Inhibition of Neuronal Nitric Oxide Synthase by 4-Amino Pteridine Derivatives: Structure−Activity Relationship of Antagonists of (6*R*)-5,6,7,8-Tetrahydrobiopterin Cofactor

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The family of nitric oxide synthases (NOS) catalyzes the conversion of L-arginine to L-citrulline and nitric oxide (NO), an important cellular messenger molecule which has been implicated in the pathophysiology of septic shock and inflammatory and neurodegenerative disease states. NOS can be maximally activated by the ubiquitous cofactor, (6*R*)-5,6,7,8-tetrahydrobiopterin (H_4Bip), and antagonists of H_4Bip may be of therapeutic importance to inhibit pathologically high NO formation. The 4-amino substituted analogue of H_4Bip was reported to be a potent NOS inhibitor. Therefore, we developed a series of novel 4-amino pteridine derivatives, antipterins, to pharmacologically target the neuronal isoform of nitric oxide synthase (NOS-I). To functionally characterize the pterin/anti-pterin interaction and establish a structure−activity relationship (SAR), we systematically altered the substituents in the 2-, 4-, 5-, 6-, and 7-position of the pteridine nucleus. Varying the substitution pattern in the 2-, 5-, and 7-position resulted in no significant inhibitory effect on enzyme activity. In contrast, bulky substituents in the 6-position, such as phenyl, markedly increased the inhibitory potency of the reduced 4-amino-5,6,7,8-tetrahydropteridines, possibly as a consequence of hydrophobic interactions within NOS-I. However, this was not the case for the aromatic 4-amino pteridines. Interestingly, chemical modification of the 4-amino substituent by dialkyl/diaralkylation together with 6-arylation of the aromatic 2,4-diamino pteridine resulted in potent and efficacious inhibitors of NOS-I, suggesting possible hydrophilic and hydrophobic interactions within NOS-I. This SAR agrees with (a) the recently published crystal structure of the oxygenase domain of the inducible NOS isoform (NOS-II) and (b) the comparative molecular field analysis of selected NOS-I inhibitors, which resulted in a 3D-QSAR model of the pterin binding site interactions. Further optimization should be possible when the full length structure of NOS-I becomes available.

Introduction

For over a decade the biomolecule nitric oxide (NO) has been of great interest to medicinal chemists from designing more effective nitrovasodilator drugs to modulating endogenous NO biosynthesis. NO is a ubiquitous modulator of both physiological and pathophysiological functions[1] that is generated endogenously[2] by the enzyme nitric oxide synthase (NOS),[3] which metabolizes L-arginine[4] substrate to L-citrulline and NO or a related *N*-oxide.[5] To date, three structurally distinct NOS isoforms have been identified.[6] The two constitutively expressed NOS are localized either in the vascular endothelium[7] (NOS-III or eNOS) and involved in the regulation of vascular tone and antithrombosis, or in neuronal tissue[8] (NOS-I or nNOS) and involved in neuromodulation.[9] The so-called inducible isoform (NOS-II or iNOS) is expressed in macrophages and many other cells upon exposure to cytokines and plays a key role in early immune responses by functioning as a cytotoxic agent.[10,11] Importantly, there is an increasing appreciation of the role of NO in contributing to the etiology of pathophysiological conditions including septic shock, inflammatory, and neurodegenerative disease states.[12] Therefore, the use of NOS inhibitors to downregulate pathologically elevated synthesis of NO has great therapeutic potential.

At the biochemical level a number of cofactors of NOS have been identified that are uniformly required for catalysis by all isoforms including NADPH and flavins (FAD, FMN).[13,14] In addition, the cofactor (6*R*)-5,6,7,8-tetrahydrobiopterin (H_4Bip) markedly stimulates NOS catalytic activity by a unique and as yet to be fully elucidated mechanism, possibly involving enzyme stabilization[15] and protection.[16] Thus, the H_4Bip binding site of NOS may represent an ideal target for selective pharmacological intervention. Prototypical NOS inhibitors were mainly analogues of their endogenous substrate, L-arginine,[4] e.g., N^{ω}-methyl-L-arginine (L-NMA), N^{ω}-methyl-L-argininemethylester (L-NMMA), N^{ω}-nitro-L-arginine (L-NNA), its methylester (L-NAME), and N^{δ}-

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Table 1. 6-Substituted 2,4-Diamino Pteridines **2**

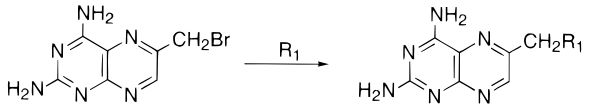
entry	R_1	UV/VIS; λ_{MAX} in nm (log ϵ), [ ] indicates a shoulder	C, H, N (MW) anal. calcd (found) for
2a	OMe	pH 1: 242 (4.18); 285 (3.65); 337 (3.97); [350 (3.92)]	$C_8H_{10}N_6O$ (206.1): C 46.60 (46.44), H 4.85 (4.69), N 40.77 (40.38)
2b	OC_2H_5	pH 1: 242 (4.19); 284 (3.67); 337 (3.98); [348 (3.92)]	$C_9H_{12}N_6O$ (220.2): C 49.11 (48.86), H 5.45 (5.24), N 38.16 (37.76)
2c	OC_6H_5	pH 1: 243 (4.13); 287 (3.61); 336 (3.90); 350 (3.82)	$C_{13}H_{12}N_6O$ (268.3): C 58.19 (57.64), H 4.51 (4.36), N 31.36 (31.40)
2d	$O(CH_2)_9CH_3$	pH 1.2: 221 (4.18); 288 (3.69); 335 (3.99); [348 (3.92)]	$C_{17}H_{28}N_6O$ (332.3): C 61.46 (61.53), H 8.42 (8.70), N 25.28 (25.13)
2e	$OCOCH_2CH_2$-C_6H_4-4CO-C_6H_5	MeOH: 203 (4.56); 260 (4.60); 372 (3.90)	$C_{23}H_{20}N_6O_3$ (428.5): C 64.48 (64.01), H 4.70 (4.94), N 19.61 (18.87)
2f	NH_2	pH 1: 244 (4.15); 290 (3.96); [346 (3.91)]	$C_7H_9N_7$ (191.1): C 43.98 (44.12), H 4.71 (5.19), N 51.29 (50.50)
2g	$N(CH_3)_2$	pH 1: 245 (4.20); 294 (3.71); 336 (3.99); [348 (3.94)]	$C_9H_{13}N_7 \times 0.25\ H_2O$ (223.6): C 48.34 (48.21), H 6.03 (6.10), N 43.83 (43.58)
2h	SMe	pH 2: 245 (4.22); 286 (3.96); 342 (3.98); [350 (3.95)]	$C_8H_{10}N_6S$ (222.2): C 43.26 (43.36), H 4.50 (4.36), N 37.82 (38.04)
2i	$NHCOCH(CH_3)_2$	pH 2: 245 (4.22); 285 (3.69); 337 (4.02); [348 (3.96)]	$C_{11}H_{15}N_7O$ (261.2): C 50.59 (50.46), H 5.47 (5.73), N 37.53 (37.29)

iminoethyl-L-ornithine (L-NIO).[17−19] Although the NOS coproduct, L-citrulline, does not autoregulate NOS function, various analogues were found to be potent NOS inhibitors,[19] e.g., L-thiocitrulline (L-TC), L-homothiocitrulline (L-HTC), and *S*-methyl-L-thiocitrulline (L-SMTC). Further modifications of the guanidino moiety of L-arginine led to the development of cyclic and acyclic amidines, guanidines, and isothioureas as active site inhibitors[20−22] with reversible and irreversible modes of action. Finally, it is a short conceptual step from a cyclic amidine to pyridyl precursors, such as 2-aminopyridines,[23] and from there to aniline derivatives[24] which show some isoform selectivity but are otherwise less potent.

Apart from the L-arginine-based NOS inhibitors, other binding sites of NOS have been pharmacologically targeted. Compounds which are thought to interfere with the flavin cofactor binding site within the reductase domain include diphenyleneiodonium (DPI)[19] and some quinazolines.[25] Within the oxygenase domain of NOS, various indazoles[19] or imidazoles[19] bind as ligands to the heme prosthetic group to inhibit catalysis while other aromatic hydroxamic acid derivatives[26] antagonize H_4Bip function. However, only a few of the NOS inhibitors that have been previously described appear to be acting exclusively at one binding site of NOS. Moreover, most of these agents also have failed to display any appreciable isoform selectivity.

We have previously shown that a novel group of pterin-derived compounds, anti-pterins, are effective inhibitors of NOS activity in intact cells and are, therefore, novel pharmacological tools to downregulate pathologically high NO formation.[27] These compounds appear to specifically interact with the pterin binding site of NOS and do not interfere with any other known cofactor/substrate binding sites.[27] In the present study we synthesized a second generation of refined H_4Bip-based NOS inhibitors with a 4-amino pteridine nucleus[27,28] and systematically varied the substitution patterns in the 2-, 4-, 5-, 6-, and 7-position. The effect of these compounds on NOS activity was taken as a biological indicator of efficacy and potency; a structure−activity relationship (SAR) was then determined. The implications of the present finding for chemical interactions within the pterin binding pocket of NOS are discussed.

Scheme 1. Synthesis of 6-Substituted 2,4-Diamino Pteridines **2**



1 **2**

Chemistry

The 6-substituted 2,4-diamino pteridines **2a-i**[30−32] were synthesized by reacting the 2,4-diamino-6-bromomethylpteridine × HBr × 2-hydroxy-propane[29] (**1**) with the nucleophile R_1 by stirring at room temperature (Scheme 1; Table 1).

The general principle for the synthesis of 2,4-diamino-6/7-aryl pteridines **6**[34] and the 2-substituted 4-amino-6-aryl pteridines **7** was based on the condensation of 2-substituted 4,5,6-triaminopyrimidine dihydrochloride derivatives **4** with different substituted phenylglyoxalmonoximes **5**[35−38] by heating in MeOH under reflux.[34] Modification of the pteridines **7b-e** in the 2-position was achieved by varying the 2-position of the pyrimidine adducts **4b**[39]**−e**, which were obtained by reduction of the nitroso-precursor **3c-e**[40] (Scheme 2). The corresponding 2-substituted 4-aminopteridine-6-carboxaldoximes **9b-d**[43] were prepared by reacting the 4,5,6-triaminopyrimidine dichlorides **4b-d** with the mesoxaldicarbaldoxime **8** (Scheme 2; Tables 2−6).

Another synthetic approach involved using initially 2,6-diamino-4-chloro-5-*p*-chlorophenylazopyrimidine **12**[44] which was prepared from *p*-chloroaniline **10** by diazotation and subsequent coupling with 2,6-diamino-4-chloropyrimidine **11**. In **12**, the chlorine atom is sufficiently activated to be nucleophilically displaced by acyclic or cyclic secondary amines **13b-f** to form the 2,6-diamino-4-dialkylamino-5-*p*-chlorophenylazopyrimidines **14b-f**, which on reduction with Raney nickel catalyst and H_2 generated the 2,5,6-triamino-4-dialkylaminopyrimidines **16b-f**. For the 4-(dimethylamino)-substituted 2,5,6-triaminopyrimidine **16a**, the direct route of PtO_2/H_2 catalyzed reduction of 2,6-diamino-4-(dimethylamino)-5-nitrosopyrimidine **15a**[41] was chosen.

Table 2. 2-Substituted 4,5,6-Triaminopyrimidines **4**[a]

entry	R_2	UV/VIS; λ_{MAX} in nm (log e), [ ] indicates a shoulder	C, H, N (MW) anal. calcd (found) for
4a[34]	NH_2		
4b[39]	H		
4c	SMe	H_2O: [231 (4.15)]; 286 (3.99)	$C_5H_{10}N_5S \times 2$ HCl (244.1): C 24.60 (24.73), H 4.54 (4.58), N 28.68 (29.01)
4d	C_6H_5	H_2O: 292 (4.01); [334 (3.80)]	$C_{10}H_{13}Cl_2N_5 \times 0.5\ H_2O$ (283.2): C 42.42 (42.36), H 4.98 (5.00), N 24.73 (24.37)
4e	$NHCH_2CH_2OH$	H_2O: 286 (4.07)	$C_6H_{15}Cl_3N_6O$ (282.3): C 24.55 (25.04), H 5.15 (5.18), N 28.63 (28.66)

[a] Compounds **4a** and **4b** were prepared according to literature.[34,39]

Scheme 2. Synthesis of 4-Amino-6/7-aryl Pteridines **6** and **7**

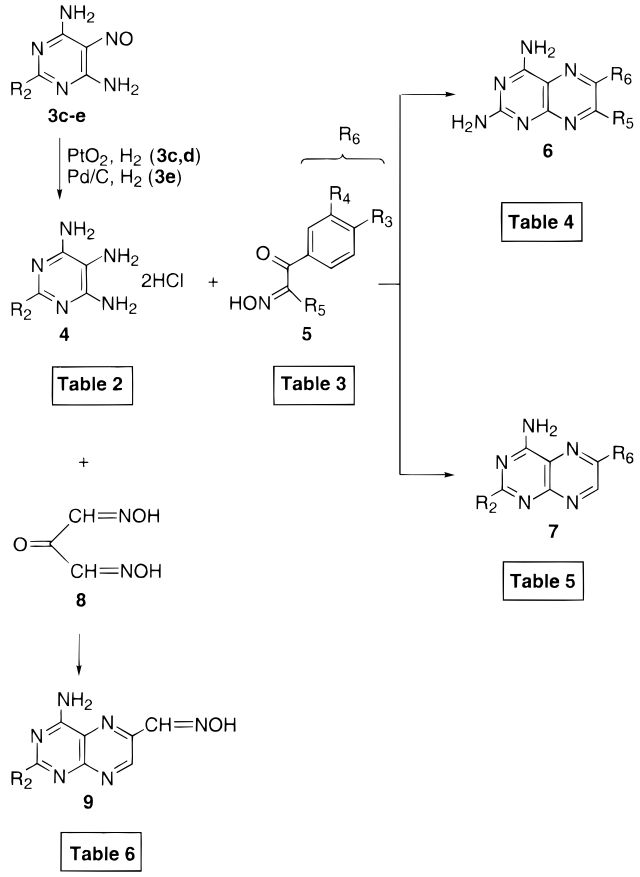


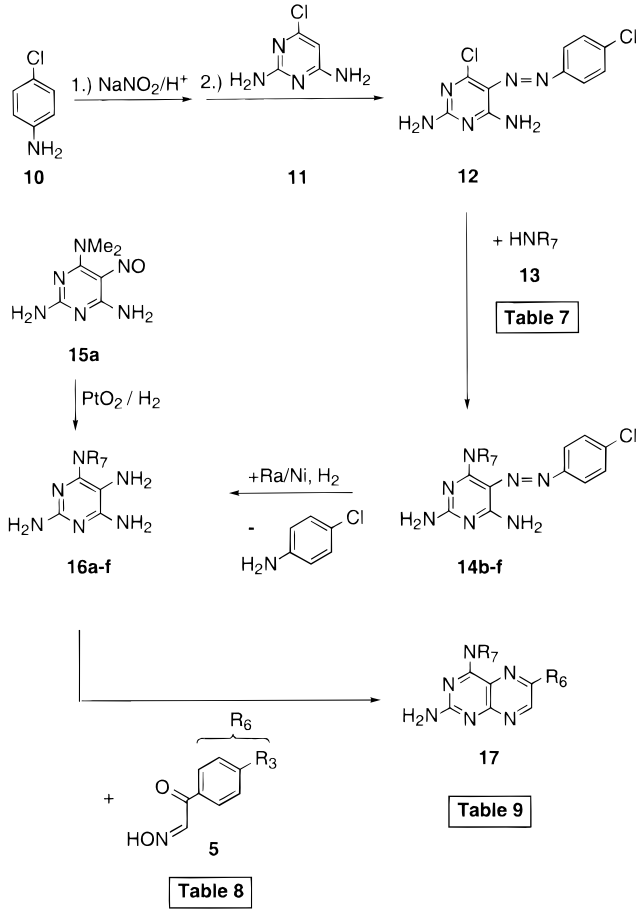
Table 3. Derivatives of Phenylglyoxalmonoxime **5**

entry	R_3	R_4	R_5
5a	H	H	H
5b	F	H	H
5c	Cl	H	H
5d	Br	H	H
5e	Me	H	H
5f	OMe	H	H
5g	OMe	OMe	H
5h	H	H	Me
5i	H	H	C_6H_5

Finally, boiling a solution of the 2,5,6-triamino-4-dialkylaminopyrimidines **16** with the arylglyoxalmonoximes **5** yielded the 2-amino-4-dialkylamino-6-aryl pteridines **17** with either C_6H_5- (**17aa-fa**) or *p*-$MeOC_6H_4$- (**17af-ff**) substitution in the R_6-position of the 2-amino-4-dialkylaminopteridine (Scheme 3; Tables 7−9).

4-Amino-2-methylaminopteridine (**20c**)[42] and its 6,7-dimethyl (**20a**) and 6,7-diphenyl derivative (**20b**) were prepared by condensation of the 4,5,6-triamino-2-methylaminopyrimidine **18** with the 1,2-dicarbonyl compounds **19** (19a: diacetyl; 19b: benzil; 19c: glyoxal) and then catalytically reduced with PtO_2 catalyst under H_2 atmosphere to generate the corresponding 5,6,7,8-tetrahydropteridines **21a-c**.[42] In an analogous manner, various 6-substituted 2,4-diaminopteridines (**2b,6aa**[34] or **20d**[33]**,g**[45]**,h**[34]) were converted by catalytic reduction into their 5,6,7,8-tetrahydro-derivatives **21d-h**. Mild acylation led to monosubstitution in the 5-position to give **22da** and **22db** (Scheme 4; Tables 10−13).

Scheme 3. Synthesis of 2-Amino-4-dialkylamino-6-aryl Pteridines **17**



2,4-Diamino-7-benzylpteridine (**24**) was prepared by condensation of 2,4,5,6-tetraaminopyrimidine (**4a**) with benzylglyoxal (**23**[46]). Bromination in the side chain and subsequent hydrolysis generated 2,4-diamino-7-benzoylpteridine (**25**) (Scheme 5).

2,4-Diamino-7-aminomercaptopteridine (**27**) was obtained by treatment of 2,4-diamino-7-mercaptopteridine (**26**[47]) with chloramine (Scheme 6).

Table 4. 2,4-Diamino-6/7-substituted Pteridines **6**[a]

entry	R_6	R_5	UV/VIS; λ_{MAX} in nm (log ϵ), [ ] indicates a shoulder	C, H, N (MW) anal. calcd (found) for
6aa[34]	C_6H_5	H	pH 2: 266 (4.48); 364 (411)	
6ab	p-FC_6H_4	H	pH 1: 264 (4.41); 365 (4.05)	$C_{12}H_9FN_6 \times 2\ H_2O$ (292.2): C 49.31 (49.05), H 4.48 (3.88), N 28.75 (28.85)
6ac	*p*-ClC_6H_4	H	pH 2: 268 (4.42); [294 (4.32)]; 365 (4.02)	$C_{13}H_{12}ClN_6$ (272.6): C 52.85 (52.73), H 3.33 (3.42), N 30.83 (29.98)
6ad	*p*-BrC_6H_4	H	DMF: 288 (4.50); [321 (4.20)]; 400 (4.10)	$C_{12}H_9BrN_6 \times 2\ H_2O$ (353.1): C 40.81 (41.02), H 3.71 (3.93), N 23.80 (23.91)
6ae	*p*-MeC_6H_4	H	pH 2: 268 (4.48); 369 (4.10)	$C_{13}H_{12}N_6$ (252.2): C 61.89 (61.98), H 4.79 (4.95), N 33.31 (32.55)
6af	*p*-$MeOC_6H_4$	H	pH 2: 273 (4.42); [291 (4.35)]; 379 (4.07)	$C_{13}H_{12}N_6O$ (277.2): C 56.31 (56.63), H 4.73 (4.61), N 30.31 (29.98)
6ag	3,4-Di-$MeOC_6H_3$	H	DMF: 285 (4.38); 317 (4.35); 408 (4.08)	$C_{14}H_{14}N_6O_2 \times 2\ H_2O$ (334.3): C 50.30 (49.90), H 5.43 (5.05), N 25.14 (25.18)
6ah	C_6H_5	Me	pH 1: 253 (4.32); 348 (4.09)	$C_{13}H_{12}N_6 \times 1.5\ H_2O$ (279.3): C 55.90 (55.85), H 5.41 (5.05), N 30.09 (30.31)
6ai[34]	C_6H_5	C_6H_5		

[a] Compounds **6aa** and **6ai** were prepared according to literature.[34]

Table 5. Varying the Substituents in the 2- and 6-Position of the 4-Amino Pteridines **7**

entry	R_2	R_6	UV/VIS; λ_{MAX} in nm (log ϵ), [ ] indicates a shoulder	C, H, N (MW) anal. calcd (found) for
7ba	H	C_6H_5	pH 1: 240 (4.26); 275 (4.23); 362 (4.09)	$C_{12}H_9N_5$ (223.2): C 64.56 (64.49), H 4.06 (3.98), N 31.37 (30.80)
7be	H	*p*-MeC_6H_4	pH 1: 244 (4.25); 284 (4.27); 371 (4.10)	$C_{13}H_{11}N_5$ (273.3): C 65.81 (65.99), H 4.67 (4.70), N 29.52 (30.17)
7bf	H	*p*-$MeOC_6H_4$	pH 1: 251 (4.15); 297 (4.30); 385 (4.08)	$C_{13}H_{11}N_5O$ (253.3): C 61.64 (61.61), H 4.38 (4.29), N 27.65 (27.35)
7ca	SMe	C_6H_5	DMF: 289 (4.52); 314 (4.06); 380 (4.10); [394 (4.06)]	$C_{13}H_{11}N_5S$ (269.3): C 57.97 (58.03), H 4.12 (4.14), N 26.00 (26.49)
7ce	SMe	*p*-MeC_6H_4	DMF: 291 (4.57); [320 (4.10)]; 383 (4.16); [401 (4.06)]	$C_{14}H_{13}N_5S$ (283.4): C 59.34 (59.51), H 4.62 (4.65), N 24.72 (25.14)
7cf	SMe	*p*-$MeOC_6H_4$	DMF: 296 (4.45); [320 (4.20)]; 389 (4.10); [404 (4.05)]	$C_{14}H_{13}N_5OS$ (299.4): C 56.17 (56.35), H 4.38 (4.39), N 23.39 (23.88)
7da	C_6H_5	C_6H_5	DMF: 259 (4.32); 293 (4.65); 377 (4.24); [397 (4.11)]	$C_{18}H_{13}N_5$ (299.3): C 72.23 (72.13), H 4.38 (4.45), N 23.40 (23.50)
7df	C_6H_5	*p*-MeC_6H_4	DMF: 259 (4.33); 305 (4.53); 390 (4.31); [409 (4.21)]	$C_{19}H_{15}N_5O$ (329.4): C 69.29 (69.25), H 4.59 (4.63), N 21.26 (21.55)
7ea	$NHCH_2CH_2OH$	C_6H_5	pH 1: 269 (4.49); 386 (4.13)	$C_{14}H_{14}N_6O$ (282.3): C 59.56 (59.81), H 5.00 (4.96), N 29.77 (29.48)
7ee	$NHCH_2CH_2OH$	*p*-MeC_6H_4	pH 1: 269 (4.49); 368 (4.13)	$C_{15}H_{16}N_6O$ (296.3): C 60.80 (60.84), H 5.44 (5.49), N 28.36 (28.56)
7ef	$NHCH_2CH_2OH$	*p*-$MeOC_6H_4$	pH 1: 278 (4.50); 384 (4.12)	$C_{15}H_{16}N_6O_2$ (312.3): C 57.68 (57.64), H 5.16 (5.21), N 26.91 (26.83)

Table 6. Varying the Substituents in the 2-Position of the 4-Aminopteridine-6-carbox-aldoxime **9**[a]

entry	R_2	UV/VIS; λ_{MAX} in nm (log ϵ), [ ] indicates a shoulder	C, H, N (MW) anal. calcd (found) for
9b	H	pH 12: 249 (4.05); 294 (4.27); [320 (4.04)]; 376 (4.01)	$C_7H_6N_6O$ (190.2): C 43.39 (43.49), H 3.33 (3.12), N 43.37 (43.18)
9c[43]	SMe	pH 12: [227 (3.84)]; 292 (4.31); [316 (4.18)]; 390 (3.99)	
9d	C_6H_5	pH 12: 294 (4.43); [329 (4.19)]; 387 (4.20)	$C_{13}H_{10}N_6O$ (266.3): C 58.64 (58.49), H 3.79 (3.65), N 31.56 (31.17)

[a] Compound **9c** was prepared according to literature.[43]

Table 7. Dialkylamines **13**

entry	R_7
13b	$(C_2H_5)_2$
13c	$(CH_2C_6H_5)_2$
13d	$(C_2H_4)_2O$
13e	$(CH_2)_5$
13f	$(C_2H_4)_2NCH_3$

Table 8. Phenyl- or Anisyl-Substituted Glyoxalmonoximes **5**

entry	R_3
5aa	H
5fb	OMe

Structure−Activity Relationship

To establish a structure−activity relationship (SAR) of the NOS pterin/anti-pterin binding site, the pteridine substitution pattern and functionalities within the scaffolds of general formula **I**−**IV** (4-amino substituted pteridine derivatives, Tables 14−17) were systematically varied. This yielded several new, potent derivatives as inhibitors of NOS-I activity. Indeed, some inhibitors were found to be very effective over a narrow concentration range and inhibited total enzyme activity whereas others, despite being potent, were incomplete enzyme inhibitors.

Methyl substitution (**21c**) at the 2-amino group of the 2,4-diamino-5,6,7,8-tetrahydropteridine derivative (**21d** of **I**; see Table 14, ranked according to degree of enzyme

Table 9. 2-Amino-4-dialkylamino-6-aryl Pteridines **17**

entry	R_7	R_6	UV/VIS; λ_{MAX} in nm (log ϵ), [ ] indicates a shoulder	C, H, N (MW) anal. calcd (found) for
17aa	$(Me)_2$	C_6H_5	pH 1: [227 (4.08)]; 275 (4.36); 366 (4.08)	$C_{14}H_{14}N_6 \times 0.5\ H_2O$ (275.3): C 61.08 (61.38), H 5.49 (5.43), N 30.53 (30.27)
17af	$(Me)_2$	*p*-$MeOC_6H_4$	pH 1: 289 (4.44); 381 (4.44)	$C_{15}H_{16}N_6O$ (296.3): C 60.80 (60.79), H 5.44 (5.53), N 28.36 (28.62)
17ba	$(C_2H_5)_2$	C_6H_5	MeOH: [209 (4.42); 236 (4.18)]; 291 (4.44); [317 (3.97)]; 395 (4.02); [411 (3.97)]	$C_{16}H_{18}N_6 \times H_2O$ (312.4): C 61.52 (61.44), H 6.45 (6.07), N 26.90 (26.62)
17bf	$(C_2H_5)_2$	*p*-$MeOC_6H_4$	MeOH: 208 (4.49); [240 (4.11)]; 298 (4.51); [321 (4.15)]; 401 (4.07)	$C_{17}H_{20}N_6O \times H_2O$ (342.4): C 59.63 (59.91), H 6.48 (6.22), N 24.54 (25.16)
17ca	$(CH_2C_6H_5)_2$	C_6H_5	MeOH: 207 (4.62); [246 (4.14)]; 288 (4.46); [317 (3.98)]; 388 (4.06); [415 (3.88)]	$C_{26}H_{22}N_6 \times 2\ H_2O$ (454.4): C 68.71 (68.15), H 5.77 (5.01), N 18.49 (18.51)
17cf	$(CH_2C_6H_5)_2$	*p*-$MeOC_6H_4$	MeOH: 207 (4.63); [246 (4.09)]; 298 (4.44); [322 (4.11)]; 406 (4.02)	$C_{27}H_{24}N_6O$ (448.4): C 72.30 (71.89), H 5.39 (4.83), N 18.74 (18.84)
17da	$(C_2H_4)_2O$	C_6H_5	MeOH: [212 (4.40)]; [237 (4.18)]; 291 (4.50); 399 (4.07)	$C_{16}H_{16}N_6O \times H_2O$ (326.4): C 58.89 (59.03), H 5.56 (5.30), N 25.75 (26.29)
17df	$(C_2H_4)_2O$	*p*-$MeOC_6H_4$	MeOH: 210 (4.41); [241 (4.10)]; 299 (4.49); 404 (4.05)	$C_{17}H_{18}N_6O_2 \times HCl \times H_2O$ (392.8): C 51.98 (51.69), H 5.39 (5.70), N 21.39 (22.08)
17ea	$(CH_2)_5$	C_6H_5	MeOH: 204 (4.50); [245 (4.24)]; 293 (4.48); [318 (4.04)]; 399 (4.13); [418 (4.00)]	$C_{17}H_{18}N_6 \times 2\ H_2O$ (342.4): C 59.63 (59.11), H 6.48 (5.98), N 24.54 (25.96)
17ef	$(CH_2)_5$	*p*-$MeOC_6H_4$	MeOH: 209 (4.49); [240 (4.18)]; 300 (4.50); [322 (4.15)]; 406 (4.04)	$C_{18}H_{20}N_6O \times 0.5\ H_2O$ (345.4): C 62.59 (62.13), H 6.12 (5.84), N 24.33 (25.50)
17fa	$(C_2H_4)_2NCH_3$	C_6H_5	MeOH: 209 (4.46); [242 (4.15)]; 288 (4.48); [318 (3.97)]; 393 (4.04); [413 (3.97)]	$C_{17}H_{19}N_7 \times 0.5\ H_2O$ (330.4): C 61.80 (61.80), H 6.10 (5.94), N 29.67 (29.69)
17ff	$(C_2H_4)_2NCH_3$	*p*-$MeOC_6H_4$	MeOH: [212 (4.55)]; [245 (4.16)]; 299 (4.52); 407 (4.10)	$C_{18}H_{21}N_7O \times 0.5\ H_2O$ (360.4): C 59.98 (59.68), H 6.15 (6.27), N 27.20 (26.73)

Scheme 4. Synthesis of 2,4-Diamino- and 4-Amino-2-methylamino-5,6,7,8-tetrahydropteridines **21** and Formylation/Benzylation Products **22d**

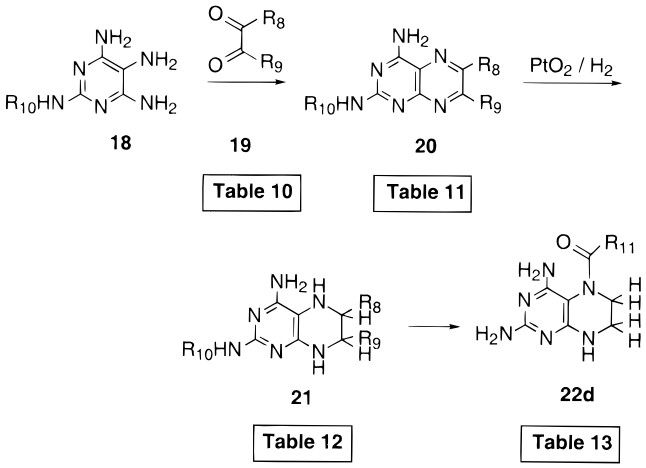


Table 10. 2,3-Diketones **19**

entry	$R_8 = R_9$
19a	Me
19b	C_6H_5
19c	H

inhibition observed with 100 μM anti-pterin) was without effect on NOS-I activity. In contrast, additional 6,7-diphenyl (**21b**) or 6,7-dimethyl substitution (**21a**) resulted in compounds that began to inhibit NOS activity (down to 86 ± 23 and 57 ± 5%, respectively) at the tested concentration. A similar degree of enzyme inhibition was achieved by N-5 acylations alone (**22da,db**; Table 14).

Interestingly, 4-amino-5,6,7,8-tetrahydrobiopterin (**21g**), which differs from the naturally occurring H_4-Bip only in the 4-amino group, was found to be a potent and effective inhibitor (down to 38 ± 2% of V_{max}; IC_{50} = 6 μM). Further inhibition was observed by substituting the dihydroxypropyl side chain in the 6-position, with either a phenyl (**21f**, inhibition down to 17 ± 1% of V_{max}; IC_{50} = 6 μM) or an ether substituent (**21e**, inhibition down to 3 ± 1% of V_{max}; IC_{50} = 30 μM). This resulted in some of our most potent NOS inhibitors based on a reduced 4-amino-5,6,7,8-tetrahydropteridine scaffold **I**. Noteworthy, monophenyl substitution in the 7-position (**21h**) resulted in a weaker NOS inhibitor (39 ± 1% of V_{max}; IC_{50} = 24 μM) compared to the 6-substituted analogue (**21f**, inhibition down to 17 ± 1% of V_{max}; IC_{50} = 6 μM).

In contrast, in the heteroaromatic series, the 7-(**24, 25,27**) and 6-mono-(**6aa**)[34] and 6,7-disubstituted-(**6ah, 6ai**[34]) 2,4-diamino pteridines (general formula **II**, Table 15) were only slightly better NOS inhibitors compared to the inactive 6,7-unsubstituted analogue (**20d**). Furthermore, varying the substitution pattern in the para-position of the 6-phenyl group with either electron-withdrawing (**6ab-ad**) or electron-donating (**6ae-ag**) substituents did not influence the degree of enzyme inhibition. Similar results were obtained for substituents in the ortho- and meta-position (data not shown). However, a significant decrease in NOS activity (16 ± 1% of V_{max}; IC_{50} = 30 μM) could be obtained by using a long-chain aliphatic substituent in the 6-position such as the decyloxymethyl ether **2d** compared, for example, to the ethyloxymethyl ether **2b**, which was otherwise an effective substituent in the 4-amino-5,6,7,8-tetrahydropteridine-series (**I**; Table 14; e.g., 21e).

The above findings consequently prompted us to change further the chemical substituent in the 6-position. Introduction of an ester substituent containing a long side chain (**2e**) generated a NOS inhibitor that was slightly less effective (29 ± 6% of V_{max}) and potent (IC_{50}: 48 μM; $p < 0.05$) whereas substitution with either a phenoxymethyl (**2c**), an aminomethyl (**2f**), a dimethylaminomethyl (**2g**), an isobutyroylaminomethyl (**2i**), or a methylthiomethyl (**2h**) was without effect on NOS-I activity (Table 15). These findings apparently argue against any hydrophilic interaction between a substituent in the 6-position of the aromatic 2,4-diamino pteridine nucleus (**II**) and the pterin binding pocket. Hydro-

Table 11. Aromatic 4-Amino-6/7-alkyl-(aryl-) Pteridines[a]

entry	R_8	R_9	R_{10}	UV/VIS; λ_{MAX} in nm (log ϵ), [ ] indicates a shoulder	C, H, N (MW) anal. calcd (found) for
20a	Me	Me	Me	pH 1: 203 (4.28); 224 (4.21); 245 (4.19); 282 (3.68); 341 (4.08)	$C_9H_{12}N_6$ (204.2): C 52.93 (52.37), H 5.92 (5.92), N 41.16 (41.17)
20b	C_6H_5	C_6H_5	Me	pH 1: 204 (4.68); [228 (4.43)]; 269 (4.36); 374 (4.26)	$C_{19}H_{16}N_6 \times 0.5\ H_2O$ (337.4): C 67.63 (68.10), H 5.08 (5.01), N 24.91 (24.64)
20c[42]	H	H	Me		
20d[33]	H	H	H		
2b	$CH_2OC_2H_5$	H	H	pH 1: 242 (4.19); 284 (3.67); 337 (3.98); [348 (3.92)]	$C_9H_{12}N_6O$ (220.2): C 49.11 (48.86), H 5.45 (5.24), N 38.16 (37.76)
6aa[34]	C_6H_5	H	H		
20g[45]	$CHOHCHOHCH_3$	H	H		
20h[34]	H	C_6H_5	H		

[a] Compounds **6aa** and **20c**,**d**,**g**,**h** were prepared according to literature.[34,42,33,45,34]

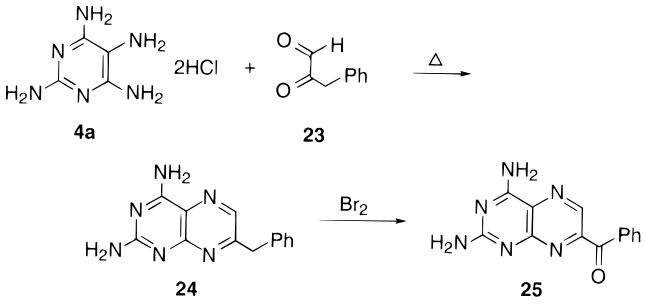
Table 12. Reduced 4-Amino-6/7-alkyl-(aryl)-5,6,7,8-tetrahydropteridines **21**

entry	R_8	R_9	R_{10}	UV/VIS; λ_{MAX} in nm (log ϵ), [ ] indicates a shoulder	C, H, N (MW) anal. calcd (found) for
21a	Me	Me	Me	pH 0: 277 (4.11)	$C_9H_{16}N_6 \times 2$ HCl (281.1): C 38.45 (38.39), H 6.46 (6.42), N 29.90 (29.44)
21b	C_6H_5	C_6H_5	Me	pH 0: 277 (4.13)	$C_{19}H_{20}N_6 \times 2$ HCl $\times H_2O$ (423.3): C 53.90 (53.68), H 5.67 (5.54), N 19.86 (20.02)
21c	H	H	Me	pH 0: 277 (4.07)	$C_7H_{12}N_6 \times 2$ HCl $\times H_2O$ (271.2): C 30.90 (31.02), H 5.20 (5.48), N 30.90 (30.68)
21d	H	H	H	MeOH: [209 (4.15)]; 224 (4.25); 299 (3.95)	$C_6H_{10}N_6 \times 2$ HCl (239.1): C 30.14 (30.41), H 5.06 (5.20), N 35.15 (34.95)
21e	$CH_2OC_2H_5$	H	H	pH 0: 220 (4.72); 275 (4.09)	$C_9H_{16}N_6O \times 3$ HCl $\times H_2O$ (351.4): C 30.75 (30.70), H 5.97 (5.75), N 23.90 (23.96)
21f	C_6H_5	H	H	pH 0: 209 (4.39); 218 (4.38); 273 (4.15)	$C_{12}H_{14}N_6 \times 2$ HCl $\times H_2O$ (333.2): C 43.25 (42.89), H 4.84 (5.31), N 25.22 (24.81)
21g	$CHOHCHOHCH_3$	H	H	MeOH: 219 (4.37); 290 (4.02)	$C_9H_{16}N_6O_2 \times 2$ HCl $\times H_2O$ (331.2): C 32.64 (32.50), H 6.09 (6.07), N 25.37 (25.10)
21h	H	C_6H_5	H	pH 0: 216 (4.38); 273 (4.17)	$C_{12}H_{14}N_6 \times 2$ HCl $\times 0.5\ H_2O$ (324.2): C 44.45 (44.76), H 5.29 (5.25), N 25.92 (25.87)

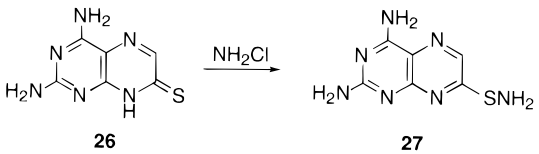
Table 13. Substitution in 5-Position of **21d**

entry	R_{11}
22da	H
22db	C_6H_5

Scheme 5. Synthesis of the 2,4-Diamino-7-benzyl (**24**) and 7-benzoylpteridines (**25**)



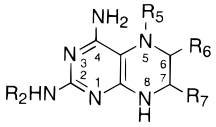
Scheme 6. Synthesis of 2,4-Diamino-7-aminomercaptopteridine (**27**)



phobic interactions, due to a long aliphatic side chain like in **2d,e**, are thus most likely responsible for NOS-I inhibition at this 6-position.

In the case of inactive compounds of the aromatic 4-amino pteridine scaffold (general formula **II**, Table 15: **6aa**,**ae,af**), additional changes in the 2-position of the pyrimidine moiety failed to produce NOS inhibitors (general formula **III**, Table 16). These changes included the replacement of the 2-amino group with either a hydrogen (**7ba,be,bf, 9b**), a methylthio (**7ca,ce,cf, 9c**), a phenyl (**7da,df, 9d**), or a 2-hydroxyethylamino substituent (**7ea,ee,ef**). However, modification at the 4-amino substituent by dialkyl/diaralkylation of the heteroaromatic 2,4-diamino pteridine nucleus (general structure **IV**; Table 17), e.g., dimethylation (**17a**), combined with an anisyl (**17af**, IC_{50}: 74 μM) but not a phenyl (**17aa**)

Table 14. Inhibition of NOS-I by 4-Amino-5,6,7,8-tetrahydropteridine Derivatives **I**

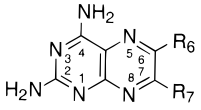


(**I**)

compd (entry)	R_2	R_5	R_6	R_7	NOS activity (% of V_{max})[a]	IC_{50} (μM)[b]
21d	H	H	H	H	102 ± 8	
21c	CH_3	H	H	H	108 ± 23	
21b	CH_3	H	C_6H_5	C_6H_5	86 ± 23	
21a	CH_3	H	CH_3	CH_3	57 ± 5	
22da	H	CHO	H	H	61 ± 1	300
22db	H	COC_6H_5	H	H	59 ± 6	
21g	H	H	$CHOHCHOHCH_3$	H	38 ± 2	6
21h	H	H	H	C_6H_5	39 ± 1	24
21f	H	H	C_6H_5	H	17 ± 1	6
21e	H	H	$CH_2OCH_2CH_3$	H	3 ± 1	30

[a] Inhibition of H_4Bip (2 μM) stimulated NOS total activity at an inhibitor concentration of 100 μM. [b] The SEM of the IC_{50} was generally lower than 5% and omitted in the table.

Table 15. Effect of Substituents in the 6- and the 7-Position on Inhibition of NOS-I by Aromatic 2,4-Diamino Pteridine Derivatives **II**

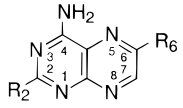


(**II**)

compd (entry)	R_6	R_7	NOS activity (% of V_{max})[a]	IC_{50} (μM)
20d	H	H	104 ± 4	
24	H	$CH_2C_6H_5$	89 ± 6	
25	H	COC_6H_5	90 ± 7	
27	H	SNH_2	71 ± 1	
6aa	C_6H_5	H	85 ± 24	
6ab	4-FC_6H_4	H	87 ± 11	
6ac	4-ClC_6H_4	H	94 ± 5	
6ad	4-BrC_6H_4	H	67 ± 11	
6ae	4-$CH_3C_6H_4$	H	113 ± 12	
6af	4-$CH_3OC_6H_4$	H	109 ± 19	
6ag	3,4-$(CH_3O)_2C_6H_3$	H	78 ± 10	
6ah	C_6H_5	CH_3	79 ± 11	
6ai	C_6H_5	C_6H_5	62 ± 4	
2b	$CH_2OCH_2CH_3$	H	115 ± 5	
2c	$CH_2OC_6H_5$	H	87 ± 28	180
2d	$CH_2O(CH_2)_9CH_3$	H	16 ± 1	30
2e	$CH_2OCO(CH_2)_2C_6H_4COC_6H_4$	H	29 ± 6	48
2f	CH_2NH_2	H	94 ± 3	
2g	$CH_2N(CH_3)_2$	H	111 ± 3	
2h	CH_2SCH_3	H	103 ± 4	
2i	$CH_2NHCOCH(CH_3)_2$	H	101 ± 0.3	

[a] Inhibition of H_4Bip (2 μM) stimulated NOS total activity at an inhibitor concentration of 100 μM.

Table 16. Effect of Additional Substituents in the 2-Position on Inhibition of NOS-I by Aromatic 4-Amino-6-Substituted Pteridine Derivatives **III**



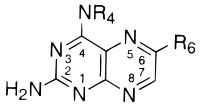
(**III**)

compd (entry)	R_2	R_6	activity (% of V_{max})[a]
7ba	H	C_6H_5	79 ± 6
7be	H	4-$CH_3C_6H_4$	104 ± 4
7bf	H	4-$CH_3OC_6H_4$	101 ± 8
9b	H	CH=NOH	81 ± 10
7ca	SCH_3	C_6H_5	104 ± 18
7ce	SCH_3	4-$CH_3C_6H_4$	96 ± 13
7cf	SCH_3	4-$CH_3OC_6H_4$	104 ± 14
9c	SCH_3	CH=NOH	79 ± 11
7da	C_6H_5	C_6H_5	86 ± 11
7df	C_6H_5	4-$CH_3OC_6H_4$	105 ± 13
9d	C_6H_5	CH=NOH	93 ± 6
7ea	$NHCH_2CH_2OH$	C_6H_5	100 ± 27
7ee	$NHCH_2CH_2OH$	4-$CH_3C_6H_4$	114 ± 9
7ef	$NHCH_2CH_2OH$	4-$CH_3OC_6H_4$	112 ± 5

[a] Inhibition of H_4Bip (2 μM) stimulated NOS total activity at an inhibitor concentration of 100 μM. Since the degree of NOS inhibition with 100 μM anti-pterin was small, no IC_{50} values were determined.

group in 6-position, led to inhibitors with moderate potency, whereas diethylation (**17b**) at the 4-amino group, combined with an anisyl group in the 6-position (**17bf**, IC_{50}: 45 μM) increased inhibitory effectiveness further down to 2% of V_{max}. Dibenzylation at the 4-amino substituent together with either a phenyl group (**17ca**, IC_{50}: 3 μM) or an anisyl group (**17cf**, IC_{50}: 5 μM) in the 6-position of **IV** led to complete inhibition of

Table 17. Effect of Additional Disubstitution at the 4-Amino Position on Inhibition of NOS-I by Aromatic 2,4-Diamino-6-aryl Pteridine Derivatives **IV**



(**IV**)

compd (entry)	R_4	R_6	NOS activity (% of V_{max})[a]	IC_{50} (μM)[b]
17aa	$(CH_3)_2$	C_6H_5	92 ± 11	
17af	$(CH_3)_2$	4-$CH_3OC_6H_4$	13 ± 4	74
17ba	$(C_2H_5)_2$	C_6H_5	75 ± 3	
17bf	$(C_2H_5)_2$	4-$CH_3OC_6H_4$	2 ± 0.1	45
17ca	$(CH_2C_6H_5)_2$	C_6H_5	0 ± 0.05	3
17cf	$(CH_2C_6H_5)_2$	4-$CH_3OC_6H_4$	0 ± 0.05	5
17da	$(C_2H_4)_2O$	C_6H_5	41 ± 8	82
17df	$(C_2H_4)_2O$	4-$CH_3OC_6H_4$	5 ± 0.1	34
17ea	$(CH_2)_5$	C_6H_5	0 ± 0.05	62
17ef	$(CH_2)_5$	4-$CH_3OC_6H_4$	7 ± 0.2	50
17fa	$(C_2H_4)_2NCH_3$	C_6H_5	83 ± 1	
17ff	$(C_2H_4)_2NCH_3$	4-$CH_3OC_6H_4$	84 ± 5	

[a] Inhibition of H_4Bip (2 μM) stimulated NOS total activity at an inhibitor concentration of 100 μM. [b] IC_{50} values were only determined for those compounds which inhibited NOS total activity at least by 50%.

NOS activity (to 0% of V_{max}) with IC_{50} values in the very low micromolar range. The integration of the 4-amino group into an alicyclic ring such as a morpholine (**17da,df**), piperidine (**17ea,ef**) or 4-methyl piperazine ring (**17fa,ff**) system, combined with a phenyl- (**17da,-ea,fa**) or an anisyl- (**17df,ef,ff**) group in 6-position, respectively, was successful and generated, in the case of the first two heterocyclic substituents, highly effective inhibitors with complete enzyme inhibition to 0% of V_{max} (**17df**, IC_{50}: 34 μM; **17ef**, IC_{50}: 62 μM; **17ef**, IC_{50}: 50 μM). In contrast, introducing a second basic group, as in the 4-methyl piperazine derivative (**17fa,ff**: still ~80% of V_{max}), decreased the NOS inhibitory potency of **IV**.

Thus, given the lower IC_{50} values of the nucleus **IV** (down to 3 μM, see Table 17; with a disubstituted 4-amino group) compared to **II** (Table 15; **6aa**,**ae**,**af** with an unsubstituted 4-amino group), this suggested an increased interaction between enzyme and inhibitor, possibly due to an improved fit within the pterin binding pocket. Furthermore, it became apparent that in nearly all cases, substitution at the 6-position of **IV** by an anisyl, contrary to a phenyl ring, led to an increase in potency with respect to NOS-I inhibition.

Discussion

We systematically developed, screened, and refined a novel class of 4-amino pteridine-based NOS inhibitors as possible therapeutic agents in NOS-mediated pathophysiology, e.g., stroke, septic shock, and inflammatory and neurodegenerative disease states. Previous studies from other investigators[28] and our laboratory[27] suggested that the 4-position of H_4Bip may be an important functional target for developing potent inhibitors of NOS. Indeed, a 4-amino substituted H_4Bip derivative was taken as a prototypical inhibitor[27,28] and, therefore, as a lead compound on which this investigation is based.

Initial investigations focused on varying the chemical substitution pattern in the 6-position of the aromatic

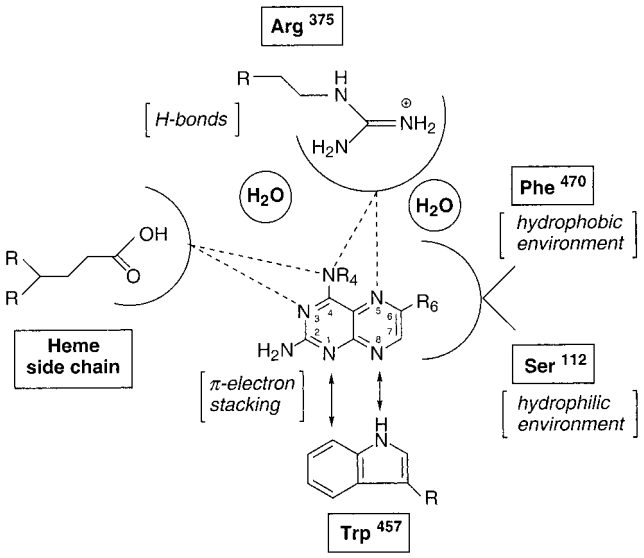


Figure 1. Possible anti-pterin (**17aa-ff**) and NOS-I interactions within the pterin binding site as predicted from a published crystal structure of NOS-II oxygenase dimer.[48]

4-amino pteridine nucleus **II** (Table 15; e.g., **6aa**,**ae**,**af**), either alone or combined with modifications in the 2-position **III** (Table 16). However, this approach did not result in any compounds (Tables 15 and 16) with an inhibitory effect on NOS-I activity, suggesting that these substituents in the case of **II** and **III** do not seem to play a role in the modulation of NOS-I enzyme activity. Similar conclusions can be drawn for the 7-position, since specific chemical modifications at this position of various aromatic 2,4-diamino pteridine derivatives **II** (Table 15; **24**, **25**, **27**) also failed to produce NOS inhibitors. This latter interpretation agrees well with the recently published crystal structure of the oxygenase domain of murine NOS-II, cocrystallized with (6*R*)-5,6,7,8-tetrahydrobiopterin.[48] In this study, hydrophilic interactions were observed between the 6-(1,2-dihydroxypropyl) side chain of the bound H_4Bip and both Phe[470] and Ser[112] of NOS-II and, conceivably, may also be of relevance for NOS-I anti-pterin interactions. However, our kinetic studies with numerous 6-substituted aromatic 2,4-diamino pteridine derivatives **II** (Table 15; **2b-i**, **6aa-ag**) reveal that this 6-substituent/NOS interaction is probably of little functional significance. Even in the situation where increased π-electron stacking of the aromatic **II** (Table 15; e.g., **2b**, **6aa**) with Trp[457] should be favored (Figure 1), no significant inhibitory effect of those compounds on NOS activity was observed. In striking contrast, the corresponding reduced 2,4-diamino-5,6,7,8-tetrahydropteridine derivatives **I** (Table 14; **21e,f**) were found to be potent NOS inhibitors. Therefore the lack of effectiveness of **II** may be due to the planar conformation of the pyrazine ring, which probably fails to effectively insert in the H_4Bip binding pocket. The present findings also reveal an important role for the 6-position of the reduced **I** since the addition of an aromatic ring to the otherwise inactive, 6-unsubstituted **21d** produced a potent NOS inhibitor, **21f**, possibly related to additional hydrophobic interactions of the 6-phenyl of **21f**, e.g., with Phe[470] within the H_4Bip binding pocket of NOS (Figure 1).

In the study by Crane and co-workers,[48] an intimate hydrophilic interaction was observed in the catalytic center of NOS-II between the 4-oxo group of H_4Bip and both (a) the propionic acid side chain of heme and (b) the iminium group of the guanidine from Arg[375]. Given the high degree of sequence homology between NOS-II and NOS-I within the catalytic center, these interactions would be expected to be directly applicable to the NOS-I used in our study. Consistent with this, the present findings indicate that the 4-position has a key function within the H_4Bip binding pocket of NOS-I in supporting catalysis. We therefore investigated further the effects on enzyme inhibition of chemical modifications at the 4-position that enhance the ability of the anti-pterins to form more effective bonding interactions with either the hydrogen-donating heme side chain and/or structural arginine (Arg[375]) elements or, alternatively, may involve displacement of adjacent crystal water localized near the heme (Figure 1). This was indeed the case, as further dialkylation of the 4-amino substituent of the aromatic 4-amino derivatives **IV** generated the most potent NOS inhibitors (Table 17; $R_4 = Me_2$: **17aa,af**, $R_4 = Et_2$: **17ba,bf**, $R_4 = (CH_2C_6H_5)_2$: **17ca,cf**, a morpholine (**17da,df**) and piperidine ring system (**17ea, ef**)).

Substitution at the 6-position of aromatic 2,4-diamino pteridine type **II** derivatives with either a phenyl (Table 15; **6aa**: 85 ± 24% of V_{max}) or an anisyl group (Table 15; **6af**: 109 + 19% of V_{max}) alone had no effect on enzyme inhibition. However, the combination of **II** (see above) with chemical substituents which increase either the hydrophilicity and/or the hydrophobicity associated with steric interactions due to the introduction of bulky substituents (Table 17; e.g., **17ca,cf**) in the 4-position, produced potent inhibitors of NOS-I activity (general formula **IV** in Table 17; **17aa-ff**). We propose that this 4-amino modification of **IV** increased the accessibility of the anti-pterins (**II**) into the H_4Bip binding pocket of NOS as a consequence of additional hydrophobic interactions between the aromatic substituent in the 6-position, phenyl or anisyl, e.g., with the phenyl ring of Phe[470] (Figure 1). Interestingly, this effect could be markedly potentiated by the introduction of an anisyl group in the 6-position of **IV** (Table 17; **17af-ef**), possibly due to an additional polar interaction with Ser[112] from NOS. Thus, the apparent lack of potency of the aromatic 2-amino-4-dialkylamino-6-phenyl pteridines **17aa,ba, da** (73−92% of V_{max}) versus the 6-anisyl substituted compounds **17af-ef** (inhibition down to 0% of V_{max}) is a striking feature, which is so far difficult to explain on the basis of the known experimental data. The underlying mechanism for the stronger binding of the 2-amino-4-dialkylamino-(**17af,bf**)- and 2-amino-4-morpholino-(**17df**)-6-anisyl pteridine compounds to NOS requires further investigation, ideally at the structural level. Furthermore, the 2-amino-4-NMe-piperazino-6-aryl pteridines **17fa,ff** seem to offer an additional counteractive hydrophilic 4-NMe interaction, which is probably not compensated by secondary effects analogous to the compounds **17af,bf,df**. The 4-morpholino substituent of **17da,df**, which contains a hydrophilic oxygen in the 4-position of the heteroparaffinic ring, occupies an intermediate position in the potency spectrum. In the 4-piperidinopteridine derivatives of **17ea,ef**, this additional hydrophilic interaction is lacking, indicating

that the hydrophobic structural feature at this part of the molecule is crucial for NOS-I inhibition potential.

In conclusion, the addition of either a bulky aromatic substituent or an aliphatic side chain in the 6-position of the reduced 2,4-diamino-5,6,7,8-tetrahydropteridine derivatives **I** resulted in potent NOS inhibitors, possibly due to hydrophobic interactions, e.g., with Phe^{470}, unlike the aromatic 2,4-diamino-6-substituted pteridine **II**. In contrast to **II**, the ability of the aromatic 2-amino-4-dialkyl/diaralkyl-6-substituted pteridine compounds **IV** to inhibit NOS-I activity appears to be due to increased hydrophilic and hydrophobic interactions of the 4-substituent within the binding pocket compared to the naturally occurring (6*R*)-5,6,7,8-tetrahydrobiopterin cofactor. Interestingly, anisylation at C-6 markedly potentiated the inhibitory effectiveness of **IV**. The present findings using the 4-amino pteridine derivatives **I**−**IV**, support an intimate localization of the pterin binding pocket adjacent to the heme and the L-arginine binding site in the crystal structure of NOS-II. From our structure−activity relationship study, we suggest that the upper portion of the pterin molecule (position 4, 5, and 6 of H_4Bip) is responsible for efficient insertion and binding of H_4Bip into the pterin binding pocket of NOS. In addition, the present SAR agrees with comparative molecular field analysis (CoMFA) of a data set of selected NOS-I inhibitors, which resulted in a statistically significant and predictive 3D-QSAR model of the pterin binding site interactions (unpublished observation). Thus, further chemical alterations of H_4Bip at these positions may represent an approach to novel, pterin-based NOS antagonists with increased potency. Importantly, highly species- and isoform-specific effects were observed (unpublished observation), raising the possibility to specifically target a particular isoform of NOS by using the anti-pterin approach.

Experimental Section

Chemistry. Thin-layer chromatography (TLC): precoated silica gel thin-layer sheets F 1500LS254 and cellulose thin-layer sheets F 1440LS254 from Schleicher and Schüll (Dassel, Germany). Detection by UV light λ: 254 and 366 nm. Flash chromatography (FC): silica gel (Baker, 30−60 μm), 0.3−0.4 bar. High-performance liquid chromatography (HPLC): Merck-Hitachi (Darmstadt, Germany) L-6200, D-2000 chromatointegrator. UV/VIS: Perkin-Elmer (Überlingen, Germany), Lambda 15; lMAX in nm (log ϵ); [ ] shoulder. Products were dried either at room temperature under high vacuum or in an oven at 100 °C. Mp: Gallenkamp MFB 595 (England) or Büchi 510 (Switzerland) melting point apparatus; no corrections.

Synthesis. General Procedure A: 6-Substituted 2,4-Diamino Pteridines (2). 2,4-Diamino-6-bromomethylpteridine × HBr × 2-hydroxypropane (**1**)[29] (1.00 g, 2.5 mmol) was reacted with the appropriate nucleophile by stirring at room temperature. The resulting precipitate was collected and purified by recrystallization.

2,4-Diamino-6-methoxymethylpteridine (2a).[30] Sodium (0.69 g, 3 mmol) was dissolved in absolute MeOH (50 mL) before compound **1** (1 g) was added, and the mixture stirred for 6 h. The solution was then evaporated to one-third of the volume, and the precipitate (0.394 g) was collected and recrystallized from DMF/H_2O with charcoal. Yield: 0.19 g (36%). Mp: 256−257 °C.

2,4-Diamino-6-ethoxymethylpteridine (2b). Method was analogous to the preceding procedure, using absolute EtOH (50 mL) and sodium with stirring for 1 h and recrystallization from DMF (15 mL). Yield: 0.18 g (32%). Mp: 240−241 °C.

2,4-Diamino-6-phenoxymethylpteridine (2c).[31] Phenol (0.56 g, 6 mmol) and potassium *tert*-butoxide (0.67 g, 6 mmol) were dissolved in dimethylacetamide (DMA) (30 mL), compound **1** was added, and the mixture was stirred for 5 days. The precipitate (0.31 g) was then collected and recrystallized from DMF (10 mL). Yield: 0.21 g (31%). Mp: 287−288 °C.

2,4-Diamino-6-*n*-decyloxymethylpteridine (2d). Sodium hydride (1.00 g, 80%) was added over a period of 1 h to a mixture of DMA (30 mL) and *n*-decanol (5 mL) with continuous stirring before compound **1** (1 g) was added. Stirring was continued at room temperature for 6 h and then diluted with H_2O (100 mL) and kept cool (0−4 °C) for 2 days. The precipitate was collected and recrystallized from EtOH/concentrated NH_3 (16:1). Yield: 0.40 g (50%). Mp: 231−232 °C.

6-[3-(4-Benzoylphenyl)propionyloxymethyl]-2,4-diaminopteridine (2e). 3-(4-Benzoylphenyl)propionic acid (0.50 g, 2 mmol)[32] was dissolved in DMF (10 mL) and then triethylamine (0.4 mL), and compound **1** (0.40 g, 1 mmol) was added with stirring. After 4 h it was filtered from a small precipitate, the filtrate was evaporated with silica gel (1.00 g), and then the crude product was purified on silica gel with a CH_2Cl_2/MeOH gradient (0−4% MeOH). The main fraction was evaporated, the residue treated with H_2O/MeOH (2:3) and filtered again, and the residue was dried to give a yellow powder. Yield: 0.03 g (8%). Mp: 208−210 °C (decomp.).

2,4-Diamino-6-aminomethylpteridine (2f). A solution of compound **1** (1.00 g) in DMF (20 mL) was cooled to −50 °C and then a slow stream of gaseous NH_3 bubbled through this solution for 2 h. Slow warming to room temperature was achieved by removing the cooling. Stirring was continued overnight, and the solution was then evaporated to dryness in high vacuum. The residue was treated with MeOH (20 mL) to give a yellow powder. The crude product was dissolved in hot 0.1 N AcOH (45 mL), treated with charcoal, and filtered, and the filtrate was adjusted to pH 12 by 2 N NaOH. On cooling, a yellow precipitate was obtained which, upon washing with EtOH and drying at 100 °C, gave a yellow powder. Yield: 0.29 g (60%). Mp: >350 °C.

2,4-Diamino-6-dimethylaminomethylpteridine (2g). Method was analogous to the preceding procedure, but in 2-methoxyethanol (20 mL) with dimethylamine gas. The crude product was further purified by dissolving in 1 N AcOH (20 mL), and a precipitate was obtained at pH 12 with 2 N NaOH. Yield: 0.25 g (60%). Mp: 276−277 °C.

2,4-Diamino-6-methylthiomethylpteridine (2h). Sodium methylthiolate (0.50 g), compound **1** (1.00 g), and potassium *tert*-butoxide (0.05 g) were added to DMA (10 mL), and the mixture was stirred at room temperature for 2 h. It was neutralized with 1 N HCl to pH 7, diluted with H_2O (20 mL), and chilled overnight on ice. The precipitate was collected and purified by dissolving in hot 1 N AcOH (40 mL), treated with charcoal, and precipitated by neutralization to pH 9 with ammonia, which gave upon filtration a yellow crystal powder. Yield: 0.38 g (67%). Mp: 290−295 °C (decomp.).

2,4-Diamino-6-isobutyroylaminomethylpteridine (2i). 2,4-Diamino-6-aminomethylpteridine (**2e**) (0.80 g, 4.2 mmol) was suspended in ethyl acetate (50 mL), and then isobutyric anhydride (10 mL) and 4-(dimethylamino)pyridine (0.05 g) were added. After it was heated for 2 h at 80 °C with stirring, the solution was evaporated in a vacuum. The residue was dissolved in EtOAc and purified on silica gel following elution with EtOAc. The main fraction was collected and evaporated, and the product was recrystallized from EtOAc/ether (2:1) to give 0.88 g (52%) of yellow crystals of 2,4-bisisobutyroylamino-6-isobutyroyl-aminomethylpteridine. Mp: 192−193 °C. This intermediate (0.40 g, 1 mmol) was treated in MeOH (12 mL) with concentrated NH_3 (12 mL) by heating to 60 °C for 2 days. After cooling overnight, the yellow crystals were collected and dried at 60 °C. Yield: 0.14 g (77%). Mp: >215 °C (decomp.).

General Procedure B: 2,4-Diamino-6-phenylpteridines (6). 2,4,5,6-Tetraaminopyrimidine dihydrochloride (**4a**) (1.00 g, 4.7 mmol) was suspended in MeOH (50 mL) and heated to boiling, and then a solution of substituted phenylglyoxalmonoxime **5** (7 mmol) in MeOH (10 mL) was added dropwise over a 10 min period. After heating for 2 h and cooling to room

temperature, concentrated ammonia was added to pH 9 and stirred for 15 min. The usually chromatographically pure precipitate was collected to give 80−95% of a yellow solid. Further purification can be achieved by recrystallization from DMF.

2,4-Diamino-6-phenylpteridine (6aa)[34] was obtained from **4a** and phenylglyoxalmonoxime (**5a**)[35] (1.04 g). Yield: 93%. Mp: 345 °C. Lit. mp: 347−348 °C.

2,4-Diamino-6-(4-fluorophenyl)pteridine (6ab) was obtained from **4a** and *p*-fluorophenyl-glyoxalmonoxime (**5b**) (1.17 g). Yield: 1.19 g (87%). Mp: >300 °C.

2,4-Diamino-6-(4-chlorophenyl)pteridine (6ac) was obtained from **4a** and *p*-chlorophenyl-glyoxalmonoxime (**5c**)[38] (1.29 g). Yield: 1.15 g (90%). Mp: >300 °C.

2,4-Diamino-6-(4-bromophenyl)pteridine (6ad) was obtained from **4a** and *p*-bromophenyl-glyoxalmonoxime (**5d**) (1.59 g). Yield: 1.48 g (89%). Mp: >300 °C.

2,4-Diamino-6-(4-tolyl)pteridine (6ae) was obtained from **4a** and *p*-tolylglyoxalmonoxime (**5e**)[36] (1.14 g). Yield: 1.04 g (88%). Mp: >300 °C.

2,4-Diamino-6-(4-methoxyphenyl)pteridine (6af) was obtained from **4a** and *p*-anisylglyoxalmonoxime (**5f**)[37] (1.25 g). Yield: 1.15 g (91%). Mp: >300 °C.

2,4-Diamino-6-(3,4-dimethoxyphenyl)pteridine (6ag) was obtained from **4a** and 3,4-dimethoxyphenylglyoxalmonoxime (**5g**) (1.46 g). Yield: 1.32 g (87%). Mp: >280 °C (decomp.).

2,4-Diamino-7-methyl-6-phenylpteridine (6ah) was obtained from **4a** and 3-phenylmethylglyoxal-2-monoxime (**5h**) (1.15 g). Yield: 0.86 g (66%). Mp: >235 °C (decomp.).

2,4-Diamino-6,7-diphenylpteridine (6ai). See ref 34.

4-Amino-6-phenylpteridine (7ba). 4,5,6-Triaminopyrimidine dihydrochloride (**4b**)[39] (1.00 g, 5 mmol) was suspended in MeOH (30 mL) and then heated to reflux, and a solution of phenylgloxalmonoxime (**5a**) (1.13 g, 7.6 mmol) in MeOH (10 mL) was added dropwise over a 10 min period. The mixture was heated for 2 h under reflux. After cooling it was neutralized by ammonia to pH 8 and stirred for 30 min. The yellow precipitate was collected and dried to give a chromatographically pure product. Yield: 0.99 g (88%). Mp: 265−268 °C (decomp.). The substance can be recrystallized from a large volume of MeOH.

4-Amino-6-(4-tolyl)pteridine (7be). Method was analogous to the preceding procedure, using instead **4b** (1.00 g) and **5e** (1.23 g). Yield: 1.08 g (90%). Mp: 285−287 °C (decomp.).

4-Amino-6-(4-methoxyphenyl)pteridine (7bf). Method was analogous to the preceding procedure, using instead **4b** (1.00 g) and **5f** (1.35 g). Yield: 1.26 g (99%). Mp: 270−272 °C.

4,5,6-Triamino-2-methylthiopyrimidine Dihydrochloride (4c). A suspension of 4,6-diamino-2-methylthio-5-nitrosopyrimidine (**3c**)[40] (5.00 g, 27 mmol) in MeOH (125 mL) was catalytically reduced with PtO_2/H_2 in a shaking apparatus until the theoretical amount of hydrogen was consumed. The clear solution was filtered from the catalyst and evaporated to dryness, and the residue was treated with saturated methanolic HCl (120 mL) with stirring for 3 h. The precipitate was collected and washed with MeOH and ether before being dried in a desiccator over P_4O_{10} to give a colorless crystal powder. Yield: 6.12 g (93%). Mp: 252−257 °C (decomp.).

4-Amino-2-methylthio-6-phenylpteridine (7ca). Method was analogous to the procedure for **7ba**, using instead **4c** (1.00 g, 4.1 mmol) and **5a** (0.95 g, 6.15 mmol). Yield: 1.07 g (97%). Mp: 293−295 °C (decomp.). Recrystallization from DMF/H_2O (10:1) gave yellow needles.

4-Amino-2-methylthio-6-(4-tolyl)pteridine (7ce). Method was analogous to the procedure for **7ba**, using instead **4c** (1.00 g, 4.1 mmol) and **5e** (1.23 g). Yield: 1.12 g (97%). Mp: 292−293 °C (decomp.). Recrystallization was done from DMF/H_2O (10:1).

4-Amino-2-methylthio-6-(4-methoxyphenyl)pteridine (7cf). Method was analogous the procedure for **7ba**, using instead **4c** (1.00 g, 4.1 mmol) and **5f** (1.35 g). Yield: 1.18 g (96%). Mp: 291−293 °C.

4,5,6-Triamino-2-phenylpyrimidine Dihydrochloride (4d). Analogous to the procedure for **4c**, a suspension of 4,6-diamino-5-nitroso-2-phenylpyrimidine **3d**[40] (3.10 g, 14 mmol) was catalytically reduced and gave on workup a colorless powder. Yield: 3.40 g (88%). Mp: > 220 °C (decomp.).

4-Amino-2,6-diphenylpteridine (7da). Method was analogous to the procedure for **4c**, using instead **4d** (1.00 g, 3.65 mmol) and **5a** (0.82 g, 5.5 mmol). Yield: 0.96 g (88%). Recrystallization from DMF/H_2O gave yellow needles. Mp: 287−290 °C.

4-Amino-6-(4-methoxyphenyl)-2-phenylpteridine (7df). Method was analogous to the procedure for **4c**, using instead **4d** (1.00 g, 3.65 mmol) and **5f** (0.98 g, 5.5 mmol). Yield: 1.07 g (89%). Recrystallization from DMF/H_2O gave yellow needles. Mp: 292−295 °C.

4,6-Diamino-2(2-hydroxyethyl)amino-5-nitrosopyrimidine (3e). A mixture of 4,6-diamino-2-methylthio-5-nitrosopyrimidine **3d**[40] (2.00 g, 10.8 mmol) and ethanolamine (10 mL) in H_2O (50 mL) was heated with stirring for 3 h to 60 °C. After cooling, it was evaporated to half of the volume, and the precipitate was collected, washed, and dried in a desiccator. Recrystallization from EtOH/H_2O (2:1) gave violet crystals. Yield: 1.18 g (55%). Mp: 235−238 °C. UV (H_2O): [231 (3.50)]; [274 (3.59)]; 333 (4.35). Anal. Calcd for $C_6H_{10}N_6O_2$ (198.2): C, 36.36; H, 5.09; N, 42.41. Found: C, 36.43; H, 5.14; N, 42.34.

4,5,6-Triamino-2-(2-hydroxyethyl)aminopyrimidine Trihydrochloride (4e). Analogous to the procedure for **4c**, a suspension of 4,6-diamino-2-(2-hydroxyethylamino)-5-nitrosopyrimidine **3e** (5.00 g, 25.3 mmol) was catalytically reduced with Pd/C (0.20 g) under H_2 atm. Yield: 1.36 g (92%). Mp: >205 °C (decomp.).

4-Amino-2-(2-hydroxyethylamino)-6-phenylpteridine (7ea). Method was analogous to the procedure for **7ba**, using instead **4e** (1.00 g, 3.4 mmol) and **5a** (0.76 g, 5.1 mmol). Yield: 0.87 g (91%). Mp: 232−239 °C.

4-Amino-2-(2-hydroxyethylamino)-6-(4-tolyl)-pteridine (7ee). Method was analogous to the procedure for **7ba**, using instead **4e** (1.00 g, 3.4 mmol) and **5e** (0.83 g, 5.1 mmol). Yield: 0.56 g (56%). Mp: 259−263 °C (decomp.).

4-Amino-2-(2-hydroxyethylamino)-6-(4-methoxyphenyl)-pteridine (7ef). Method was analogous to the procedure for **7ba**, using instead **4e** (1.00 g, 3.4 mmol) and **5f** (0.92 g, 5.1 mmol). Yield: 0.93 g (87%). Mp: 270−275 °C.

4-Aminopteridine-6-carboxaldoxime (9b). A suspension of 4,5,6-triaminopyrimidine dihydrochloride (**4b**) (0.80 g, 4 mmol) in MeOH (20 mL) was heated under reflux. A solution of mesoxaldicarboxaldoxime (**8**) (0.70 g, 6 mmol) in MeOH (10 mL) was added slowly dropwise, and then the reaction mixture was heated for 2 h. After cooling, it was neutralized with concentrated ammonia to pH 7 and stirred for 30 min, and then the precipitate was collected, washed with MeOH, and dried at 100 °C. Yield: 0.74 g (96%). Mp: > 250 °C (decomp.).

4-Amino-2-methylthiopteridine-6-carboxaldoxime (9c).[43] Method was analogous to the preceding procedure, using instead 4,5,6-triamino-2-methylthiopyrimidine dihydrochloride (**4c**) (1.00 g, 4.1 mmol) and **8** (0.70 g, 6 mmol). Yield: 0.96 g (99%). Mp: >250 °C (decomp.). Lit. mp: 250 °C.

4-Amino-2-phenylpteridine-6-carboxaldoxime (9d). Method was analogous to the preceding procedure, using instead 4,5,6-triamino-2-phenylpyrimidine dihydrochloride (**4d**) (1.00 g, 3.7 mmol) and **8** (0.65 g, 5.5 mmol). Yield: 0.81 g (83%). Mp: > 249 °C (decomp.).

2,6-Diamino-4-chloro-5-*p*-chlorophenylazopyrimidine (12).[44] A solution of *p*-chloroaniline **10** (25.5 g, 0.2 mol) in 6 N HCl (100 mL) was cooled to 0−5 °C, and then $NaNO_2$ (13.8 g, 0.2 mol) in water (40 mL) was added dropwise with stirring. After the addition was completed, the solution was stirred for another 15 min and checked by iodine-starch-paper to give a blue color. Urea (5.00 g) was added to destroy the excess of HNO_2. The diazonium salt solution was then poured into a solution of 2,6-diamino-4-chloropyrimidine **11** (26.0 g, 0.18 mol) in water (500 mL) and stirred for 30 min. Potassium acetate (70.0 g) was then added, and the mixture was stirred for 16 h at room temperature. The resulting precipitate was

collected by suction, washed with H_2O and dried in a vacuum desiccator over P_4O_{10} to give a yellow solid. Yield: 44.0 g (81%). Recrystallization was done from DMF/H_2O. Mp: 268 °C. Lit. mp: 270 °C. Anal. Calcd for $C_{10}H_8Cl_2N_6 \times H_2O$ (301.1): C, 39.89; H, 3.35; N, 27.91. Found: C, 40.14; H, 2.95; N, 27.64.

2,6-Diamino-4-diethylamino-5-*p*-chlorophenylazopyrimidine (14b). A solution of 2,6-diamino-4-chloro-5-*p*-chlorophenylazopyrimidine (**12**) (5.00 g, 16.6 mmol) in DMF (50 mL) and diethylamine **13b** (10.0 g) was heated in an oil bath to 70 °C for 5 h. Then water (50 mL) was added, the solution cooled, and the yellow precipitate was collected, washed with water, and dried. Recrystallization was done from EtOH. Yield: 3.04 g (56%). Mp: 145−148 °C. UV (60% H_2SO_4): 213 (4.28); 266 (4.22); [334 (3.93)]; 389 (4.38); [420 (4.29)]. Anal. Calcd for $C_{14}H_{18}ClN_7 \times 0.5\ H_2O$ (328.8): C, 51.14; H, 5.82; N, 29.82. Found: C, 51.36; H, 5.54; N, 29.29.

2,6-Diamino-4-dibenzylamino-5-*p*-chlorophenylazopyrimidine (14c). Method was analogous to the preceding procedure, using instead **12** (5.00 g) and dibenzylamine **13c** (10.0 g). Recrystallization from acetone/H_2O gave yellow crystals. Yield: 5.22 g (67%). Mp: 185−186 °C. Anal. Calcd for $C_{24}H_{26}ClN_7 \times H_2O$ (465.9): C, 61.88; H, 6.06; N, 21.04. Found: C, 61.53; H, 5.83; N, 21.88.

2,6-Diamino-4-morpholino-5-*p*-chlorophenylazopyrimidine (14d). Method was analogous to the preceding procedure, using instead **12** (5.00 g) and morpholine **13d** (10.0 g). Recrystallization was done from EtOH. Yield: 5.00 g (90%). Mp: 219−221 °C. UV (60% H_2SO_4): 213 (4.30); 271 (4.17); [333 (3.86)]; 389 (4.35); [414 (4.24)]. Anal. Calcd for $C_{14}H_{16}ClN_7O$ (333.8): C, 50.38; H, 4.83; N, 29.37. Found: C, 50.55; H, 4.83; N, 29.37.

2,6-Diamino-4-piperidino-5-*p*-chlorophenylazopyrimidine (14e). Method was analogous to the preceding procedure, using instead **12** (5.00 g) and piperidine **13e** (10.0 g). Recrystallization from EtOH gave yellow crystals. Yield: 4.85 g (88%). Mp: 199−201 °C. UV (60% H_2SO_4): 213 (4.20); 271 (4.15); [339 (3.86)]; 396 (4.29); [421 (4.22)]. Anal. Calcd for $C_{15}H_{18}ClN_7$ (331.8): C, 54.30; H, 5.47; N, 26.08. Found: C, 54.10; H, 5.27; N, 28.81.

2,6-Diamino-4-*N*-methylpiperazino-5-*p*-chlorophenylazopyrimidine (14f). Method was analogous to the preceding procedure, using instead **12** (5.00 g) and *N*-methylpiperazine **13f** (10.0 g). Yield: 5.50 g (91%). Mp: 218−220 °C. UV (60% H_2SO_4): 212 (4.38); 270 (4.24); [331 (3.85)]; 389 (4.38); [416 (4.28)]. Anal. Calcd for $C_{15}H_{19}ClN_8 \times H_2O$ (364.8): C, 49.38; H, 5.80; N, 30.71. Found: C, 49.02; H, 5.64; N, 30.42.

2,5,6-Triamino-4-dimethylaminopyrimidine Dihydrochloride (16a). Method was analogous to the procedure for **4c**, using instead 2,6-diamino-4-(dimethylamino)-5-nitrosopyrimidine **15a**[41] (5.00 g, 27.5 mmol) with PtO_2 (0.50 g) as catalyst under H_2 atm. Yield: 2.68 g (56%). Mp: 210−212 °C (decomp.). UV (H_2O): [231 (4.12)]; 300 (4.08). Anal. Calcd for $C_6H_{14}Cl_2N_6$ (241.0): C, 29.89; H, 5.85; N, 34.85. Found: C, 29.73; H, 5.77; N, 34.81.

2,5,6-Triamino-4-diethylaminopyrimidine Dihydrochloride (16b). A suspension of 2,6-diamino-4-diethylamino-5-*p*-chlorophenylazopyrimidine (**14b**) (3.28 g, 10 mmol) in MeOH (70 mL) and concentrated ammonia (10 mL) was reduced in a shaking apparatus under H_2 atm in the presence of Raney nickel catalyst (3.50 g) for 2 days. The catalyst was filtered off under argon atmosphere, and then the filtrate was evaporated in a vacuum to dryness. The residue was treated with ether to remove the *p*-chloroaniline and filtered, and then the solid was stirred in methanolic HCl (10%, 50 mL) overnight. The dihydrochloride salt was collected and dried in a vacuum desiccator over KOH. Yield: 2.63 g (89%). Mp: 138−142 °C. UV (MeOH): 213 (4.19); 233 (4.22); 294 (4.10); [323 (3.54)]. Anal. Calcd for $C_8H_{16}N_6 \times 2\ HCl \times 1.5\ H_2O$ (296.2): C, 32.44; H, 6.46; N, 28.37. Found: C, 32.37; H, 6.37; N, 29.35.

2,5,6-Triamino-4-dibenzylaminopyrimidine Dihydrochloride (16c). Method was analogous to the preceding procedure, using instead **14c** (4.65 g, 10 mmol). Yield: 3.82 g (93%). Mp: 165−167 °C. UV (MeOH): 206 (4.41); 233 (4.26); 295 (4.10); [332 (3.62)]. Anal. Calcd for $C_{18}H_{20}N_6 \times 2\ HCl \times H_2O$ (411.3): C, 52.56; H, 5.88; N, 20.43. Found: C, 52.54; H, 5.94; N, 20.49.

2,5,6-Triamino-4-morpholinopyrimidine Dihydrochloride (16d). Method was analogous to the preceding procedure, using instead **14d** (3.34 g, 10 mmol). Yield: 1.81 g (64%). Mp: 215−218 °C (decomp.). UV (MeOH): 208 (4.04); 237 (4.26); 301 (4.09); [332 (3.89)]. Anal. Calcd for $C_8H_{14}N_6 \times 2$ HCl (283.2): C, 33.93; H, 5.70; N, 29.68. Found: C, 34.04; H, 5.42; N, 29.47.

2,5,6-Triamino-4-piperidinopyrimidine Dihydrochloride (16e). Method was analogous to the preceding procedure, using instead **14e** (3.32 g, 100 mmol). Yield: 2.11 g (75%). Mp: 238−242 °C. UV (MeOH): 219 (4.03); 234 (4.01); 300 (3.77); [328 (3.61)]. Anal. Calcd for $C_9H_{16}N_6 \times 2$ HCl (281.2): C, 38.44; H, 6.45; N, 29.89. Found: C, 38.11; H, 5.98; N, 29.53.

2,5,6-Triamino-4-*N*-methylpiperazinopyrimidine trihydrochloride (16f). Method was analogous to the preceding procedure, using instead **14f** (3.65 g, 100 mmol). Yield: 3.07 g (88%). Mp: 226−230 °C. (decomp.). UV (MeOH): [205 (3.88)]; 236 (4.27); 300 (3.92); [336 (3.75)]. Anal. Calcd for $C_9H_{17}N_7 \times 3\ HCl \times H_2O$ (350.7): C, 30.83; H, 6.32; N, 27.96. Found: C, 30.99; H, 6.29; N, 27.29.

General Procedure C: 2-Amino-4-dialkylamino-6-aryl Pteridines 17. A solution of arylglyoxalmonoxime **5** (7.5 mmol) in MeOH (10 mL) was added dropwise to a boiling solution of 2,5,6-triamino-4-dialkylaminopyrimidine dihydrochloride salt **16** (5 mmol) in MeOH (20 mL), and then the mixture was heated under reflux for 3 h. After cooling, the suspension or solution was made alkaline by concentrated ammonia to pH 9−10, and the resulting precipitate was filtered off, washed with water, and dried. Recrystallization was done from EtOH and DMF/H_2O, respectively, to give a yellow solid.

2-Amino-4-dimethylamino-6-phenylpteridine (17aa). Method was analogous to the general procedure, using instead **16a** (0.80 g, 3.3 mmol) and **5a** (0.74 g, 5 mmol). Yield: 0.76 g (86%). Mp: 247−250 °C.

2-Amino-4-dimethylamino-6-(4-methoxyphenyl)-pteridine (17af). Method was analogous to the general procedure, using instead **16a** (0.80 g) and **5f** (0.89 g, 5 mmol). Yield: 0.80 g (81%). Mp: 280−284 °C (decomp.).

2-Amino-4-diethylamino-6-phenylpteridine (17ba). According to the general procedure, **17ba** was obtained from **16b** (1.48 g) and phenylglyoxalmonoxime (**5a**) (1.12 g).Yield: 0.87 g (56%). Mp: 203−205 °C.

2-Amino-4-diethylamino-6-(4-methoxyphenyl)pteridine (17bf). According to the general procedure, **17bf** was obtained from **16b** (1.48 g) and *p*-methoxyphenylglyoxalmonoxime (**5f**) (1.34 g). Yield: 0.79 g (46%). Mp: 220−222 °C.

2-Amino-4-dibenzylamino-6-phenylpteridine (17ca). According to the general procedure, **17ca** was obtained from **16c** (2.06 g) and phenylglyoxalmonoxime (**5a**) (1.12 g). Yield: 1.57 g (69%). Mp: 225−227 °C.

2-Amino-4-dibenzylamino-6-(4-methoxyphenyl)pteridine (17cf). According to the general procedure, **17cf** was obtained from **16c** (2.06 g) and *p*-methoxyphenylglyoxalmonoxime (**5f**) (1.34 g). Yield: 1.57 g (70%). Mp: 245−247 °C.

2-Amino-4-morpholino-6-phenylpteridine (17da). According to the general procedure, **17da** was obtained from **16d** (1.42 g) and phenylglyoxalmonoxime (**5a**) (1.12 g). Yield: 1.37 g (84%). Mp: 224−227 °C.

2-Amino-4-morpholino-6-(4-methoxyphenyl)pteridine (17df). According to the general procedure, **17df** was obtained from **16d** (1.42 g) and *p*-methoxyphenylglyoxalmonoxime (**5f**) (1.34 g). Yield: 1.53 g (78%). Mp: 238−240 °C.

2-Amino-4-piperidino-6-phenylpteridine (17ea). According to the general procedure, **17ea** was obtained from **16e** (1.41 g) and phenylglyoxalmonoxime (**5a**) (1.12 g). Yield: 1.37 g (80%). Mp: 209−211 °C.

2-Amino-4-piperidino-6-(4-methoxyphenyl)pteridine (17ef). According to the general procedure, **17ef** was obtained from **16e** (1.41 g) and *p*-methoxyphenylglyoxalmonoxime (**5f**) (1.34 g). Yield: 1.40 g (81%). Mp: 211−214 °C.

2-Amino-4-*N*-methylpiperazino-6-phenylpteridine(17fa). According to the general procedure, **17fa** was obtained from

16f (1.75 g) and phenylglyoxalmonoxime (**5a**) (1.12 g). Yield: 0.96 g (58%). Mp: 245−247 °C.

2-Amino-4-*N*-methylpiperazino-6-(4-methoxyphenyl)-pteridine (17ff). According to the general procedure, **17ff** was obtained from **16f** (1.75 g) and *p*-methoxyphenylglyoxalmonoxime (**5f**) (1.34 g). Yield: 1.55 g (83%). Mp: 228−230 °C.

4-Amino-6,7-dimethyl-2-methylaminopteridine (20a). A solution of 4,5,6-triamino-2-methylaminopyrimidine dihydrochloride (**18**) (2.25 g, 0.01 mol) in water (50 mL) was treated with diacetyl **19a** (1.50 g) in dioxane (10 mL) and then heated under reflux for 1 h. The warm solution was neutralized with concentrated ammonia to pH 9, and the precipitate was collected after cooling. Yield: 1.75 g (86%). Mp: 268 °C (decomp.).

4-Amino-2-methylamino-6,7-diphenylpteridine (20b). Analogous to the preceding procedure, **20b** was obtained from **18** (2.25 g, 0.01 mol) and benzil **19b** (2.50 g, 0.01 mol) in EtOH (50 mL). Yield: 2.88 g (88%). Mp: 303 °C.

4-Amino-2-methylaminopteridine (20c). See ref 42.

2,4-Diaminopteridine (20d). See ref 33.

2,4-Diamino-6-(1,2-dihydroxypropyl)pteridine (20g). See ref 45.

2,4-Diamino-7-phenylpteridine (20h). See ref 34.

4-Amino-2-methylamino-6,7-dimethyl-5,6,7,8-tetrahydropteridine Dihydrochloride (21a). A solution of **20a** (0.61 g, 3 mmol) in TFA (25 mL) was reduced catalytically with PtO_2 (0.10 g)/H_2 in a shaking apparatus until the uptake of hydrogen ceased. The catalyst was filtered off, the filtrate was evaporated to dryness, and the residue was treated with methanolic HCl (10%, 20 mL) by stirring for several hours. The colorless crystal powder was collected, washed with ether, and dried in a vacuum desiccator. Yield: 0.59 g (68%). Mp: 265 °C.

4-Amino-2-methylamino-6,7-diphenyl-5,6,7,8-tetrahydropteridine Dihydrochloride (21b). Analogous to the preceding procedure, **21b** was obtained from **20b** (0.98 g, 3 mmol). Yield: 0.88 g (69%). Mp: 245 °C.

4-Amino-2-methylamino-5,6,7,8-tetrahydropteridine Dihydrochloride (21c). Analogous to the preceding procedure, **21c** was obtained from **20c** (0.54 g, 3 mmol). Yield: 0.50 g (61%). Mp: 275 °C.

2,4-Diamino-5,6,7,8-tetrahydropteridine Dihydrochloride (21d).[33] A suspension of 2,4-diaminopteridine **20d**[33] (3.00 g, 18.5 mmol) in MeOH (700 mL) was reduced catalytically with PtO_2 (0.90 g)/H_2 in a shaking apparatus for 3 days. The suspension was filtered under argon atmosphere, concentrated HCl (10 mL) was added to the filtrate, and the mixture was concentrated to 100 mL whereby a precipitate was formed, then diluted with ether (100 mL), and cooled for 3 h. Finally, the solid was collected, washed with ether, and dried in a vacuum desiccator. Yield: 3.93 g (89%). Mp: 253 °C (decomp.).

2,4-Diamino-6-ethoxymethyl-5,6,7,8-tetrahydropteridine Trihydrochloride (21e). A solution of 2,4-diamino-6-ethoxymethylpteridine (**2b**) (0.91 g, 4.1 mmol) in trifluoroacetic acid (100 mL) was reduced catalytically with PtO_2 (0.10 g)/H_2 in a shaking apparatus for 16 h. The catalyst was filtered off, the filtrate evaporated, and the residue was treated with methanolic HCl (20 mL). The precipitate was collected, recrystallized from MeOH/concentrated HCl with charcoal to give a colorless powder. Yield: 0.47 g (38%). Mp: > 130 °C (decomp.).

2,4-Diamino-6-phenyl-5,6,7,8-tetrahydropteridine Dihydrochloride (21f). A solution of 2,4-diamino-6-phenylpteridine (**6aa**)[34] (4.28 g, 0.02 mol) in TFA (50 mL) was reduced catalytically with PtO_2 (0.20 g)/H_2 in a shaking apparatus until the uptake of hydrogen ceased. The catalyst was filtered off, the filtrate evaporated to dryness, and then the residue was treated with methanolic HCl (10%, 50 mL) by stirring for 3 h. The resulting precipitate was collected, dissolved in MeOH, and then diluted with the same volume of ether. A colorless precipitate was formed. After cooling, the solid was collected and dried in a vacuum desiccator. Yield: 3.85 g (58%). Mp: 248 °C.

2,4-Diamino-6-(1,2-dihydroxypropyl)-5,6,7,8-tetrahydropteridine Dihydrochloride (21g). A suspension of 2,4-diamino-6-(1,2-dihydroxypropyl)pteridine (**20g**)[45] (0.40 g, 1.7 mmol) in MeOH (50 mL) was reduced catalytically with PtO_2 (0.05 g)/H_2 in a shaking apparatus until 2 mol of H_2 were consumed (2 days). Methanolic HCl (20%, 5 mL) was added, then the catalyst was filtered off and evaporated to dryness, and the residue was treated again with methanolic HCl (5 mL) for 3 h with stirring and cooling. The colorless precipitate was collected, washed with ether, and dried in a vacuum desiccator over KOH. Yield: 0.26 g (47%). Mp: >300 °C.

2,4-Diamino-7-phenyl-5,6,7,8-tetrahydropteridine Dihydrochloride (21h). According to the preceding procedure, **21h** was obtained from 2,4-diamino-7-phenylpteridine (**20h**)[34] (4.28 g, 0.02 mol). Yield: 5.00 g (75%). Mp: 240−242 °C.

2,4-Diamino-5-formyl-5,6,7,8-tetrahydropteridine (22da). See ref 33.

2,4-Diamino-5-benzoyl-5,6,7,8-tetrahydropteridine (22db). See ref 33.

2,4-Diamino-7-benzylpteridine (24). A solution of 2,4,5,6-tetraaminopyrimidine dihydrochloride (**4a**) (5.00 g, 23 mmol) in H_2O (250 mL) was adjusted with concentrated ammonia to pH 9. Benzylglyoxal (**23**)[46] (3.85 g, 25 mmol) in EtOH (100 mL) was added, and then the mixture was heated under reflux for 1 h. The precipitate was collected after cooling, washed with H_2O and acetone, and then dried at 100 °C to give a yellow powder. Yield: 3.65 g (60%). Mp: 260−264 °C (decomp.). UV (pH 8): 217 (4.15); 255 (4.32); [279 (3.72)]; 366 (3.97). Anal. Calcd for $C_{13}H_{12}N_6$ (252.3): C, 61.89; H, 4.79; N, 33.31. Found: C, 61.87; H, 4.98; N, 32.95.

2,4-Diamino-7-benzoylpteridine (25). 2,4-Diamino-7-benzylpteridine (**24**) (4.03 g, 16 mmol) in AcOH (150 mL) was heated to 70 °C in an oil bath, and then bromine (10.2 g, 64 mmol) was added dropwise with stirring. The mixture was heated under reflux for 1 h whereby a precipitate began to separate out after 15 min. After cooling, the solid was collected and dried (3.70 g). The filtrate was concentrated to 50 mL and allowed to stand overnight before a second crop was collected (1.70 g). The combined crude reaction product was then heated in a mixture of H_2O (80 mL) and dioxane (120 mL) for 1 h. The solution was cooled to 50 °C and then neutralized by NaOH (5%) to pH 9 to give a yellow precipitate (2.53 g). Recrystallization was done from dioxane/H_2O. Yield: 2.28 g (54%). Mp: 288−290 °C. UV (pH 7): 226 (4.33); 249 (4.31); [272 (4.22)]; 394 (3.89). Anal. Calcd for $C_{13}H_{10}N_6O$ (266.3): C, 58.64; H, 3.79; N, 31.56. Found: C, 58.29; H, 3.82; N, 31.72.

2,4-Diamino-7-aminomercaptopteridine (27). A solution of 2,4-diamino-7-mercaptopteridine (**26**)[47] (0.98 g, 5 mmol) in 1 N NaOH (25 mL) was cooled to 0 °C, and then a cold solution of chloramine (prepared from concentrated ammonia (18 mL) and NaOCl (9 mL, 13%)) was added slowly with stirring. After 15 min, the resulting precipitate was collected, washed with H_2O and EtOH, and dried to give a yellowish crystal powder. Yield: 0.52 g (55%). Mp: >300 °C (decomp.). UV (pH 1): 205 (4.26); 224 (4.16); 250 (3.94); [356 (4.09)]; 369 (4.10). Anal. Calcd for $C_6H_7N_7S \times H_2O$ (227.2): C, 31.72; H, 3.99; N, 43.16. Found: C, 31.99; H, 3.53; N, 43.06.

Biology. [2,3,4,5-^{3}H]L-Arginine hydrochloride (2.85 Tbq mmol^{-1}) was purchased from Amersham (Freiburg, Germany); H_4Bip, from Dr. B. Schircks Laboratories (Jona, Switzerland); β-nicotinamide adenine dinucleotide phosphate tetrasodium-salt tetrahydrate (NADPH), from Applichem GmbH (Darmstadt, Germany); flavin adenine dinucleotide (FAD), flavin mononucleotide (FMN), and glutathione (GSH), from Boehringer-Mannheim (Mannheim, Germany); L-arginine hydrochloride, from Fluka (Buchs, Germany); 2′,5′-adenosine diphosphate (ADP)-sepharose and calmodulin (CaM)-sepharose 4 B, from Pharmacia (Freiburg, Germany). All other chemicals were of the highest purity grade available and obtained from Sigma Chemicals (Deisenhofen, Germany). Water was deionized to 18 MΩ cm (Milli-Q; Millipore, Eschborn, Germany), deoxygenated by gassing with argon.

Enzyme Purification. Native NOS-I (nNOS) was isolated from porcine brain cerebellum by ammonium sulfate precipita-

tion and 2′,5′-ADP-sepharose affinity chromatography and eluted with excess 2′-adenosine monophosphate (AMP) as previously described.[49] To exclude the possibility that the purification procedure per se influenced the inhibitory profile of the anti-pterins, native NOS-I was additionally purified by diethyl aminoethyl (DEAE) ion exchange chromatography (200 mM, NaCl elution buffer; 20−25 mL min-1, flow rate) instead of ammonium sulfate precipitation and subsequent 2′,5′-ADP-sepharose affinity chromatography. Finally, the enzyme was further purified in the presence of Ca^{2+} using CaM-sepharose 4 B and eluted by chelating Ca^{2+} with excess EGTA.[7] The yield of this purification method was 0.50 mg of enzyme from 1.00 kg of tissue with greater than 90% purity and a specific activity of up to 670 nmol L-citrulline mg^{-1} min^{-1}. Protein concentrations were determined spectrophotometrically according to a modified Bradford method[50] using bovine serum albumin as a standard and a Microplate reader (Molecular Devices; Sunnyvale, CA). The purity was established from densitometric scanning of Coomassie-stained SDS−PAGE gels using NIH Image software (National Institutes of Health; Bethesda, MD). NOS-I immunoreactive bands were examined by Western blot analysis using an ECL enhanced chemiluminescense kit (Amersham; Braunschweig, Germany) and a NOS-I specific antibody (Transduction Laboratories; Hamburg, Germany).

Enzyme Activity. NOS-I enzyme activity was determined from the calcium/calmodulin-dependent conversion of [^{3}H]L-arginine to [^{3}H]L-citrulline.[3,49] NOS-I was incubated for 15 min at pH = 7.2 and 37 °C in a mixture containing 2 μM H_4Bip, 50 nM CaM, 1 mM $CaCl_2$, 5 μM FAD, 10 μM FMN, 250 μM 3-[(3-cholamidopropyl)-dimethylammonio]-2-hydroxy-1-propanesulfonat (Chapso), 50 mM triethanolamine (TEA), 1 mM NADPH, 7 mM GSH, and 50 μM L-arginine + [2,3,4,5-^{3}H]L-arginine, and the reaction was stopped by adding ice-cold acetate buffer (pH = 5.5). [^{3}H]L-Citrulline was separated from the mixture by cation exchange chromatography, and the amount of radioactivity was determined by liquid scintillation counting. The effect of the anti-pterins, at an initial inhibitor concentration of 100 μM, on NOS-I stimulated total activity in the presence of 2 μM H_4Bip was examined. Dose−response curves of the most effective inhibitors (>50% inhibition of stimulated NOS activity) in the range from 0 to 1000 μM were constructed, and the corresponding IC_{50} values were determined by nonlinear regression analysis (UltraFit software Biosoft, Cambridge, U.K., or GraphPad software, Prism, Version 2.0a, San Diego, CA).

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