.72 (br s, 1H exchangeable with D_2O). IR ν: 1669 cm^{-1}. MS *m/z* (CI): 246 $(M-NO_2)^+$, 293 $(M+H)^+$, 310 $(M+NH_4)^+$. *Anal*. Calcd for $C_{12}H_9FN_4O_4$: C, 49.32; H, 3.10; N, 19.17. Found: C, 49.41; H, 3.19; N, 19.01.

2-Amino-5,6-dihydro-5-(3-fluorophenyl)-6-nitrofuro[2,3-*d*]pyrimidin-4(3*H*)-one (8m) Yield 60% (879 mg), mp >260 °C. ^{1}H-NMR (DMSO-d_6) δ: 4.79 (d, 1H, J=2.3), 6.53 (d, 1H, J=2.3), 6.53 (br s, 2H exchangeable with D_2O), 7.12—7.65 (m, 4H), 10.39 (br s, 1H exchangeable with D_2O). IR ν: 1670 cm^{-1}. MS *m/z* (CI): 246 $(M-NO_2)^+$, 293 $(M+H)^+$, 310 $(M+NH_4)^+$. *Anal*. Calcd for $C_{12}H_9FN_4O_4$: C, 49.32; H, 3.10; N, 19.17. Found: C, 49.53; H, 3.21; N, 19.01.

2-Amino-5,6-dihydro-5-(4-fluorophenyl)-6-nitrofuro[2,3-*d*]pyrimidin-4(3*H*)-one (8n) Yield 61% (891 mg), mp >260 °C. ^{1}H-NMR (DMSO-d_6) δ: 4.73 (d, 1H, J=2.5), 6.50 (d, 1H, J=2.5), 7.16 (br s, 2H exchangeable with D_2O), 7.03—7.15 (m, 4H), 10.90 (br s, 1H exchangeable with D_2O). IR ν: 1689 cm^{-1}. MS *m/z* (CI): 246 $(M-NO_2)^+$, 293 $(M+H)^+$, 310 $(M+NH_4)^+$. *Anal*. Calcd for $C_{12}H_9FN_4O_4$: C, 49.32; H, 3.10; N, 19.17. Found: C, 49.55; H, 3.22; N, 19.31.

2-Amino-5-(2,6-difluorophenyl)-5,6-dihydro-6-nitrofuro[2,3-*d*]pyrimidin-4(3*H*)-one (8o) Yield 64% (993 mg), mp >260 °C. ^{1}H-NMR (DMSO-d_6) δ: 5.00 (d, 1H, J=2.0), 6.76 (d, 1H, J=2.0), 7.01 (br s, 2H exchangeable with D_2O), 7.10—7.53 (m, 3H), 10.77 (br s, 1H exchangeable with D_2O). IR ν: 1685 cm^{-1}. MS *m/z* (CI): 264 $(M-NO_2)^+$, 311 $(M+H)^+$, 328 $(M+NH_4)^+$. *Anal*. Calcd for $C_{12}H_8F_2N_4O_4$: C, 46.46; H, 2.60; N, 18.06. Found: C, 46.59; H, 2.65; N, 17.87.

2-Amino-5,6-dihydro-5-(2,5-dimethoxyphenyl)-6-nitrofuro[2,3-*d*]pyrimidin-4(3*H*)-one (8p) Yield 58% (969 mg), mp >260 °C. ^{1}H-NMR (DMSO-d_6) δ: 3.66 (s, 3H), 3.80 (s, 3H), 4.80 (d, 1H, J=2.1), 6.50 (d, 1H, J=2.1), 6.52 (d, 1H, J=8.5), 6.66 (d, 1H, J=4.6), 6.80—7.06 (m, 1H), 6.93 (br s, 2H exchangeable with D_2O), 11.06 (br s, 1H exchangeable with D_2O). IR ν: 1687 cm^{-1}. MS *m/z* (CI): 288 $(M-NO_2)^+$, 335 $(M+H)^+$, 352 $(M+NH_4)^+$. *Anal*. Calcd for $C_{14}H_{14}N_4O_6$: C, 50.30; H, 4.22; N, 16.76. Found: C, 50.41; H, 4.39; N, 16.60.

2-Amino-5,6-dihydro-5-(2,6-dimethoxyphenyl)-6-nitrofuro[2,3-*d*]pyrimidin-4(3*H*)-one (8q) Yield 50% (836 mg), mp >260 °C. ^{1}H-NMR (DMSO-d_6) δ: 3.73 (s, 6H), 5.11 (d, 1H, J=2.9), 6.35 (d, 1H, J=2.9), 6.60—7.36 (m, 3H), 6.83 (br s, 2H exchangeable with D_2O), 10.53 (br s, 1H exchangeable with D_2O). IR ν: 1683 cm^{-1}. MS *m/z* (CI): 288 $(M-NO_2)^+$, 335 $(M+H)^+$, 352 $(M+NH_4)^+$. *Anal*. Calcd for $C_{14}H_{14}N_4O_6$: C, 50.30; H, 4.22; N, 16.76. Found: C, 50.43; H, 4.38; N, 16.53.

2-Amino-5-(5-chloro-2-methoxyphenyl)-5,6-dihydro-6-nitrofuro[2,3-*d*]pyrimidin-4(3*H*)-one (8r) Yield 58% (983 mg), mp >260 °C. ^{1}H-NMR (DMSO-d_6) δ: 3.83 (s, 3H), 4.86 (d, 1H, J=2.9), 6.36 (d, 1H, J=2.9), 6.93—7.13 (m, 1H), 7.10 (br s, 2H exchangeable with D_2O), 7.40 (d, 1H, J=8.2), 7.52 (br d, 1H, J=8.2), 10.83 (br s, 1H exchangeable with D_2O). IR ν: 1677 cm^{-1}. MS *m/z* (CI): 292, 294 $(M-NO_2)^+$, 339, 341 $(M+H)^+$. *Anal*. Calcd for $C_{13}H_{11}ClN_4O_5$: C, 46.10; H, 3.27; N, 16.54. Found: C, 46.23; H, 3.37; N, 16.43.

2-Amino-5,6-dihydro-5-(4-hydroxyphenyl)-6-nitrofuro[2,3-*d*]pyrimidin-4(3*H*)-one (8s) Yield 57% (827 mg), mp >260 °C; ^{1}H-NMR (DMSO-d_6) δ: 4.53 (d, 1H, J=2.1), 6.33 (d, 1H, J=2.1), 6.80—6.86 (br d, 2H, AA′BB′ system, J=8.1), 6.95 (br s, 2H exchangeable with D_2O), 6.96—7.02 (br d, 2H, AA′BB′ system, J=8.1), 9.40 (sh, 1H exchangeable with D_2O), 10.81 (br s, 1H exchangeable with D_2O). IR ν: 1670 cm^{-1}. MS *m/z* (CI): 244 $(M-NO_2)^+$, 291 $(M+H)^+$, 308 $(M+NH_4)^+$. *Anal*. Calcd for $C_{12}H_{10}N_4O_5$: C, 49.66; H, 3.47; N, 19.30. Found: C, 49.41; H, 3.21; N, 19.27.

2-Amino-5-phenylfuro[2,3-*d*]pyrimidin-4(3*H*)-one (9a) A solution of *Z*-(2-chloro-2-nitroethenyl)benzene (**7a**) (918 mg, 5 mmol) in a mixture of anhydrous ethanol (33 ml) and butanone (17 ml) was placed in a dried, two-necked, 100 ml round-bottomed flask fitted with a septum inlet and a water condenser. 2-Amino-4,6-dihydroxypyrimidine (**6**) (690 mg, 5.5 mmol) was then added in one portion under an argon atmosphere. The mixture was stirred at room temperature for ten minutes and DBU (3.80 g, 3.74 ml, 25 mmol) was introduced *via* a syringe. The reaction mixture was smoothly refluxed, with stirring, using an oil bath. When the starting material **7a** had completely disappeared as judged by thin layer chromatography (eluent CH_2Cl_2/methanol: 9/1), three hours in this case, the mixture was allowed to cool to room temperature and filtered by suction through a short pad of Celite. The Celite was thoroughly rinsed with a CH_2Cl_2/methanol 9/1 mixture and the combined volatile materials were evaporated *in vacuo*. The crude product was chromatographed over a silica gel column (300 g, eluent CH_2Cl_2/methanol: 9/1). Removal of the solvent followed by washing of the obtained solid in boiling ethanol gave satisfactorily pure **9a** in 61% yield (693 mg), mp >260 °C. ^{1}H-NMR (DMSO-d_6) δ: 6.66 (br s, 2H exchangeable with D_2O), 7.33—7.43 (m, 3H), 7.80 (s, 1H), 7.96—8.06 (m, 2H), 10.83 (br s, 1H exchangeable with D_2O). IR ν: 1680 cm^{-1}. MS *m/z* (CI): 228 $(M+H)^+$, 245 $(M+NH_4)^+$. *Anal*. Calcd for $C_{12}H_9N_3O_2$: C, 63.43; H, 3.99; N, 18.49. Found: C, 63.27; H, 3.82; N, 18.62.

The 2-amino-5-arylfuro[2,3-*d*]pyrimidin-4(3*H*)-ones **9b**—**t** were synthesized from **7b**—**t** in a manner similar to that described above for the preparation of **9a**. When the reaction time or the employed solvent was different of those used for **9a**, they are especially mentioned.

2-Amino-5-(2-methoxyphenyl)furo[2,3-*d*]pyrimidin-4(3H)-one (9b) Yield 46% (591 mg), mp >260 °C. ^{1}H-NMR (DMSO-d_6) δ: 3.83 (s, 3H), 6.68 (br s, 2H exchangeable with D_2O), 6.86—7.06 (m, 3H), 7.48 (dd, 1H, J=1.9, 8.1), 7.83 (s, 1H), 10.76 (br s, 1H exchangeable with D_2O). IR ν: 1672 cm^{-1}. MS *m/z* (CI): 258 $(M+H)^+$, 275 $(M+NH_4)^+$. *Anal*. Calcd for $C_{13}H_{11}N_3O_3$: C, 60.70; H, 4.31; N, 16.33. Found: C, 60.61; H, 4.25; N, 16.49.

2-Amino-5-(3-methoxyphenyl)furo[2,3-*d*]pyrimidin-4(3*H*)-one (9c) Yield 60% (772 mg), mp >260 °C. ^{1}H-NMR (DMSO-d_6) δ: 3.80 (s, 3H), 6.60 (br s, 2H exchangeable with D_2O), 6.73—6.86 (m, 1H), 7.20 (t, 1H, J=7.7), 7.43—7.53 (m, 2H), 7.73 (s, 1H), 11.0 (br s, 1H exchangeable with D_2O). IR ν: 1674 cm^{-1}. MS *m/z* (CI): 258 $(M+H)^+$, 275 $(M+NH_4)^+$. *Anal*. Calcd for $C_{13}H_{11}N_3O_3$: C, 60.70; H, 4.31; N, 16.33. Found: C, 60.81; H, 4.49; N, 16.17.

2-Amino-5-(4-methoxyphenyl)furo[2,3-*d*]pyrimidin-4(3*H*)-one (9d) Yield 54% (694 mg), mp >260 °C. ^{1}H-NMR (DMSO-d_6) δ: 3.76 (s, 3H), 6.62 (br s, 2H exchangeable with D_2O), 6.83—6.93 (br d, 2H, AA′BB′ system, J=7.6), 7.66 (s, 1H), 7.83—7.93 (br d, 2H, AA′BB′ system, J=7.6), 11.26 (br s, 1H exchangeable with D_2O). IR ν: 1666 cm^{-1}. MS *m/z* (CI): 258 $(M+H)^+$, 275 $(M+NH_4)^+$. *Anal*. Calcd for $C_{13}H_{11}N_3O_3$: C, 60.70; H, 4.31; N, 16.33. Found: C, 60.83; H, 4.47 N, 16.13.

2-Amino-5-(3,4,5-trimethoxyphenyl)furo[2,3-*d*]pyrimidin-4(3*H*)-one (9e) Yield 68% (1.08 g), mp >260 °C. ^{1}H-NMR (DMSO-d_6) δ: 3.70 (s, 3H), 3.83 (s, 6H), 6.72 (br s, 2H exchangeable with D_2O), 7.45 (s, 2H), 7.87 (s, 1H), 10.78 (br s, 1H exchangeable with D_2O). IR ν: 1668 cm^{-1}. MS *m/z* (CI): 318 $(M+H)^+$, 335 $(M+NH_4)^+$. *Anal*. Calcd for $C_{15}H_{15}N_3O_5$: C, 56.78; H, 4.77; N, 13.24. Found: C, 56.81; H, 4.77 N, 13.22.

2-Amino-5-(2-chlorophenyl)furo[2,3-*d*]pyrimidin-4(3*H*)-one (9f) Yield 59% (772 mg), mp >260 °C. ^{1}H-NMR (DMSO-d_6) δ: 6.79(br s, 2H exchangeable with D_2O), 7.17—7.57 (m, 4H), 7.93 (s, 1H), 10.75 (br s, 1H exchangeable with D_2O). IR ν: 1675 cm^{-1}. MS *m/z* (CI): 262, 264 $(M+H)^+$, 279, 281 $(M+NH_4)^+$. *Anal*. Calcd for $C_{12}H_8C_lN_3O_2$: C, 55.08; H, 3.08; N, 16.06. Found: C, 55.27; H, 3.01; N, 16.17.

2-Amino-5-(3-chlorophenyl)furo[2,3-*d*]pyrimidin-4(3*H*)-one (9g) Yield 64% (837 mg), mp >260 °C. ^{1}H-NMR (DMSO-d_6) δ: 6.73(br s, 2H exchangeable with D_2O), 7.33—7.46 (m, 3H), 7.92 (s, 1H), 8.20—8.29 (m, 1H), 11.03 (br s, 1H exchangeable with D_2O). IR ν: 1685 cm^{-1}. MS *m/z* (CI): 262, 264 $(M+H)^+$, 279, 281 $(M+NH_4)^+$. *Anal*. Calcd for $C_{12}H_8C_lN_3O_2$: C, 55.08; H, 3.08; N, 16.06. Found: C, 55.21; H, 3.17; N, 15.89.

2-Amino-5-(4-chlorophenyl)furo[2,3-*d*]pyrimidin-4(3*H*)-one (9h) Yield 65% (850 mg), mp >260 °C. ^{1}H-NMR (DMSO-d_6) δ: 6.70 (br s, 2H exchangeable with D_2O), 7.33—7.43 (br d, 2H, AA′BB′ system, J=8.8), 7.80 (s, 1H), 8.00—8.10 (br d, 2H, AA′BB′ system, J=8.8), 10.93 (br s, 1H exchangeable with D_2O). IR ν: 1691 cm^{-1}. MS *m/z* (CI): 262, 264 $(M+H)^+$, 279, 281 $(M+NH_4)^+$. *Anal*. Calcd for $C_{12}H_8C_lN_3O_2$: C, 55.08; H, 3.08; N, 16.06. Found: C, 55.23; H, 3.15; N, 15.94.

2-Amino-5-(3-nitrophenyl)furo[2,3-*d*]pyrimidin-4(3*H*)-one (9i) Yield 40% (544 mg), mp >260 °C. ^{1}H-NMR (DMSO-d_6) δ: 6.79 (br s, 2H exchangeable with D_2O), 7.66 (t, 1H, J=8.3), 8.00 (s, 1H), 8.03—8.06 (m, 1H), 8.39—8.45 (m, 1H), 8.95—9.05 (m, 1H), 11.00 (br s, 1H exchangeable with D_2O). IR ν: 1689 cm^{-1}. MS *m/z* (CI): 273 $(M+H)^+$, 290 $(M+NH_4)^+$. *Anal*. Calcd for $C_{12}H_8N_4O_4$: C, 52.95; H, 2.96; N, 20.58. Found: C, 53.05; H, 3.19; N, 20.27.

2-Amino-5-(4-nitrophenyl)furo[2,3-*d*]pyrimidin-4(3*H*)-one (9j) Yield 44% (599 mg), mp >260 °C. ^{1}H-NMR (DMSO-d_6) δ: 6.85 (br s, 2H exchangeable with D_2O), 8.10 (s, 1H), 8.16—8.26 (br d, 2H, AA′BB′ system, J=7.5), 8.33—8.43 (br d, 2H, AA′BB′ system, J=7.5), 10.95 (br s, 1H exchangeable with D_2O). IR ν: 1688 cm^{-1}. MS *m/z* (CI): 273 $(M+H)^+$, 290 $(M+NH_4)^+$. *Anal*. Calcd for $C_{12}H_8N_4O_4$: C, 52.95; H, 2.96; N, 20.58. Found: C, 53.13; H, 3.12; N, 20.35.

2-Amino-5-(3,4-dimethoxyphenyl)furo[2,3-*d*]pyrimidin-4(3*H*)-one (9k) Yield 54% (776 mg), mp >260 °C. ^{1}H-NMR (DMSO-d_6) δ: 3.80 (s, 3H), 3.86 (s, 3H), 6.66 (br s, 2H exchangeable with D_2O), 7.13—7.19 (m, 2H), 7.33 (s, 1H), 7.42—7.59 (m, 1H), 10.86 (br s, 1H exchangeable with D_2O). IR ν: 1688 cm^{-1}. MS *m/z* (CI): 288 $(M+H)^+$, 305 $(M+NH_4)^+$. *Anal*. Calcd for $C_{14}H_{13}N_3O_4$: C, 58.53; H, 4.56; N, 14.63. Found: C, 58.71; H, 4.67; N, 14.81.

2-Amino-5-(2-fluorophenyl)furo[2,3-*d*]pyrimidin-4(3*H*)-one (9l) Yield 60% (736 mg), mp >260 °C. ^{1}H-NMR (DMSO-d_6) δ: 6.76 (br s, 2H exchangeable with D_2O), 7.20—7.26 (m, 2H), 7.30 (s, 1H), 7.66 (br s, 1H), 8.16—8.36 (m, 1H), 10.93 (br s, 1H exchangeable with D_2O). IR ν: 1682 cm^{-1}; MS *m/z* (CI): 246 $(M+H)^+$, 263 $(M+NH_4)^+$. *Anal*. Calcd for $C_{12}H_8FN_3O_2$: C, 58.78; H, 3.29; N, 17.14. Found: C, 58.93; H, 3.19; N, 17.03.

2-Amino-5-(3-fluorophenyl)furo[2,3-*d*]pyrimidin-4(3*H*)-one (9m) Yield 63% (772 mg), mp >260 °C. ^{1}H-NMR (DMSO-d_6) δ: 6.67 (br s, 2H exchangeable with D_2O), 7.21—7.39 (m, 3H), 7.87 (s, 1H), 8.31—8.41 (m, 1H), 10.97 (br s, 1H exchangeable with D_2O). IR ν: 1669 cm^{-1}. MS *m/z* (CI): 246 $(M+H)^+$, 263 $(M+NH_4)^+$. *Anal*. Calcd for $C_{12}H_8FN_3O_2$: C, 58.78; H, 3.29; N, 17.14. Found: C, 58.59; H, 3.21; N, 17.27.

2-Amino-5-(4-fluorophenyl)furo[2,3-*d*]pyrimidin-4(3*H*)-one (9n) Yield 68% (834 mg), mp >260 °C. ^{1}H-NMR (DMSO-d_6) δ: 6.53 (br s, 2H exchangeable with D_2O), 7.00 (br t, 1H, J=6.8), 7.61 (s, 1H), 7.80—8.00 (m, 3H), 10.83 (br s, 1H exchangeable with D_2O). IR ν: 1667 cm^{-1}. MS *m/z* (CI): 246 $(M+H)^+$, 263 $(M+NH_4)^+$. *Anal*. Calcd for $C_{12}H_8FN_3O_2$: C, 58.78; H, 3.29; N, 17.14. Found: C, 58.87; H, 3.35; N, 17.03.

2-Amino-5-(2,6-difluorophenyl)furo[2,3-*d*]pyrimidin-4(3*H*)-one (9o) Yield 57% (750 mg), mp >260 °C. ^{1}H-NMR (DMSO-d_6) δ: 6.70 (br s, 2H exchangeable with D_2O), 7.16—7.25 (m, 1H), 7.30 (s, 1H), 7.63—7.70 (m, 1H), 8.16—8.31 (m, 1H), 10.91 (br s, 1H exchangeable with D_2O). IR ν: 1677 cm^{-1}. MS *m/z* (CI): 264 $(M+H)^+$, 281 $(M+NH_4)^+$. *Anal*. Calcd for $C_{12}H_7F_2N_3O_2$: C, 54.76; H, 2.68; N, 15.96. Found: C, 54.71; H, 2.75; N, 15.85.

2-Amino-5-(2,5-dimethoxyphenyl)furo[2,3-*d*]pyrimidin-4(3*H*)-one (9p) Yield 46% (661 mg), mp >260 °C. ^{1}H-NMR (DMSO-d_6) δ: 3.79 (s, 3H), 3.83(s, 3H), 6.67 (br s, 2H exchangeable with D_2O), 6.86—7.00 (m, 2H), 7.80 (s, 1H), 8.06 (m, 1H), 10.87 (br s, 1H exchangeable with D_2O). IR ν: 1689 cm^{-1}. MS *m/z* (CI): 288 $(M+H)^+$. *Anal*. Calcd for $C_{14}H_{13}N_3O_4$: C, 58.53; H, 4.56; N, 14.63. Found: C, 58.71; H, 4.37; N, 14.55.

2-Amino-5-(2,6-dimethoxyphenyl)furo[2,3-*d*]pyrimidin-4(3*H*)-one (9q) Yield 48% (689 mg), mp >260 °C. ^{1}H-NMR (DMSO-d_6) δ: 3.86 (s, 3H), 3.91(s, 3H), 6.76 (br s, 2H exchangeable with D_2O), 7.13—7.46 (m, 3H), 7.66 (s, 1H), 10.29 (br s, 1H exchangeable with D_2O). IR ν: 1691 cm^{-1}; MS *m/z* (CI): 288 $(M+H)^+$. *Anal*. Calcd for $C_{14}H_{13}N_3O_4$: C, 58.53; H, 4.56; N, 14.63. Found: C, 58.79; H, 4.31; N, 14.43.

2-Amino-5-(5-chloro-2-methoxyphenyl)furo[2,3-*d*]pyrimidin-4(3*H*)-one (9r) Yield 65% (948 mg), mp >260 °C. ^{1}H-NMR (DMSO-d_6) δ: 3.76 (s, 3H), 6.63 (br s, 2H exchangeable with D_2O), 6.96—7.06 (m, 2H), 7.17 (d, 1H, J=6.8), 7.80 (s, 1H), 10.76 (br s, 1H exchangeable with D_2O). IR ν: 1687 cm^{-1}; MS *m/z* (CI): 292, 294 $(M+H)^+$. *Anal*. Calcd for $C_{13}H_{10}ClN_3O_3$: C, 53.53; H, 3.46; N, 14.41. Found: C, 53.27; H, 3.61; N, 14.19.

2-Amino-5-(4-hydroxyphenyl)furo[2,3-*d*]pyrimidin-4(3*H*)-one (9s) Yield 42% (510 mg), mp >260 °C. ^{1}H-NMR (DMSO-d_6) δ: 6.65 (br s, 2H exchangeable with D_2O), 7.33—7.39 (br d, 2H, AA′BB′ system, J=8.8), 7.80 (s, 1H), 7.95—8.02 (br d, 2H, AA′BB′ system, J=8.8), 9.33 (sh, 1H exchangeable with D_2O), 10.83 (br s, 1H exchangeable with D_2O). IR ν: 1679 cm^{-1}. MS *m/z* (CI): 244 $(M+H)^+$, 261 $(M+NH_4)^+$. *Anal*. Calcd for $C_{12}H_9N_3O_3$: C, 59.26; H, 3.73; N, 17.28. Found: C, 59.13; H, 3.53; N, 17.50.

Methyl 4-(2-Amino-4(3*H*)-oxofuro[2,3-*d*]pyrimidine-5-yl)benzoate (9t) Yield 78% (1.11 g), mp >260 °C. ^{1}H-NMR (DMSO-d_6) δ: 3.80 (s, 3H), 6.73 (br s, 2H exchangeable with D_2O), 7.86—7.96 (br d, 2H, AA′BB′ system, J=8.4), 7.93 (s, 1H), 8.06—8.16 (br d, 2H, AA′BB′ system, J=8.4), 10.96 (br s, 1H exchangeable with D_2O). IR ν: 1720, 1680 cm^{-1}. MS *m/z* (CI): 286 $(M+H)^+$, 303 $(M+NH_4)^+$. *Anal*. Calcd for $C_{14}H_{11}N_3O_4$: C, 58.95; H, 3.89; N, 14.73. Found: C, 58.71; H, 3.83; N, 14.95.

2-Amino-5,6-dihydro-5-(3,4,5-trimethoxyphenyl)furo[2,3-*d*]pyrimidin-4(3H)-one (10) A solution of 2-amino-5-(3,4,5-trimethoxyphenyl)-furo[2,3-*d*]pyrimidin-4(3*H*)-one (**9e**) (32 mg, 0.1 mmol) in dioxane (7 ml) was stirred for 16 h under hydrogen in the presence of 5% Pd–C (12 mg) at atmospheric pressure and at room temperature. After filtration through a pad of Celite, the filtrate was evaporated *in vacuo*. The obtained residue was further chromatographed over a silica gel column (10g, eluent CH_2Cl_2/methanol: 93/7) to give the desired compound **10** in 90% yield (29 mg), mp >260 °C. ^{1}H-NMR (DMSO-d_6) δ: 3.63 (s, 3H), 3.73 (s, 6H), 4.10—4.45 (m, 2H), 4.78 (t, 1H, J=7.8), 6.48 (s, 2H), 6.68 (br s, 2H exchangeable with D_2O), 10.60 (br s, 1H exchangeable with D_2O). IR ν: 1668 cm^{-1}. MS *m/z* (CI): 320 $(M+H)^+$, 337 $(M+NH_4)^+$. *Anal*. Calcd for $C_{15}H_{17}N_3O_5$: C, 56.42; H, 5.37; N, 13.16. Found: C, 56.71; H, 5.19; N, 13.23.

2-Amino-4-chloro-5-(3,4,5-trimethoxyphenyl)furo[2,3-*d*]pyrimidine (11) Diethylaniline hydrochloride (315 mg, 1.7 mmol) and freshly distilled phosphorus oxychloride (7.5 g, 12.3 ml, 80 mmol) were successively added to **9e** (317 mg, 1 mmol). The mixture was heated overnight at 60 °C under an argon atmosphere using an oil bath. After cooling, the excess $POCl_3$ was evaporated under reduced pressure. The residue was taken up with CH_2Cl_2 (25 ml) and crushed ice (25 g). The whole was stirred at room temperature for 1 h. After decantation, the aqueous layer was extracted with CH_2Cl_2 (3×25 ml). The combined organic phases were then successively washed with 1 N hydrochloric acid (15 ml), aqueous saturated $NaHCO_3$ (2×10 ml), and ice-water (2×20 ml). After drying ($MgSO_4$), filtration, and removal of the solvent *in vacuo*, the residue was flash-chromatographed over a silica gel column (60g, eluent CH_2Cl_2/methanol : 96/4) to give the desired compound **11** in 74% yield (248 mg), mp 247—249 °C (recrystallized from ethanol). ^{1}H-NMR (DMSO-d_6) δ: 3.71 (s, 3H), 3.83 (s, 6H), 6.86 (s, 2H), 7.29 (br s, 2H exchangeable with D_2O), 7.89 (br s, 1H). MS *m/z* (CI): 336, 338 $(M+H)^+$. *Anal*. Calcd for $C_{15}H_{14}C_lN_3O_4$: C, 53.66; H, 4.20; N, 12.52. Found: C, 53.45; H, 4.11; N, 12.79.

2,4-Diamino-5-(3,4,5-trimethoxyphenyl)furo[2,3-d]pyrimidine (12) A solution of the chloro derivative **11** (168 mg, 0.5 mmol) in saturated methanolic ammonia (10 ml) was heated in a stainless steel bomb at 100 °C for 5 days using an oil bath. Ammonia and methanol were evaporated under reduced pressure. The residue was then purified by silica gel chromatography (60 g, eluent CH_2Cl_2/methanol : 85/15) to provide the diamino derivative **12** in 65% yield (103 mg), mp >260 °C (washed with ethanol). ^{1}H-NMR (DMSO-d_6) δ: 3.70 (s, 3H), 3.82 (s, 6H), 6.00 (br s, 2H exchangeable with D_2O), 6.10 (br s, 2H exchangeable with D_2O), 6.73 (s, 2H), 7.53 (s, 1H). MS *m/z* (CI): 317 $(M+H)^+$. *Anal*. Calcd for $C_{15}H_{16}N_4O_4$: C, 56.96; H, 5.10; N, 17.71. Found: C, 56.73; H, 4.93; N, 17.99.

2,4-Diamino-5,6-dihydro-5-(3,4,5-trimethoxyphenyl)furo[2,3-*d*]pyrimidine (13) The 5,6-dihydro derivative **13** was prepared starting from **12** (32 mg, 0.1 mmol) by a procedure similar to that described for **10** in 90% yield (29 mg), mp >260 °C (washed with ethanol). ^{1}H-NMR (DMSO-d_6) δ: 3.73 (s, 3H), 3.83 (s, 6H), 4.23—4.28 (m, 1H), 4.31—4.38 (m, 1H), 4.73 (t, 1H, J=8.7), 5.77 (br s, 2H exchangeable with D_2O), 5.94 (br s, 2H exchangeable with D_2O), 6.51 (s, 2H). MS *m/z* (CI): 319 $(M+H)^+$. *Anal*. Calcd for $C_{15}H_{18}N_4O_4$: C, 56.60; H, 5.70; N, 17.60. Found: C, 56.35; H, 5.81; N, 17.65.

6-Hydroxy-5-[1-(3,4,5-trimethoxyphenyl)-1-ethyl]-2,4-pyrimidinediamine (14) The pyrimidinediamine **14** was obtained in 68% yield (28 mg) starting from **12** (32 mg, 0.1 mmol) in a similar manner to that employed for

10, but using 10% Pd–C (12 mg) instead of 5% Pd–C (in this case, a trace of **13** was also detected by TLC), mp 259—260 °C. ^{1}H-NMR (DMSO-d_6) δ: 1.49 (d, 3H, J=7.2), 3.61 (s, 3H), 3.71 (s, 6H), 4.02 (q, 1H, J=7.2), 5.44 (br s, 2H exchangeable with D_2O), 6.00 (br s, 2H exchangeable with D_2O), 6.71 (s, 2H), 9.80 (br s, 1H exchangeable with D_2O). MS *m/z* (CI): 321 $(M+H)^+$.

2-Amino-5,6-dihydro-5-(2,5-dimethoxyphenyl)furo[2,3-*d*]pyrimidin-4(3*H*)-one (15) The furo[2,3-*d*]pyrimidin-4(3*H*)-one **15** was prepared starting from **9p** (28 mg, 0.1 mmol) in a similar manner to that employed for **10**. Yield 88% (25 mg), mp >260 °C. ^{1}H-NMR (DMSO-d_6) δ: 3.80 (s, 3H), 3.87 (s, 3H), 4.15—4.43 (m, 2H), 4.66 (t, 1H, J=8.5), 6.66 (br s, 2H exchangeable with D_2O), 6.87—6.99 (m, 2H), 8.06 (m, 1H), 10.40 (br s, ^{1}H exchangeable with D_2O). IR ν: 1669 cm^{-1}. MS *m/z* (CI): 290 $(M+H)^+$, 307 $(M+NH_4)^+$. *Anal*. Calcd for $C_{14}H_{15}N_3O_4$: C, 58.13; H, 5.23; N, 14.53. Found: C, 58.35; H, 5.13; N, 14.63.

4-(2-Amino-4(3*H*)-oxofuro[2,3-*d*]pyrimidine-5-yl)benzoic acid (16) The ester **9t** (285 mg, 1 mmol) was suspended in aqueous 1 N sodium hydroxide (4 ml) and the mixture was stirred at room temperature. The solid disappeared gradually and the solution obtained after 3 h was acidified with acetic acid (0.8 ml). The formed precipitate was suction-filtered and successively washed with water (3 ml) and diethyl ether (3 ml) to afford satisfactorily pure **16** in 73% yield (178 mg), mp >260 °C. ^{1}H-NMR (DMSO-d_6) δ: 3.38 (br s, 1H exchangeable with D_2O), 6.91 (br s, 2H exchangeable with D_2O), 7.92—7.95 (br d, 2H, AA′BB′ system, J=7.6), 7.98 (s, 1H), 8.12—8.15 (br d, 2H, AA′BB′ system, J=7.6), 11.23 (br s, 1H exchangeable with D_2O). IR ν: 1732, 1675 cm^{-1}. MS *m/z* (CI): 272 $(M+H)^+$. *Anal*. Calcd for $C_{14}H_{11}N_3O_4$: C, 57.57; H, 3.34; N, 15.49. Found: C, 57.29; H, 3.17; N, 15.73.

Diethyl *N*-[4-(2-Amino-4(3*H*)-oxofuro[2,3-*d*]pyrimidin-5-yl)benzoyl]-L-glutamate (17) 4-chloro-4,6-dimethoxytriazine (560 mg, 3.18 mmol) was added to a solution of **16** (862 mg, 3.18 mmol) and *N*-methylmorpholine (322 mg, 0.35 ml, 3.18 mmol) in dry DMF (10 ml). This mixture was stirred at room temperature for 2 h before 4-chloro-4,6-dimethoxytriazine (110 mg, 0.63 mmol) and *N*-methylmorpholine (151 mg, 0.165 ml, 1.5 mmol) were added again (this two-fold addition process provided better yields than the direct use of reagents in excess). The solution was stirred for 30 additional minutes and *N*-methylmorpholine (322 mg, 0.35 ml, 3.18 mmol), then diethyl L-glutamate hydrochloride (0.76 g, 3.5 mmol) were successively added. The reaction mixture was stirred at room temperature for 16 h and silica gel (3 g) was added. The suspension was evaporated to dryness under reduced pressure. The obtained residue was loaded on a dry silica gel column (60 g) and eluted with a mixture CH_2Cl_2/methanol : 94/6. The fractions corresponding to the product were pooled, then evaporated *in vacuo* to give satisfactorily pure **17** in 90% yield (1.30 g), mp >260 °C. ^{1}H-NMR (DMSO-d_6) δ: 1.16—1.23 (m, 6H), 1.99—2.14 (m, 2H), 2.45 (t, 2H, J=7.2), 4.06—4.14 (m, 4H), 4.39—4.50 (m, 1H), 6.81 (br s, 2H exchangeable with D_2O), 7.88—7.91 (br d, 2H, AA′BB′ system, J=7.9), 7.98 (s, 1H), 8.09—8.15 (br d, 2H, AA′BB′ system, J=7.9), 8.68 (d, 1H exchangeable with D_2O, J=7.2,), 11.00 (br s, 1H exchangeable with D_2O). IR ν: 1720, 1675 cm^{-1}. MS *m/z* (CI): 457 $(M+H)^+$. *Anal*. Calcd for $C_{22}H_{24}N_4O_7$: C, 57.89; H, 5.30; N, 12.27. Found: C, 57.51; H, 5.19; N, 12.63.

***N*-[4-(2-Amino-4(3*H*)-oxofuro[2,3-*d*]pyrimidin-5-yl)benzoyl]-L-glutamic acid (18)** The above diester **17** (46 mg, 0.1 mmol) was suspended in aqueous 1 N sodium hydroxide (3 ml) and the mixture was stirred at room temperature. The solid disappeared gradually and the solution obtained after 3 h was acidified with acetic acid (0.7 ml). The measured pH (test paper) was about 3. The precipitate that formed was collected by filtration and was thoroughly washed with water (4 ml) and diethyl ether (3 ml) to afford, after drying *in vacuo*, satisfactorily pure **18** in 72% yield (29 mg), mp >260 °C. ^{1}H-NMR (DMSO-d_6) δ: 1.88—2.00 (m, 2H), 2.24—2.27 (m, 2H), 4.15—4.20 (m, 1H), 7.30 (br s, 2H exchangeable with D_2O), 7.81—7.84 (br d, 2H, AA′BB′ system, J=8.1), 7.90 (s, 1H), 8.10—8.13 (br d, 2H, AA′BB′ system, J=8.1), 8.51 (d, 1H exchangeable with D_2O, J=7.5), 11.20 (br s, 1H exchangeable with D_2O), 11.80 (br s, 2H exchangeable with D_2O). IR ν: 1740, 1675 cm^{-1}. MS *m/z* (ESI): 423 $(M+Na)^+$. *Anal*. Calcd for $C_{18}H_{16}N_4O_7$: C, 54.00; H, 4.03; N, 13.99. Found: C, 54.41; H, 4.31; N, 13.63.

Diethyl *N*-[4-(2-amino-5,6-dihydro-4(3*H*)-oxofuro[2,3-*d*]pyrimidin-5-yl)benzoyl]-L-glutamate (19) A solution of the diester **17** (46 mg, 0.1 mmol) in dioxane (5 ml) was stirred overnight, under hydrogen in the presence of 5% Pd–C (16 mg) at atmospheric pressure and at room temperature. After filtration through a pad of Celite and rinsing of the solid several times with ethanol, the filtrate was evaporated *in vacuo*. The residue was then subjected to column chromatography on silica gel (8 g, CH_2Cl_2 /methanol : 92/8) to provide **19** with satisfactory purity in 88% yield (40 mg), mp >260 °C. ^{1}H-NMR (DMSO-d_6) δ: 1.15—1.36 (m, 6H), 2.00—2.18 (m, 2H), 2.50 (t, 2H, J=7.8), 4.07—4.18 (m, 4H), 4.21—4.29 (m, 1H), 4.34—4.44 (m, 1H), 4.84 (dd, 1H, J=4.0, 9.1), 4.96 (t, 1H, J=9.1), 6.80 (br s, 2H exchangeable with D_2O), 7.27—7.30 (br d, 2H, AA′BB′ system, J=7.9), 7.80—7.83 (br d, 2H, AA′BB′ system, J=7.9), 8.67 (d, 1H exchangeable with D_2O, J=7.5), 10.42 (br s, 1H exchangeable with D_2O). IR ν: 1725, 1681 cm^{-1}. MS *m/z* (CI): 459 $(M+H)^+$. *Anal*. Calcd for $C_{22}H_{26}N_4O_7$: C, 57.64; H, 5.72; N, 12.22. Found: C, 57.35; H, 5.59; N, 12.69.

***N*-[4-(2-amino-5,6-dihydro-4(3*H*)-oxofuro[2,3-*d*]pyrimidin-5-yl)benzoyl]-L-glutamic acid (20)** This compound was synthesized in 75% yield (30 mg) starting from the diester **19** (46 mg, 0.1 mmol) in a similar manner to that employed for **18**. This diacid **20** was also prepared in 72% yield (29 mg) by an alternative procedure starting from **18** (46 mg, 0.1 mmol) using the methodology employed to prepare **19** from **17**, but using a mixture of CH_2Cl_2/methanol : 85/15 (instead of 80/20) for the column chromatography purification, mp >260 °C. ^{1}H-NMR (DMSO-d_6) δ: 1.99—2.18 (m, 2H), 2.49 (t, 2H, J=7.1), 4.23—4.27 (m, 1H), 4.39—4.45 (m, 1H), 4.79 (dd, 1H, J=4.0, 8.9), 4.95 (t, 1H, J=8.9) 7.01 (br s, 2H exchangeable with D_2O), 7.39—7.45 (br d, 2H, AA′BB′ system, J=8.6), 7.52—7.58 (br d, 2H, AA′BB′ system, J=8.6), 8.67 (d, 1H exchangeable with D_2O, J=7.7), 10.59 (br s, 1H exchangeable with D_2O), 11.53 (br s, 2H exchangeable with D_2O). IR ν: 1745, 1675 cm^{-1}. MS *m/z* (ESI): 425 $(M+Na)^+$. *Anal*. Calcd for $C_{18}H_{18}N_4O_7$: C, 53.73; H, 4.51; N, 13.92. Found: C, 53.24; H, 4.73; N, 14.03.

Inhibition of DHFR The standard assay for DHFR activity depends on the oxidation of the cofactor NADPH, which occurs concurrently with the reduction of the substrate dihydrofolate, and is followed by a reduction in the optical density of the reaction mixture at 340 nm. The reaction mixture, in cuvettes of 1 cm path length, was made up of: 100 μl 1 mM NADPH, 50 μl 1.2 mM dihydrofolate (DHF), tested inhibitor, enzyme, and made up to 1 ml with 50 mM imidazole buffer, pH 7.0.

The amount of enzyme was adjusted to give a reduction of OD_{340} of about 0.04 units per minute. The reaction was started by addition of DHF after a 3 min pre-incubation of the other components. The reaction was carried out at a temperature of 25 °C. Measurements were made at various inhibitor concentrations and the IC_{50} (concentration required to reduce the activity by 50%) was determined graphically.

***In vitro* Antibacterial Activity** Bacterial strains were obtained from the F. Hoffmann-La Roche collection. MICs were routinely determined by an agar dilution method[14] using Iso-Sensitest agar (Oxoid). Stock solutions of the compounds were prepared in dimethyl sulfoxide (DMSO). Agar plates containing serial twofold dilution of the tested compounds were inoculated with the help of a multipoint inoculator (Denley A400) to yield about 1×10^{-4} to 5×10^{-4} CFU (Colonies Forming Unity) per spot. The plates were evaluated after incubation for 18 h at 37 °C. The MIC was defined at the lowest concentration of a compound that prevented clearly visible growth. TMP was used as a reference compound.

In vitro* Activity against *Plasmodium falciparum Antimalarial activity was tested against *P. falciparum* according to the method described by Desjardins *et al*.[15] Strain K1 (resistant to chloroquine and pyrimethamine) and strain NF54 (sensitive to standard antimalarials) were used.

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