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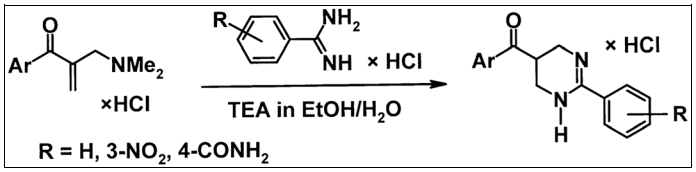

Aryl methyl ketones can be easily converted to 1-aryl-2-dimethylaminomethylpropenones that are known as interesting lead structures for drug development. By reaction of these enone Mannich bases with benzamidines, a series of new 2-aryl-5-aroyl-3,4,5,6-tetrahydopyrimidines were synthesized. These structures were characterized according to their lipophilicity. Thirty five tetrahydropyrimidines were evaluated as nitric oxide synthase (NOS) inhibitors in usual screening assays. Some interesting members of this class of compounds were forwarded to more detailed tests determining mechanism of inhibition and inhibition of NADH consumption. The investigated structures showed modest activity of NOS inhibition. However, some new tetrahydropyrimidines bearing extended aromatic substituents such as the naphthyloyl or biphenyloyl residue displayed some activity of neuronal NOS and endothelial NOS inhibition but without selectivity for an isoform and should be of interest for further modifications.

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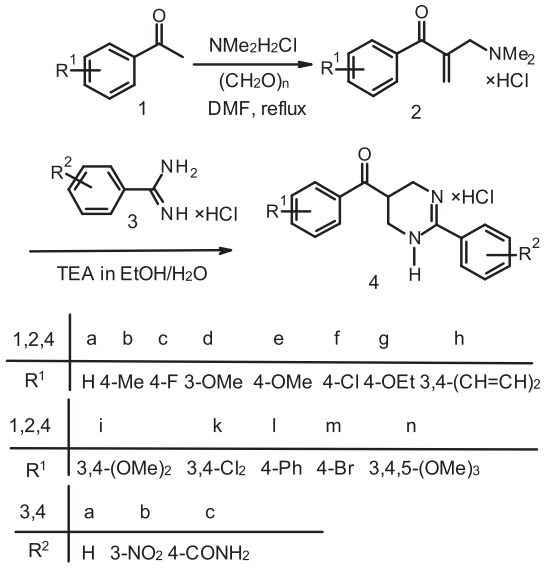
INTRODUCTION

1-Aryl-2-dimethylaminomethylprop-2-en-1-ones. 1-Aryl-2-dimethylaminomethylprop-2-en-1-ones **2** are formed in the reaction of activated aromatic methylketones **1** with fomaldehyde and amines or preformed iminium salts. First evidence for a double Mannich reaction of an activated methylene group can already be found in the work of Mannich and Baumroth as early as 1924 [1]; they found as follow-up product in the Mannich reaction of pyruvic acid with formaldehyde and dimethylammonium chloride the 4-dimethylaminomethyl-substituted 3-hydroxy-2,5-dihydrofuran-2-one. In this reaction, a dimethylaminomethyl-prop-2-en-1-one can be anticipated at least as a formal intermediate that reacts further with intramolecular cyclization. The first report of a stable and isolable 1-aryl-2-dimethylaminomethylpropenone was given by Hunziker *et al*. in 1963, presenting NMR data in D_2O [2]. In the literature, the isolation of 1-aryl-2-dimethylaminomethylprop-2-enone, also named acrylophenone, has been reported in 1970 by Greenhill and Mehta [3] as products in the Mannich reaction of an acetophenone derivative in high boiling solvents; and at the same time, some more reactions were reported by Back [4,5]. We were also interested in this class of compounds and coined the name enone Mannich bases (EMBs) for these propenones **2** [6]. These EMBs **2** (Scheme 1)—with different secondary amine groups—drew the attention of pharmacologists, for example, as analgetics [7]; and a leading series of investigations of Dimmock and coworkers [8–14] on the toxicological properties and the selective toxicity towards malignant cancer cells has to be mentioned. Recently, Zampieri *et al*. investigated EMBs **2** and bis-Mannich bases according to their antifungal and antimycobacterial activity [15], followed by another (re-)investigation of antifungal activity by Mete *et al*. [16]. The group of Lesieur reported on anti-microtubular activity and inhibition of human platelet aggregation and some antifungal potential [17–20]. The antifungal inhibition power of the free bases of **2**, with variations in the amino part using cyclic aliphatic and aromatic amines, was also investigated by Ogata *et al*. [21], and Loiseau and Depreux investigated the interaction with microtubules [22]. A major drawback of the suitability of **2** as a drug is its high reactivity towards nucleophiles [26], which has to be considered in any pharmacological study [7,12].

EMBs **2** (Table 1) were tested as epidermal growth factor receptor inhibitors by Traxler *et al*. [23] and are intermediates in the formation of pyrido[2,3-*d*]pyrimidines [24,25], which showed a promising potential for NO synthase inhibition. EMBs **2** are also versatile precursors to a large number of interesting heterocycles [26–28]. We could demonstrate the access to tetrahydropyrimidines (THPs) **4** starting from EMBs **2**, as shown in Scheme 1 [29].

Scheme 1



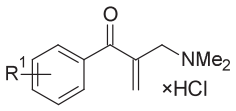
NOS inhibitors. Nitric oxide, NO, acts as a regulator of the vascular tone, a neurotransmitter and a cytotoxic agent. NO also plays important roles in the pathogenesis of several diseases [30,31]. In particular, NO contributes to inflammatory and autoimmune diseases such as rheumatoid arthritis, osteoarthritis, inflammatory bowel disease, and multiple sclerosis [32–36]. Therefore, nitric oxide synthases (NOS), the enzymes that produce NO by oxidation of L-arginine, are potential targets for new therapeutic agents. In mammals, three isoforms of NOS have been identified. The constitutive neuronal NOS (nNOS) and endothelial NOS (eNOS) are Ca^{++}-dependent enzymes that play key roles in the nervous and cardiovascular systems, whereas the inducible nitric oxide synthase (iNOS) produces large quantities of NO following immunological challenge [37–39]. NOSs are homodimeric enzymes, and each NOS subunit contains an NH_2-terminal oxygenase domain that bears binding sites for the substrate L-arginine, the heme prosthetic group and the cofactor (6*R*)-5,6,7,8-tetrahydro-L-biopterin (H_4B), and a CO_2H-terminal reductase domain that contains binding sites for the flavins, flavin mononucleotide, flavin adenine dinucleotide, and the cofactor NADPH. These two domains are fused by a Ca^{++}-dependent calmodulin (CaM)-binding sequence. CaM-binding to NOS activates both intradomain as well as interdomain electron transfers and is required for maximal NO-forming activity [37–39].

There is much evidence that NOS inhibitors could be useful therapeutical agents in the treatment of diseases such as diabetes, congestive heart failure, atherosclerosis, migraine, asthma, cerebral ischemia and Parkinson's disease [30,31,40–43]. However, they must be selective towards nNOS or iNOS to avoid interferences with the vital functions of eNOS involved in the control of vascular tone. Recent studies in the synthesis of new NOS inhibitors have thus focused on the development of either nNOS or iNOS selective inhibitors [44–47].

Several structurally diverse classes of NOS inhibitors have been investigated. Analogs of the natural substrate L-arginine, dipeptides, and peptidomimetics derived from

Table 1

Synthesis and properties of the enone Mannich bases **2a–n** used in this work.



2

2	R^1	Yield, %	Mp, °C	logD(7.4)	References
a	H	36 [7][a]	158 [7]	1.25	[7,18]
b	4-Me	38 [7][a]	160 [7]	1.52	[7]
c	4-F	36	169	1.00	[15], this work
d	3-OMe	20	138	1.22	[25], this work
e	4-OMe	44 [7][a]	153 [7]	1.25	[7,8]
f	4-Cl	40	154	1.70	[18]
g	4-OEt	16	162	1.85	[25], this work
h	3,4-(CH=CH)$_2$	60	159	2.26	[25], this work
i	3,4-(OMe)$_2$	41 [7][a]	165 [7]	1.05	[7]
k	3,4-Cl$_2$	26 [7][a]	177	2.40	[7,8]
l	4-Ph	39	180	2.82	[6], this work
m	4-Br	38 [7][a]	181	1.82	[7]
n	3,4,5-(OMe)$_3$	40	188	1.71	[25], this work

[a]Using method A in reference [7].

N^{ω}-nitro-L-arginine (L-NNA) have been studied, some of them exhibiting selectivities of up to three orders of magnitude over either eNOS or iNOS inhibition [48–53]. In the meantime, a great number of non-amino acid compounds have been tested as either nNOS or iNOS inhibitors. Substituted indazoles (with 7-nitroindazole, as the lead compound) or imidazoles are potent NOS inhibitors but their selectivity remains low, at least *in vitro* [54,55]. Potent non-amino acid inhibitors with isothiourea and amidine functions have been reported [56–64]. 2-Aminopyridines have been described as NOS inhibitors, and selective nNOS inhibitors containing this scaffold were recently reported [65,66]. However, simple 2-amino-4-methylpyridine derivatives, which are reported to be highly potent inhibitors of all three isoforms, can exhibit high toxicity and should not be suitable for further development [65]. The most recent developments in the field of NO synthase inhibition have been summarized in two reviews on new structures and new patents [67,68].

We were interested in the synthesis of new THPs **4** as potential inhibitors for NOSs and considered them more suitable for drug development than the starting EMBs **2**. These THPs exhibit the typical phamacophoric centers found in the EMBs **2**, that is, the aroyl moiety and a cyclic aliphatic spacer, and a basic cyclic amidine that could mimic the guanidinium function of L-arginine. The presence of a strongly basic moiety able to interact with a key-glutamate residue of the active site of NOS is a pre-requisite for NOS inhibition. Evans reported for 1,3,5,6-tetrahydropyrimidines, which are substituted in 2-position, a pK_a value of 13.0 in the case of an aliphatic substituent and a value of 12.8 for an aromatic substituent [69]. One can thus consider THPs **4** as strong bases which are permanently charged under physiological conditions. These compounds were evaluated as inhibitors of the three purified NOS isoforms from various species. Only two of them exhibited good inhibitory effects with selectivity for one isoform of NOS thus opening the way to further SAR studies.

RESULTS AND DISCUSSION

Chemistry. EMBs **2a–n** are accessible through the heating of aryl methyl ketones **1a–n** and paraformaldehyde and dimethylamine in *N*,*N*-dimethylformamide in moderate yield (Scheme 1 and Table 1) [7]. The yields can be improved using dimethyl(methylene)ammonium chloride (Eschenmoser's salt), a preformed iminium salt [7, method B], no further investigations using other catalysts were performed, as the starting materials are readily available in bulk quantities. Tetrahydropyrimidines **4aa–4nc** were prepared by condensation of benzamidines **3a–c** with enone Mannich bases **2a–n** in ethanol/water mixtures using trimethyl amine as base [29]. After the addition of the nucleophile to the enone structure, the dimethylamino group was eliminated followed by ring closure to form the heterocycles **4** in moderate yields (Table 2). All new compounds were fully characterized by the usual analytical methods. It has to be mentioned that the protonated tetrahydropyrimidinium ring in **4** shows symmetry, that is, for **4fb**, one chemical shift of the nitrogen atom was determined at −267.1 ppm relative to nitromethane by ^{15}N NMR. In all other cases, the NH-proton was very broad and did not allow any inverse measurements. A large number of benzamidines are commercially available or can be synthesized in only one step via Pinner synthesis from the corresponding nitriles. As starting moieties, we chose the simple unsubstituted benzamidine **3a**, benzamidines **3b** with an electron deficient nitro group in *meta* position, and **3c** with an additional amide group in *para* position.

Determination of $logD_{7.4}$. LogD values of the EMBs **2** and THPs **4** were determined experimentally by using a gradient elution HPLC method and reference compounds [70]. Under the test conditions (pH 7.4), the structures **2** and **4** are considered to be permanently charged. The results are listed in Tables 1 and 2. Interestingly, quite similar values were found for the EMBs and the corresponding THPs formed with the unsubstituted benzamidine and with the nitro group. Carboxamide substituted THPs show on average a smaller value of about 0.5 logD units.

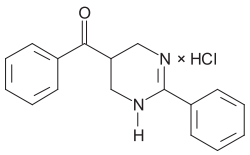
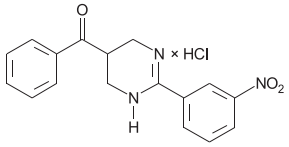
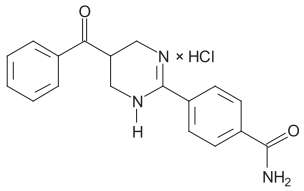
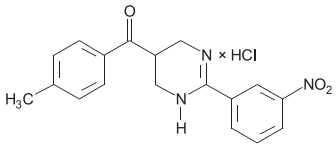
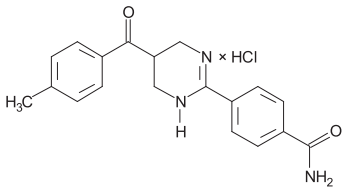
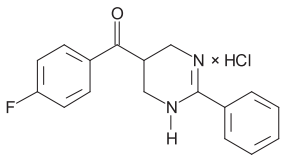
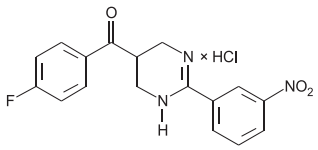
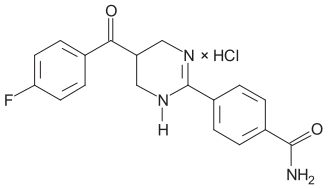
Biological testing – Griess assay. In a first series of experiments, NOS inhibition data were obtained for all compounds introduced at 10, 100, and 1000 μM (final concentration) using recombinant NOS isoforms from different species and the colorimetric Griess assay for nitrite determination [71]. From these results, IC_{50} values were estimated by linear regression (Table 3). Experiments including the known inhibitor of NOS N^{ω}-nitro-L-arginine (L-NNA) were performed for comparative purposes and are also listed in Table 3. By using this assay, only a few of the tested THPs **4** were found to be active, that is, the chloro derivative **4fb**, the naphthoyl derivatives **4ha–c**, and the benzoyl derivative **4la**, all with a preference for eNOS inhibition, in comparison with nNOS and iNOS.

Biological testing – hemoglobine assay. We also determined the inhibitory effects of some selected THPs on the activity of NOS using the classical spectrophotometric hemoglobin assay for NO [72,73]. The results of this test system are summarized in Table 4. All new compounds were tested at a concentration of 1 mM, and the data are based on the percentage of the complete system without THP. This complementary test confirmed the results of the Griess assay. Compounds **4fb**, the 4-chloro benzoyl derivative, and **4la**, the biphenyl derivative, displayed selectivity in inhibition of eNOS, whereas the naphthyl derivative **4ha** and the biphenyl derivative **4la** predominantly inhibited nNOS. However, none of the tested compounds significantly inhibited iNOS.

For the more promising THPs, IC_{50} values were determined for nNOS inhibition in the presence of different

Table 2

Synthesis and characterization of tetrahydropyrimidines **4aa–4nc**.

4	Structure	Yield, %	Mp, °C	logD (7.4)
aa		53 [29]	>300 [29]	1.33
ab		48 [29]	>300 [29]	1.31
ac		51	299	0.78
bb		42	>300	1.82
bc		15	264	1.31
ca		39	>300	1.50
cb		36	>300	1.47
cc		52	288	0.88

(*Continued*)

Table 2
(*Continued*)

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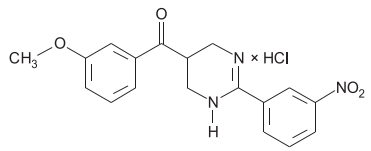
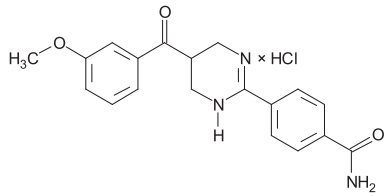
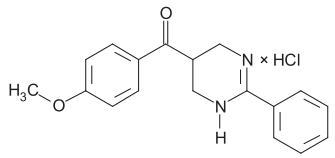
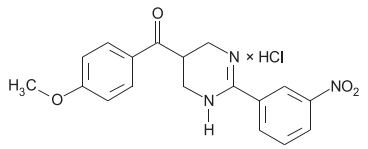
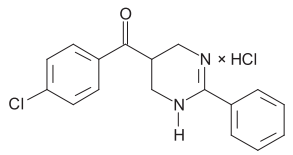
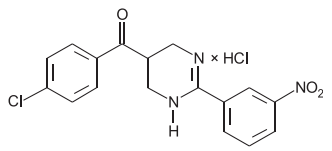
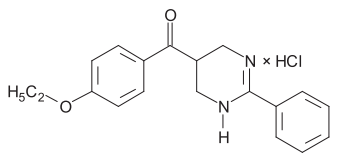
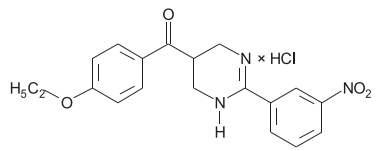
db		40	204	1.59
dc		40	178	1.10
ea		49 [29]	280 [29]	1.60
eb		40 [29]	299 [29]	1.57
fa		44	>280	2.00
fb		40	280	1.96
ga		32	298	2.13
gb		32	>300	2.05

(*Continued*)

Table 2
(*Continued*)

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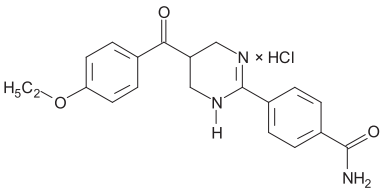
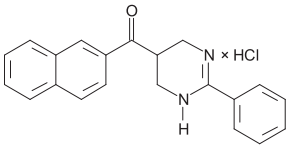
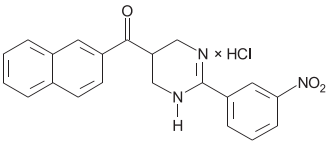
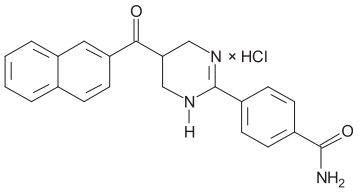
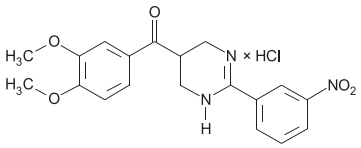
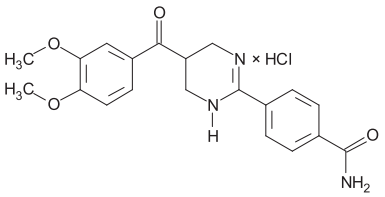
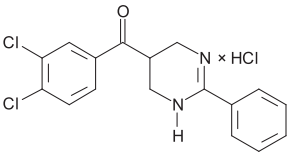
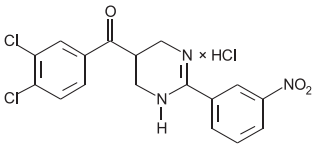
gc		34	259	1.61
ha		31	>290	2.44
hb		63	281	2.35
hc		32	279	2.02
ib		44	260	1.30
ic		40	265	0.93
ka		44 [29]	>300 [29]	2.52
kb		45 [29]	297 [29]	2.55

(*Continued*)

Table 2
(*Continued*)

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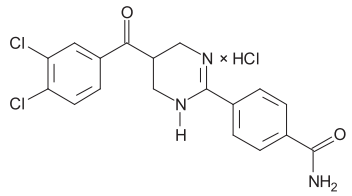
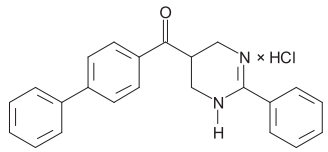
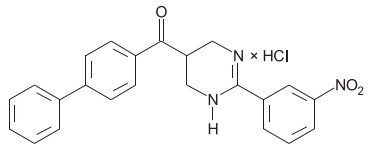
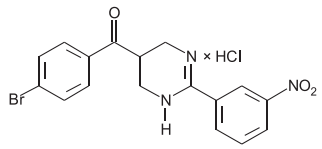
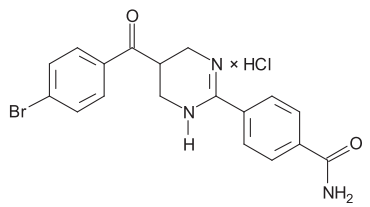
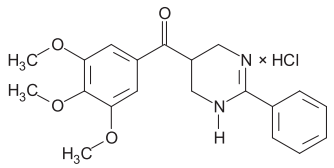
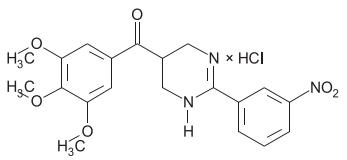
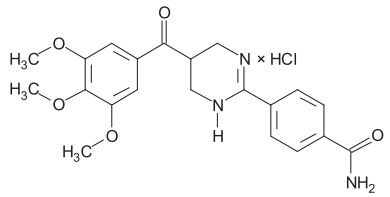
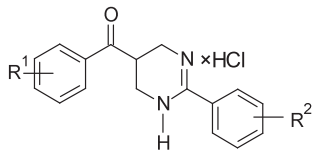
kc		62	226	2.06
la		30	283	2.84
lb		53	>280	2.88
mb		47	230	2.06
mc		42	272	1.60
na		30	278	1.61
nb		32	269	1.59
nc		26	263	1.15

Table 3

Inhibition of nitrite formation by recombinant NOS isoforms in the presence of tetrahydropyrimidines **4aa–4nc** and the reference compound L-NNA.[a]



4

			IC_{50}/mM		
Compound	R^1	R^2	eNOS (rat)	iNOS (murine)	nNOS (bovine)
4aa	H	H	>1	>1	>1
4ab	H	3-NO_2	>1	>1	>1
4ac	H	4-$CONH_2$	>1	>1	>1
4bb	4-Me	3-NO_2	>1	0.73	>1
4bc	4-Me	4-$CONH_2$	>1	>1	>1
4ca	4-F	H	>1	>1	>1
4cb	4-F	3-NO_2	0.92	>1	0.68
4cc	4-F	4-$CONH_2$	>1	>1	>1
4db	3-OMe	3-NO_2	>1	>1	0.81
4dc	3-OMe	4-$CONH_2$	>1	>1	0.68
4ea	4-OMe	H	>1	>1	>1
4eb	4-OMe	3-NO_2	>1	>1	>1
4fa	4-Cl	H	>1	>1	>1
4fb	4-Cl	3-NO_2	0.34	0.90	0.51
4ga	4-OEt	H	>1	>1	>1
4gb	4-OEt	3-NO_2	>1	>1	0.82
4gc	4-OEt	4-$CONH_2$	>1	>1	>1
4ha	3,4-(CH=CH)$_2$	H	0.19	>1	0.71
4ha	(human enzymes)		0.34	>1	0.47
4hb	3,4-(CH=CH)$_2$	3-NO_2	0.12	0.85	0.45
4hb	(human enzymes)		0.12	>1	0.33
4hc	3,4-(CH=CH)$_2$	4-$CONH_2$	0.37	>1	>1
4hc	(human enzymes)		0.74	>1	0.86
4ib	3,4-$(OMe)_2$	3-NO_2	>1	0.96	>1
4ic	3,4-$(OMe)_2$	4-$CONH_2$	>1	>1	>1
4ka	3,4-Cl_2	H	>1	>1	>1
4kb	3,4-Cl_2	3-NO_2	>1	>1	0.55
4kc	3,4-Cl_2	4-$CONH_2$	0.45	>1	0.46
4la	4-Ph	H	0.06	>1	0.46
4lb	4-Ph	3-NO_2	>1	>1	>1
4mb	4-Br	3-NO_2	0.94	>1	>1
4mc	4-Br	4-$CONH_2$	0.86	>1	>1
4na	3,4,5-$(OMe)_3$	H	>1	>1	>1
4nb	3,4,5-$(OMe)_3$	3-NO_2	>1	>1	>1
4nc	3,4,5-$(OMe)_3$	4-$CONH_2$	>1	>1	>1
L-NNA			0.012	0.44	0.007
L-NNA	(human enzymes)		0.027	0.09	0.005

NOS, nitric oxide synthase; eNOS, endothelial nitric oxide synthase; nNOS, neuronal nitric oxide synthase; iNOS, inducible nitric oxide synthase.
[a]Average relative standard deviation 10%, $n = 4$.

amounts of L-arginine (Table 5). The significant increase of the IC_{50} values when increasing L-arginine concentration from 10 to 100 μM suggests that there was competition between the substrate and the tested THPs.

NADPH consumption. In order to obtain further information on the effects of some THPs on the activity of NOSs, we have tested their effects on the NADPH consumption catalyzed by nNOS. These data will give information on their effects on the electron transfer from the reductase domain to the heme domain and the possible generation of superoxide anion and hydrogen peroxide by nNOS[74]. The results are given in % of the complete system without THP addition (Table 6). In the absence and in the presence of a saturating concentration of substrate, **4ha** and **4la** were found to inhibit the NADPH oxidase activity of nNOS; however, no clear effects were observed for **4lb** and **4kb**.

Table 4

Inhibitory effects of some THPs **4** on the activity of three NOS isoforms measured by the hemoglobin assay.[a]

	% activity of the complete system[b]		
Compound	eNOS	iNOS	nNOS
4aa	84	101	120
4ab	61	105	132
4ca	104	103	84
4cb	68	105	95
4cc	88	95	107
4dc	75	94	90
4ea	98	94	114
4eb	61	110	111
4fa	46	98	74
4fb	9	95	51
4ga	82	91	105
4gb	79	106	89
4gc	96	104	118
4ha	16	107	5
4ic	90	99	107
4ka	68	77	81
4kb	31	92	13
4la	0	71	0
4lb	24	93	10

THPs, tetrahydropyrimidines; NOS, nitric oxide synthase; eNOS, endothelial nitric oxide synthase; nNOS, neuronal nitric oxide synthase; iNOS, inducible nitric oxide synthase.
[a]Results are stated as % of the complete system. All compounds were tested at 1 mM (final concentration).
[b]Estimated relative error 20%, $n = 2–3$.

Table 5

Determination of IC_{50} values of selected THPs **4** on nNOS activity in the presence of 10 or 100 μM L-arginine. The activity of nNOS was measured by the hemoglobin assay.

Compounds	IC_{50}/μM[a]	
[L-Arg]/μM	10	100
4lb	800	1100
4la	200	450
4kb	600	800

L-Arg, L-arginine; THPs, tetrahydropyrimidines; nNOS, neuronal nitric oxide synthase
[a]Means from two experiments.

Table 6

Effects of some THPs **4** on NADPH consumption by nNOS.[a]

Compounds	without L-Arg	with L-Arg (100 μM)
4ha	25 ± 11	35 ± 25
4la	17 ± 5	44 ± 11
4lb	48	116 ± 58
4kb	117 ± 35	66 ± 30
L-**NNA**	27	21

L-Arg, L-arginine; THPs, tetrahydropyrimidines; nNOS, neuronal nitric oxide synthase.
[a]All compounds were tested at 1 mM, final concentration. Results are means from 2 to 3 experiments.

CONCLUSIONS

We have shown that a large number of 2,5-disubstituted tetrahydropyrimidines are accessible via two preparatively and synthetically simple steps employing basic building blocks, namely acetophenones **1** and benzamidines **3**, which are commercially available with large structural diversity. The THPs **4** thus available contain three typical pharmacological moieties, the aromatic residue, the aliphatic chain, and a basic group, here a cyclic *N*,*N*′-disubstituted amidinium ion. The structures might be of general interest because of their pharmacological profile, which still needs to be explored in detail.

We investigated the potential of these compounds to inhibit NO synthases and found some of the more lipophilic members ($logD_{7.4} > 2$) to be modest inhibitors of eNOS and nNOS. The most potent compounds bear a large aromatic group (biphenyl or naphthyl) and a small aromatic substituent on the amidine residue, that is, **4ha** and **4la**. Our data suggest that these compounds are competitive inhibitors with the substrate L-arginine and could inhibit NADPH oxidation by nNOS. Compared with other nonpeptoid NOs inhibitors, the inhibition potency as well as the selectivity is moderate. As lead compounds, however, they open the way to further structural modifications to get more active compounds.

EXPERIMENTAL

Lipophilicity. The logD values were determined by an HPLC method with gradient elution. A Waters Terra X column C8 MS was employed (4.6×50 mm, 3.5 μm particle size) together with a C8 Phenomenex precolumn (3×2 mm). The solvent system consisted of the following two mixtures: Solvent A 5% of acetonitrile and 95% (by volume) of 25 mM ammonium acetate, pH 7.4. Solvent B consisted of 95% of acetonitrile and 5% of the buffer, respectively. The solvents were eluted starting with 100% of A, changing linearly within 50 min to 100% of solvent B with a flow rate of 0.5 mL. Detection was performed at 240 nm, and the column was thermostated to 30°C. The following reference compounds were used (logP values in brackets [70]): acetanilide (1.0), acetophenone (1.7), anisole (2.1), ethyl benzoate (2.4), benzophenone (3.6), and diphenyl ether (4.2). The dead volume was determined by using uracil, and the calculated k values were correlated in a linear model with the reported logP values.

Chemistry. General: Starting materials were of standard quality and were employed as obtained from commercial suppliers and were used without further purification. All NMR spectra were recorded and evaluated in DMSO-d_6 with a Bruker Avance III 300 spectrometer, at 300 K and at 300 MHz for ^{1}H, 75 MHz for ^{13}C,^{1}H-broadband-decoupled spectra and for the DEPT pulse sequence (135°-tip angle), at 282 MHz for ^{19}F, and at 30.5 MHz for ^{15}N. Chemical shifts δ were calibrated with internal DMSO-d_6 (^{1}H at 2.50 ppm for the center of the DMSO-d_5 quintet, ^{13}C at 39.5 ppm for the DMSO-d_6 septet) or with external $CFCl_3$ in DMSO-d_6 ($\delta(^{19}F) = 0.0$ ppm) or with internal nitro methane ($\delta(^{15}N) = 0.0$ ppm). Coupling constants J are given in Hertz. The carbon resonances are given together with the phase of the signal in the corresponding 135-DEPT ^{13}C NMR spectrum. EI nominal resolution mass spectra were recorded by using an HP5989 MS

engine A with ionization energy of 70 eV and a direct inlet probe equipped with a tungsten wire. Elemental analyses were recorded with a Heraeus C,H,N analyzer at the Institute of Inorganic Chemistry at the University of Kiel, high resolution mass spectra (HR-ESI(+)-MS) were recorded with electrospray ionization with a Bruker Apex II FT ICR system at the Institute of Analytical Chemistry at the University of Leipzig. Generally, up to three peaks are analyzed: additionally to the quasi-molecular ion $[M+H]^+$, the value of the $[M+1+H]^+$ or $[M+2+H]^+$, and, if available, of the dimeric cluster $[2M+H]^+$ is given.

General procedure for the synthesis of EMBs 2. About 50 mmol of the corresponding substituted acetophenone **1a–m** was heated to 130°C for 1.5 h in 50 mL of DMF together with 2.2 equivalents of dimethylammonium chloride and 3.3 equivalents of paraformaldehyde. The solvent was then evaporated in vacuo, and the residue was triturated for 30 min with acetone at 0°C. If necessary, the suspension was left standing overnight at 6–8°C for complete crystallization. The solid was filtered off and dissolved in 100 mL of water. The pH of this solution was adjusted to eight using sodium bicarbonate. This solution was extracted three times with 100 mL of dichloromethane, the organic layer was dried over sodium sulfate, and then the solvent was evaporated. The oily remainder was triturated with a mixture of 20 mL of ethanol and 10 mL of concentrated hydrochloric acid. Finally, after twice adding and evaporating 40 mL of toluene, the solid was recrystallized from acetone/isopropanol mixtures.

Compounds. The complete analytical characterization of the following enone Mannich bases is reported elsewhere [7]: 1-Phenyl-2-(dimethylaminomethyl)-prop-2-en-1-one hydrochloride (**2a**), 1-(4-methylphenyl)-2-(dimethylaminomethyl)-prop-2-en-1-one hydrochloride (**2b**), 1-(4-methoxyphenyl)-2-(dimethylaminomethyl)-prop-2-en-1-one hydrochloride (**2e**), 1-(3,4-dimethoxyphenyl)-2-(dimethylaminomethyl)-prop-2-en-1-one hydrochloride (**2i**), 1-(3,4-dichlorophenyl)-2-(dimethylaminomethyl)-prop-2-en-1-one hydrochloride (**2k**), 1-(4-bromophenyl)-2-(dimethylaminomethyl)-prop-2-en-1-one hydrochloride (**2m**).

2-(Dimethylaminomethyl)-1-(4-fluorophenyl)-prop-2-en-1-one hydrochloride (2c) [15]. 6.9 g (50 mmol) of **1c** afforded 3.47 g (14 mmol, 28%) of white crystals, mp 169°C. Anal., %: Calcd for $C_{12}H_{15}NOFCl$ C 59.14, H, 6.21, N 5.75; found C 59.11, H 6.31, N 5.64. ^{1}H-NMR δ: 2.78 (d, $J=4.8$, 6H, NMe_2), 4.10 (d, $J=5.2$, 2H, NCH_2), 6.19/6.79 (2 × s, 2 × 1H, $=CH_2$), 7.40 (m, 2H, H-3′/5′), 7.91 (m, 2H, H-2′/6′), 11.17 (br s, 1H, NH). ^{13}C NMR δ: 42.1 (+), 55.4 (−), 115.6 (+, $J_{CF}=21.9$), 132.5 (+, $J_{CF}=9.5$), 132.5, 132.6 ($J_{CF}=2.2$), 136.5 (−), 164.8 ($J_{CF}=251.8$), 193.9. ^{19}F NMR δ: −105.9 (tt, $J=9.2/5.1$, 1F). MS *m/z*: 207 (M^+, 3), 190 (30), 123 (18), 112 (4), 95 (20), 84 (6), 75 (12), 58 (100), 44 (21), 42 (38).

2-(Dimethylaminomethyl)-1-(3-methoxyphenyl)-prop-2-en-1-one hydrochloride (2d). 7.5 g (50 mmol) of **1d** afforded 2.53 g (10 mmol, 20%) of white crystals, mp 138°C. ^{1}H NMR δ: 2.78 (br s, 6H, NMe_2), 3.83 (s, 3H, OMe), 4.11 (br s, 2H, NCH_2), 6.21/6.79 (2 × s, 2 × 1H, $=CH_2$), 7.25 (ddd, $J=8.0/2.7/1.1$, 1H, H-6), 7.29 (dd, $J=2.7/1.6$, 1H, H-2), 7.36 (ddd, $J=7.5/1.5/1.2$, 1H, H-4), 7.48 (dd, $J=7.7/7.7$, 1H, H-5), 11.11 (br s, 1H, NH). ^{13}C NMR δ: 42.1 (+), 55.3 (−), 55.4 (+), 114.2 (+), 118.7 (+), 121.9 (+), 129.6 (+), 136.87 (−), 136.91, 137.3, 159.1, 195.0. HR–ESI (+)-MS: Calcd for $[C_{13}H_{17}NO_2+H]^+$ 220.1332, found 220.1334; calcd for $[^{13}C^{12}C_{12}H_{17}NO_2+H]^+$ 221.1366, found 221.1367.

1-(4-Chlorophenyl)-2-(dimethylaminomethyl)-prop-2-en-1-one hydrochloride (2f) [18]. 7.7 g (6.5 mL, 50 mmol) of **1f**, 5.1 g (170 mmol) of paraformaldehyde and 9.0 g (110 mmol) of dimethylammonium chloride in 25 mL of DMF afforded 3.4 g (13 mmol, 40%) of white crystals, mp 154°C. ^{1}H NMR δ: 2.77 (br s, 6H, NMe_2), 4.09 (br s, NCH_2), 6.20/6.80 (2 × s, 2 × 1H, $=CH_2$), 7.63 (d, 2H, $J=8.6$, H-3′/H-5′), 7.82 (d, $J=8.6$, 2H, H-2′/6′), 11.15 (br s, 1H, NH). ^{13}C NMR δ: 42.2 (+), 55.3 (−), 128.6 (+), 131.4 (+), 134.7, 136.8, 137.0 (br, −), 137.7, 194.2. MS *m/z*: 223 (M^+, 4), 222 (9), 206 (67), 145 (10), 139 (22), 111 (15), 64 (9), 58 (100), 44 (15), 42 (17).

2-(Dimethylaminomethyl)-1-(4-ethoxyphenyl)-prop-2-en-1-one hydrochloride (2g). 8.2 g (50 mmol) of **1g** and 5.1 g (170 mmol) of paraformaldehyde and 9.0 g (110 mmol) of dimethylammonium chloride in 25 mL of DMF afforded 2.2 g (8 mmol, 16%) of white crystals, mp 162°C. ^{1}H NMR δ: 1.36 (t, $J=6.9$, 3H, Me), 2.58 (br s, 6H, NMe_2), 4.08 (br s, 2H, NCH_2), 4.14 (q, $J=6.9$, 2H, OCH_2), 6.12/6.66 (2 × s, 2 × 1 H, $=CH_2$), 7.07 (d, $J=8.7$, 2H, H-3′/H-5′), 7.82 (d, $J=8.6$, 2H, H-2′/6′), 10.97 (br s, 1H, NH). ^{13}C NMR δ: 14.4 (+), 42.1 (+), 55.7 (−), 63.6 (−), 114.2 (+), 128.1, 132.1 (+), 134.8 (−), 136.9, 162.5, 193.7. MS *m/z*: 233 (M^+, 12), 216 (73), 149 (15), 121 (19), 93 (10), 84 (11), 65 (15), 58 (100), 44 (19), 42 (26). HR−ESI(+)-MS: Calcd for $[C_{14}H_{19}NO_2+H]^+$ 234.1489, found 234.1488; calcd for $[^{13}C^{12}C_{13}H_{19}NO_2+H]^+$ 235.1523, found 235.1522.

2-(Dimethylaminomethyl)-1-(2′-naphthyl)-prop-2-en-1-one hydrochloride (2h). 8.5 g (50 mmol) of **1h** and 5.1 g (170 mmol) of paraformaldehyde and 9.0 g (110 mmol) of dimethylammonium chloride in 25 mL of DMF afforded 8.2 g (30 mmol, 60%) of white crystals, mp 159°C. *Anal.*, %: calcd for $C_{16}H_{18}NOCl$ C 69.68, H 6.58, N 5.08; found C 69.58, H 6.65, N 4.95. ^{1}H NMR δ: 2.83 (d, $J=4.4$, 6H, NMe_2), 4.17 (d, $J=4.8$, 2H, NCH_2), 6.30/6.83 (2 × s, 2 × 1 H, $=CH_2$), 7.69 (m, 2H, ArH), 7.86 (dd, $J=8.5/1.7$, 1H, ArH), 8.02–7.17 (m, 3H, ArH), 8.48 (s, 1H, ArH) 10.91 (br s, 1H, NH). ^{13}C NMR δ: 42.4 (+), 56.0 (−), 125.0 (+), 127.0 (+), 127.6 (+), 128.3 (+), 128.6 (+), 129.5 (+), 131.4 (+), 131.8, 133.2, 134.8, 136.5 (br, −), 137.1, 195.3. MS *m/z*: 239 (M^+, 7), 238 (7), 222 (19), 196 (5), 155 (7), 127 (15), 84 (7), 77 (6), 58 (100), 44 (31). HR–ESI(+)-MS: Calcd for $[C_{16}H_{17}NO+H]^+$ 240.1383, found 240.1384; calcd for $[^{13}C^{12}C_{15}H_{17}NO+H]^+$ 241.1417, found 241.1418.

1-(4-Biphenyl)-2-(dimethylaminomethyl)-prop-2-en-1-one hydrochloride (21) [6]. 9.8 g (50 mmol) of **1l** and 5.1 g (170 mmol) of paraformaldehyde and 9.0 g (110 mmol) of dimethylammonium chloride in 25 mL of DMF afforded 5.8 g (19 mmol, 39%) of white crystals, mp 180°C. ^{1}H NMR δ: 2.79 (br s, 6H, NMe_2), 4.12 (br s, NCH_2), 6.25/6.78 (2 × s, 21 × 1H, $=CH_2$), 7.44 (m, 1H, H-4″), 7.52 (m, 2H, H-3‴/5″), 7.77 (d, $J=7.0$, 2H, H-2″/6″), 7.88 (m, 4H, H-2′/3′(5′/6′), 11.00 (br s, 1H, NH). ^{13}C NMR δ: 42.4 (+), 55.6 (−), 126.7 (+),126.9 (+),128.4 (+),129.1 (+),130.3 (+), 134.7, 136.4 (br, −), 137.0, 144.4, 194.8. MS *m/z*: 265 (M^+, 14), 264 (18), 248 (100), 181 (20), 152 (26), 112 (10), 84 (11), 58 (97), 44 (19), 42 (17).

1-(3,4,5-Trimethoxyphenyl)-2-(dimethylaminomethyl)-prop-2-en-1-one hydrochloride (2n). 4.2 g (50 mmol) of **1n** and 5.1 g (170 mmol) of paraformaldehyde and 9.0 g (110 mmol) of dimethylammonium chloride in 25 mL of DMF

afforded 2.5 g (8 mmol, 40%) of yellowish crystals, mp 188°C. ^{1}H NMR δ: 2.77 (d, J = 4.8, 6H, NMe_2), 3.77/3.86 (2 × s, 1 × 3H, 1 × 6H, 3 × OMe), 4.12 (d, J = 5.0, 2H, NCH_2), 6.25/6.75 (2 × s, 2 × 1H, =CH_2), 7.13 (s, 2H, H-2′/H-6′) 11.22 (br s, 1H, NH). ^{13}C NMR δ: 42.2 (+), 55.9 (−), 56.1 (+), 60.1 (+), 107.4 (+), 130.9 (+), 136.1, 136.7 (−), 141.8, 152.6, 194.2. HR–ESI(+)-MS: Calcd for $[C_{15}H_{21}NO_4 + H]^+$ 280.1543, found 280.1543; calcd for $[^{13}C^{12}C_{14}H_{21}NO_4 + H]^+$ 281.1577, found 281.1578.

General procedure for the synthesis of the THP 4aa–4nc. 1 mmol of EMB **2** and 1.1 mmol of the benzamidinium hydrochloride **3** were dissolved in about 10 mL of a 1:1 mixture of water and ethanol, then 0.6 mL of triethylamine was added and the mixture was brought to reflux for 30 min. After cooling, the solvents were evaporated in vacuum, and the remainder was suspended and refluxed with 15 mL of isopropanol. After filtration, the solid was washed with 5 mL of isopropanol and dried in vacuo. The benzamidines **3a–c** are commercially available. The characterization of the following THP **4** and their analytical data are reported elsewhere [29]: 5-Benzoyl-2-phenyl-1,4,5,6-tetrahydropyrimidine hydrochloride (**4aa**), 5-benzoyl-2-(3-nitrophenyl)-1,4,5,6-tetrahydropyrimidine hydrochloride (**4ab**), 5-(4-methoxybenzoyl)-2-phenyl-1,4,5,6-tetrahydropyrimidine hydrochloride (**4ea**), 5-(4-methoxybenzoyl)-2-(3-nitrophenyl)-1,4,5,6-tetrahydropyrimidine hydrochloride (**4eb**), 5-(3,4-dichlorobenzoyl)-2-phenyl-1,4,5,6-tetrahydropyrimidine hydrochloride (**4ka**), 5-(3,4-dichlorobenzoyl)-2-(3-nitrophenyl)-1,4,5,6-tetrahydropyrimidine hydrochloride (**4kb**).

5-Benzoyl-2-(4′-carbamoylphenyl)-1,4,5,6-tetrahydropyrimidine hydrochloride (4ac). 258 mg (1.14 mmol) of **2a** and 248 mg (1.23 mmol) of **3c** afforded 201 mg (0.58 mmol, 51%) of **4ac** as white solid, mp 299°C. ^{1}H NMR δ: 3.61 (dd, J = 13.6, 6.7, 2H, H-4/6), 3.80 (dd, J = 13.6, 4.4, 2H, H-4/6), 4.33 (m, 1H, H-5), 7.57/8.26 (2 × br s, 2 × 1H, $CONH_2$), 7.61 (d, J = 7.8, 2H, H-3″/5″), 7.71 (m, 1H, H-4″), 7.84 (d, J = 8.3, 2H, H-3′/5′), 8.07 (2 × d, J = 8.4, 2 × 2H, H-2′/6′, H-2″/H6″), 10.50 (br s, 2H, NH). ^{13}C NMR δ: 34.8 (+), 40.8 (−), 127.8 (+), 127.9 (+), 128.5 (+), 129.0 (+), 130.1, 133.9, 134.8 (+), 138.2, 158.2, 166.5, 198.0. MS *m/z*: 307 (M^+, 1), 202 (100), 188 (6), 160 (7), 147 (24), 130 (8), 105 (44), 77 (53), 56 (61), 43 (14). HR–ESI (+)-MS: Calcd for $[C_{18}H_{17}N_3O_2 + H]^+$ 308.1394, found 308.1392; calcd for $[^{13}C^{12}C_{17}H_{17}N_3O_2 + H]^+$ 309.1428, found 309.1426; calcd for $[(C_{18}H_{17}N_3O_2)_2 + H]^+$ 615.2714, found 615.2717.

5-(4″-Methylbenzoyl)-2-(3′-nitrophenyl)-1,4,5,6-tetrahydropyrimidine hydrochloride (4bb). 480 mg (2.0 mmol) of **2b** and 332 mg (2.0 mmol) of **3b** afforded 304 mg (0.84 mmol, 42%) of **4bb** as slightly yellowish solid, mp >300°C. ^{1}H NMR δ: 2.42 (s, 3H, Me), 3.60 (dd, J = 13.5, 7.2, 2H, H-4/6), 3.80 (dd, J = 13.5, 4.6, 2H, H-4/6), 4.29 (m, 1H, H-5), 7.41 (d, J = 8.0, 2H, H-3″/5″), 7.93 (t, J = 8.1, 1H, H-5′), 8.00 (d, J = 8.2, 2H, H-2″/′6″), 8.24 (d, J = 8.0, 1H, H-6′), 8.55 (dd, J = 8.3, 2.2, 1H, H-4′), 8.64 (t, J = 2.0, 1H, H-2′), 10.81 (br s, 2H, NH). ^{13}C NMR δ: 21.1 (+), 34.6 (+), 40.9 (−), 123,1 (+), 127.5 (+), 128.6 (+), 129.3, 129.5 (+), 130.7 (+), 132.4, 134.3 (+), 144.5, 147.5, 157.1, 197.4. MS *m/z*: 323 (M^+, 1), 204 (100), 174 (37), 131 (8), 119 (51), 103 (10), 91 (42), 65 (19), 56 (57), 44 (10). HR–ESI(+)-MS: Calcd for $[C_{18}H_{17}N_3O_3 + H]^+$ 324.1343, found 324.1341; calcd for $[^{13}C^{12}C_{17}H_{17}N_3O_3 + H]^+$ 325.1377, found 325.1375; calcd for $[(C_{18}H_{17}N_3O_3)_2 + H]^+$ 647.2613, found 647.2616.

2-(4′-Carbamoylpheny)-5-(4″-methylbenzoyl)-1,4,5,6-tetrahydropyrimidine hydrochloride (4bc). 496 mg (2.05 mmol) of **2b** and 408 mg (2.04 mmol) of **3c** afforded 98 mg (0.55 mmol, 15%) of **4bc** as white solid, mp 264°C (dec.). ^{1}H NMR δ: 2.41 (s, 3H, Me), 3.60 (dd, J = 13.5, 6.8, 2H, H-4/6), 3.79 (dd, J = 13.4, 4.6, 2H, H-4/6), 4.29 (m, 1H, H-5), 7.41 (d, J = 8.0, 2H, H-3″/5″), 7.64/8.22 (2 × br s, 2 × 1H, $CONH_2$), 7.83 (d, J = 8.4, 2H, H-2′/6′), 7.99 (d, J = 8.0, 2H, H-2″/′6″), 8.08 (2 × d, J = 8.4, 2 × 2H, H-3′/5′), 10.42 (br s, 2H, NH). ^{13}C NMR δ: 21.1 (+), 34.7 (+), 40.8 (−), 127.7 (+), 127.8 (+),128.6 (+), 129.5 (+), 130.2, 132.3, 136.5, 144.5, 158.1, 167.1, 197.4. MS *m/z*: 321 (M^+, 1), 164 (87), 148 (100), 120 (29), 103 (43), 76 (16), 65 (23), 51 (20), 44 (26). HR–ESI(+)-MS: Calcd for $[C_{19}H_{19}N_3O_2 + H]^+$ 322.1550, found 322.1548; calcd for $[^{13}C^{12}C_{18}H_{19}N_3O_2 + H]^+$ 323.1584, found 323.1583; calcd for $[(C_{19}H_{19}N_3O_2)_2 + H]^+$ 643.3027, found 643.3034.

5-(4′-Fluorobenzoyl)-2-phenyl-1,4,5,6-tetrahydropyrimidine hydrochloride (4ca). 243 mg (1.0 mmol) of **2c** and 157 mg (1.0 mmol) of **3a** × **H_2O** afforded 124 mg (0.39 mmol, 39%) of **4ca** as white solid, mp >300°C. ^{1}H NMR δ: 3.60 (dd, J = 13.3, 6.5, 2H, H-4/6), 3.80 (dd, J = 13.5, 4.0, 2H, H-4/6), 4.32 (m, 1H, H-5), 7.43 (t, J = 8.8, 2H, ArH), 7.62 (m, 2H, ArH), 7.73 (m, 1H, ArH), 7.82 (d, J = 7.3, 2H, ArH), 8.19 (m, 2H, ArH), 10.52 (br s, 2H, NH). ^{13}C NMR δ: 34.9 (+), 40.7 (−), 116.0 (+, J_{CF} = 22.0), 127.6 (+), 127.9, 129.0 (+), 131.6 (+, J_{CF} = 9.8), 131.7 (J_{CF} = 2.8), 133.2, 158.8, 165.4 (J_{CF} = 253.0), 196.6. ^{19}F NMR δ: −104.4 (tt, J = 8.9/5.1, 1F). MS *m/z*: 282 (M^+, 1), 159 (100), 123 (41), 117 (10), 104 (50), 95 (42), 77 (41), 75 (21), 56 (67), 51 (22). HR–ESI(+)-MS: Calcd for $[C_{17}H_{15}N_2OF + H]^+$ 283.1241, found 283.1239; calcd for $[^{13}C^{12}C_{16}H_{15}N_2OF + H]^+$ 284.1275, found 284.1274.

5-(4′-Fluorobenzoyl)-2-(3′-nitrophenyl)-1,4,5,6-tetrahydropyrimidine hydrochloride (4cb). 243 mg (1.0 mmol) of **2c** and 166 mg (1.0 mmol) of **3b** afforded 131 mg (0.36 mmol, 36%) of **4cb** as white solid, mp >300°C. ^{1}H NMR δ: 3.61 (dd, J = 13.5, 7.2, 2H, H-4/6), 3.81 (dd, J = 13.6, 4.5, 2H, H-4/6), 4.31 (m, 1H, H-5), 7.45 (t, J = 8.8, 2H, H-3″/5″), 7.94 (t, J = 8.1, 1H, H-5′), 8.16–8.22 (m, 3H, H-2″/6″, H-6′), 8.56 (dd, J = 8.3, 2.2, 1H, H-4′), 8.62 (m, 1H, H-2′), 10.71 (br s, 2H, NH). ^{13}C NMR δ: 34.7 (+), 40.8 (−), 116.0 (+, J_{CF} = 22.0), 123.0 (+), 127.6 (+), 130.7 (+), 131.6 (+, J_{CF} = 9.9), 131.7 (J_{CF} = 2.8), 134.3 (+), 147.5, 157.1, 165.4 (J_{CF} = 253.0), 196.6. ^{19}F NMR δ: -104.3 (tt, J = 9.1/5.2, 1F). MS *m/z*: 327 (M^+, 1), 204 (95), 174 (9), 149 (15), 123 (55), 103 (27), 95 (27), 75 (28), 56 (100), 45 (16). HR–ESI(+)-MS: Calcd for $[C_{17}H_{14}N_3O_3F + H]^+$ 328.1092, found 328.1089; calcd for $[^{13}C^{12}C_{16}H_{14}N_3O_3F + H]^+$ 329.1126, found 329.1125; calcd for $[(C_{17}H_{14}N_3O_3F)_2 + H]^+$ 655.2111, found 655.2107.

2-(4′-Carbamoylphenyl)-5-(4″-fluorobenzoyl)-1,4,5,6-tetrahydropyrimidine hydrochloride (4cc). 243 mg (1.0 mmol) of **2c** and 200 mg (1.0 mmol) of **3c** afforded 192 mg (0.53 mmol, 52%) of **4cc** as white solid, mp 288°C. ^{1}H NMR δ: 3.60 (dd, J = 13.4, 6.5, 2H, H-4/6), 3.80 (dd, J = 13.7, 4.5, 2H, H-4/6), 4.32 (m, 1H, H-5), 7.43 (t, J = 8.7, 2H, ArH), 7.63/8.25 (2 × br s, 2H, $CONH_2$), 7.89 (m, 2H, ArH), 8.08 (d, J = 8.3, 2H, ArH), 8.19 (m, 2H, ArH), 10.60 (br s, 2H, NH). ^{13}C NMR δ: 34.8 (+), 40.7 (−), 116.0 (+, J_{CF} = 22.0), 127.8 (+), 127.9 (+), 130.1, 131.6 (+, J_{CF} = 2.8), 131.7 (J_{CF} = 9.6), 138.3, 158.0, 165.5 (J_{CF} = 253.1), 166.5, 196.6. ^{19}F NMR δ: −104.4 (tt, J = 8.6/5.2, 1F). MS *m/z*:

325 (M^+, 2), 202 (100), 147 (27), 123 (54), 103 (24), 95 (44), 75 (22), 65 (12), 56 (96), 44 (39). HR–ESI(+)-MS: Calcd for $[C_{18}H_{16}N_3O_2F+H]^+$ 326.1299, found 326.1296; calcd for $[^{13}C^{12}C_{17}H_{16}N_3O_2F+H]^+$ 327.1333, found 327.1331; calcd for $[(C_{18}H_{16}N_3O_2F)_2+H]^+$ 651.2526, found 651.2520.

5-(3″-Methoxybenzoyl)-2-(3′-nitrophenyl)-1,4,5,6-tetrahydropyrimidine hydrochloride (4db). 256 mg (1.0 mmol) of **2d** and 165 mg (1.0 mmol) of **3b** afforded 149 mg (0.40 mmol, 40%) of **4db** as slightly yellowish solid, mp 204°C. *Anal.*, %: calcd for $C_{18}H_{18}N_3O_4Cl$ C 57.53, H 4.83, N 11.18; found C 57.50, H 4.92, N 11.05. ^{1}H NMR δ: 3.61 (dd, J = 13.5, 7.1, 2H, H-4/6), 3.82 (dd, J = 13.5, 4.7, 2H, H-4/6), 3.85 (s, 3H, OMe), 4.31 (m, 1H, H-5), 7.31 (ddd, J = 8.2, 2.7, 1.0, 1H, H-4″), 7.53 (t, J = 8.0, 1H, H-5″), 7.56 (t, J = 2.0, 1H, H-2″), 7.69 (ddd, J = 7.7, 1.6, 1.0, 1H, H-6″), 7.94 (td, J = 8.0, 0.6, 1H, H-5′), 8.23 (ddd, J = 8.0, 1.8, 1.0, 1H, H-6′), 8.56 (ddd, J = 8.3, 2.3, 1.0, 1H, H-4′), 8.64 (t, J = 2.0, 1H, H-2′), 10.70 (br s, 2H, NH). ^{13}C NMR δ: 34.8 (+), 40.9 (−), 55.4 (+), 113.2 (+),119.8 (+),120.9 (+),123.0 (+), 127.6 (+), 129.4, 130.2 (+), 130.8 (+), 134.3 (+), 136.3, 147.5, 157.1, 159.6, 197.8. MS *m/z*: 339 (M^+, 1), 204 (100), 174 (20), 135 (21), 119 (8), 107 (17), 103 (11), 92 (14), 77 (23), 56 (57). HR–ESI(+)-MS: Calcd for $[C_{18}H_{17}N_3O_4+H]^+$ 340.1292, found 340.1288; calcd for $[^{13}C^{12}C_{17}H_{17}N_3O_4+H]^+$ 341.1326, found 341.1323; calcd for $[(C_{18}H_{17}N_3O_4)_2+H]^+$ 679.2511, found 679.2500.

2-(4′-Carbamoylphenyl)-5-(3″-methoxybenzoyl)-1,4,5,6-tetrahydropyrimidine hydrochloride (4dc). 256 mg (1.0 mmol) of **2d** and 200 mg (1.0 mmol) of **3c** afforded 150 mg (0.40 mmol, 40%) of **4dc** as white solid, mp 178°C. *Anal.*, %: calcd for $C_{19}H_{20}N_3O_3Cl$ C 61.05, H 5.39, N 11.24; found C 61.00, H 5.31, N 11.13. ^{1}H NMR δ: 3.60 (dd, J = 13.6, 6.9, 2H, H-4/6), 3.81 (m, 2H, H-4/6), 3.84 (s, 3H, OMe), 4.35 (m, 1H, H-5), 7.30 (ddd, J = 8.1, 2.4, 1.0, 1H, H-4″), 7.52 (t, J = 7.8, 1H, H-5″), 7.56 (br s, 1H, H-2″), 7.63/8.29 (2 × br s, 2 × 1H, $CONH_2$), 7.69 (ddd, J = 7.6, 1.5, 1.0, 1H, H-6″), 7.93 (d, J = 8.6, 2H, H-3′/5′), 8.09 (d, J = 8.5, 2H, H-2′/H-6′), 10.92 (br s, 2H, NH). ^{13}C NMR δ: 34.9 (+), 40.8 (−), 55.5 (+), 113.1 (+), 119.9 (+), 121.0 (+), 127.8 (+), 127.9 (+), 130.1 (+), 130.2, 136.2, 138.3, 158.1, 159.6, 166.5, 197.9. MS *m/z*: 337 (M^+, 2), 202 (100), 164 (14), 148 (24), 135 (30), 107 (20), 86 (59), 77 (27), 56 (46), 44 (42). HR–ESI(+)-MS: Calcd for $[C_{19}H_{19}N_3O_3+H]^+$ 338.1499, found 338.1496; calcd for $[^{13}C^{12}C_{18}H_{19}N_3O_3+H]^+$ 339.1533, found 339.1531; calcd for $[(C_{19}H_{19}N_3O_3)_2+H]^+$ 675.2926, found 675.2918.

5-(4″-Chlorobenzoyl)-2-phenyl-1,4,5,6-tetrahydropyrimidine hydrochloride (4fa). 260 mg (1.0 mmol) of **2f** and 157 mg (1.0 mmol) of **3a** × **H_2O** afforded 148 mg (0.44 mmol, 44%) of **4fa** as white solid, mp >280°C. ^{1}H NMR δ: 3.55 (dd, J = 13.5, 7,2, 2H, H-4/6), 3.76 (dd, J = 13.4, 4.5, 2H, H-4/6), 4.18 (m, 1H, H-5), 7.62 (m, 2H, H-3′/5′), 7.67 (d, J = 8.6, 2H, H-3″/5″), 7.73 (m, 1H, H-4′), 7.81 (m, 2H, H-2′/6′), 8.11 (d, J = 8.6, 2H, H-2″/6″), 10.06 (br s, 2H, NH). ^{13}C NMR δ: 35.0 (+), 40.7 (−), 127.6 (+), 128.0, 129.0 (+),129.1 (+), 130.4 (+), 133.2, 133.6, 138.8, 158.6, 197.1. MS *m/z*: 298 (M^+, 3), 268 (6), 159 (100), 145 (10), 139 (18), 131 (7), 111 (11), 104 (25), 77 (16), 56 (29). HR–ESI (+)-MS: Calcd for $[C_{17}H_{15}N_2O^{35}Cl+H]^+$ 299.0946, found 299.0945; calcd for $[^{13}C^{12}C_{16}H_{15}N_2O^{35}Cl+H]^+$ 300.0980, found 300.0979; calcd for $[C_{17}H_{15}N_2O^{37}Cl+H]^+$ 301.0916, found 301.0914.

5-(4″-Chlorobenzoyl)-2-(3′-nitrophenyl)-1,4,5,6-tetrahydropyrimidine hydrochloride (4fb). 260 mg (1.0 mmol) of **2f** and 166 mg (1.0 mmol) of **3b** afforded 141 mg (0.40 mmol, 40%) of **4fb** as yellow solid, mp = 280°C. ^{1}H NMR δ: 3.60 (dd, J = 13.4, 6.8, 2H, H-4/6), 3.82 (dd, J = 13.4, 4.4, 2H, H-4/6), 4.33 (m, 1H, H-5), 7.68 (d, J = 8.4, 2H, H-3″/5″), 7.93 (t, J = 8.0, 1H, H-5′), 8.11 (d, J = 8.5, 2H, H-2″/6″), 8.27 (d, J = 7.7, 1H, H-6′), 8.55 (d, J = 8.0, 1H, H-4′), 8.66 (br S, 1H, H-2′), 10.93 (br s, 2H, NH). ^{13}C NMR δ: 34.8 (+), 40.7 (−), 123.1 (+), 127.6, 129.1 (+) ,129.3, 130.4 (+), 130.7 (+), 133.6, 134,3 (+), 138.9, 147.5, 157.1, 197.0. ^{15}N NMR δ: −13.9 (NO_2), −267.1 (N1/3). MS *m/z*: 343 (M^+, <1), 204 (100), 149 (7), 149 (16), 139 (21), 131 (7), 111 (14), 103 (14), 75 (9), 56 (39). HR–ESI(+)-MS: Calcd for $[C_{17}H_{14}N_3O_3^{35}Cl+H]^+$ 344.0796, found 344.0795; calcd for $[^{13}C^{12}C_{16}H_{14}N_3O_3^{35}Cl+H]_+$ 345.0830, found 345.0829; calcd for $[(C_{17}H_{15}N_3O_3^{35}Cl)+H]_+$ 687.1520, found 687.1532.

5-(4″-Ethoxybenzoyl)-2-phenyl-1,4,5,6-tetrahydropyrimidine hydrochloride (4ga). 270 mg (1.0 mmol) of **2 g** and 157 mg (1.0 mmol) of **3a** × **H_2O** afforded 110 mg (0.32 mmol, 32%) of **4ga** as white solid, mp = 298°C. ^{1}H NMR δ: 1.36 (t, J = 6.9, 3H, Me), 3.58 (dd, J = 13.3, 6.6, 2H, H-4/6), 3.76 (dd, J = 13.5, 4.0, 2H, H-4/6), 4.16 (q, J = 7.0, 2H, OCH_2) 4.25 (m, 1H, H-5), 7.09 (d, J = 8.9, 2H, H-3″/5″), 7.62 (m, 2H, H-3′/5′), 7.73 (m, 1H, H-4′), 7.80 (d, J = 7.3, 2H, H-2′/6′), 8.06 (d, J = 8.9, 2H, H-2″/6″), 10.44 (br s, 2H, NH). ^{13}C NMR δ: 14.4 (+), 34.4 (+), 40.9 (−), 63.7 (−), 114.6 (+), 127.5 (+), 128.0, 128.9 (+), 130.9 (+), 133.2 (+), 158.6, 163.0, 196.2. MS *m/z*: 308 (M^+, 1), 278 (3), 159 (100), 149 (12), 121 (20), 104 (17), 93 (8), 77 (13), 65 (11), 56 (26). HR–ESI(+)-MS: Calcd for $[C_{19}H_{20}N_2O_2+H]^+$ 309.1598, found 309.1594; calcd for $[^{13}C^{12}C_{18}H_{20}N_2O_2+H]^+$ 310.1632, found 310.1630; calcd for $[(C_{19}H_{20}N_2O_2)_2+H]^+$ 617.3122, found 617.3115.

5-(4″-Ethoxybenzoyl)-2-(3′-nitrophenyl)-1,4,5,6-tetrahydropyrimidine hydrochloride (4gb). 270 mg (1.0 mmol) of **2 g** and 166 mg (1.0 mmol) of **3b** afforded 125 mg (0.32 mmol, 32%) of **4gb** as slightly yellowish solid, mp >300°C. ^{1}H NMR δ: 1.37 (t, J = 7.0, 3H, Me), 3.58 (dd, J = 13.4, 6.7,3H, H-4/6), 3.78 (dd, J = 13.5, 4.6, 2H, H-4/6), 4.16 (q, J = 7.0, 2H, CH_2O), 4.24 (m, 1H, H-5), 7.10 (d, J = 8.9, 2H, 2H, H-3″/5″), 7.93 (t, J = 8.1, 1H, H-5′), 8.06 (d, J = 8.9, 2H, H-2″/6″), 8.21 (d, J = 8.4, 1H, H-6′), 8.55 (m, 1H, H-4′), 8.62 (m, 1H, H-2′), 10.63 (br s, 2H, NH). ^{13}C NMR δ: 14.4 (+), 34.2 (+), 41.1 (−), 63.7 (−), 114.6 (+), 123.0 (+), 127.6 (+),129.5, 130.8 (+), 131.0 (two overlapping signals, +), 134.2 (+), 147.5, 157.2, 163.1, 196.1. MS *m/z*: 353 (M^+, <1), 204 (100), 158 (4), 149 (16), 121 (23), 103 (8), 93 (9), 76 (5), 65 (11), 56 (25). HR–ESI(+)-MS: Calcd for $[C_{19}H_{19}N_3O_4+H]^+$ 354.1448, found 354.1447; calcd for $[^{13}C^{12}C_{18}H_{19}N_3O_4+H]^+$ 355.1482, found 355.1482; calcd for $[(C_{19}H_{19}N_3O_4)_2+H]^+$ 707.2824, found 707.2821.

2-(4′-Carbamoylphenyl)-5-(4″-ethoxybenzoyl)-1,4,5,6-tetrahydropyrimidine hydrochloride (4gc). 270 mg (1.0 mmol) of **2h** and 200 mg (1.0 mmol) of **3c** afforded 128 mg (0.34 mmol, 34%) of **4hc** as white solid, mp 259°C (dec.). ^{1}H NMR δ: 1.36 (t, J = 6.9, 3H, Me), 3.42 (dd, J = 13.2, 9.3, 2H, H-4/6), 3.63 (dd, J = 13.0, 4.1, 2H, H-4/6), 3.77 (m, 1H, H-5), 4.14 (q, J = 6.9, 2H, CH_2O), 7.04 (d, J = 8.9, 2H, H-3″/5″), 7.52/8.02 (2 × br s, 2 × 1H, $CONH_2$), 7.84 (d, J = 8.5, 2H, H-2′/6′), 7.91 (d, J = 8.5, H-3′/5′), 8.01

(d, $J = 8.9$, 2H, H-2″/6″), NH-protons not observed. ^{13}C NMR δ: 14.4 (+), 37.2 (+), 44.1 (−), 63.5 (−), 114.4 (+), 126.1 (+), 127.2 (+),128.5, 130.6 (+), 135.4, 137.3, 152.9, 162.6, 198.6. MS *m/z*: 351 (M^+, 2), 304 (81), 202 (100), 184 (12), 149 (15), 103 (6), 93 (9), 65 (11), 56 (36), 44 (22). HR–ESI(+)-MS: Calcd for $[C_{20}H_{21}N_3O_3 + H]^+$ 352.1656, found 352.1653; calcd for $[^{13}C^{12}C_{19}H_{21}N_3O_3 + H]^+$ 353.1690, found 353.1690; calcd for $[(C_{20}H_{21}N_3O_3)_2 + H]^+$ 703.3239, found 703.3228.

5-(2″-Naphthoyl)-2-phenyl-1,4,5,6-tetrahydropyrimidine hydrochloride (4ha). 255 mg (1.0 mmol) of **2h** and 156 mg (1.0 mmol) of **3a** × **H_2O** afforded 110 mg (0.31 mmol, 31%) of **4ha** as white solid, mp >290°C. *Anal*., %: calcd for $C_{21}H_{19}N_2OCl$ C 71.89, H 5.46, N 7.98; found C 71.85, H 5.54, N 7.82. ^1H NMR δ: 3.68 (dd, $J = 13.6$, 6.7, 2H, H-4/6), 3.87 (dd, $J = 13.6$, 4.5, 2H, H-4/6), 4.49 (m, 1H, H-5), 7.62–7.77 (m, 5H, ArH), 7.82 (d, $J = 7.3$, 2H, ArH), 8.03–8.11 (m, 3H, ArH), 8.17 (d, $J = 7.8$, 1H, ArH), 8.90 (s, 1H, ArH), 10.46 (br s, 2H, NH). ^{13}C NMR δ: 34.9 (+), 41.0 (−), 123.7 (+), 127.1 (+), 127.6 (2 overlapping signals, 2 × +), 127.7 (+), 128.1, 128.6 (+), 129.0 (+), 129.6 (+), 130.8 (+), 132.1, 132.2, 133.3 (+), 135.3, 158.9, 197.9. MS *m/z*: 314 (M^+, 2), 159 (100), 155 (16), 145 (9), 127 (29), 117 (5), 104 (15), 77 (20), 56 (42), 41 (7). HR–ESI(+)-MS: Calcd for $[C_{21}H_{18}N_2O + H]^+$ 315.1492, found 315.1489; calcd for $[^{13}C^{12}C_{20}H_{18}N_2O + H]^+$ 316.1526, found 316.1524; calcd for $[(C_{21}H_{18}N_2O)_2 + H]^+$ 629.2911, found 629.2904.

5-(2″-Naphthoyl)-2-(3′-nitrophenyl)1,4,5,6-tetrahydropyrimidine hydrochloride (4hb). 255 mg (1.0 mmol) of **2h** and 166 mg (1.0 mmol) of **3b** afforded 250 mg (0.63 mmol, 63%) of **4hb** as pale yellowish solid, mp 281°C. *Anal*., %: calcd for $C_{21}H_{18}N_3O_3Cl$ C 63.72, H 4.58, N 10.61; found C 63.63, H 4.49, N 10.54. ^1H NMR δ: 3.67 (dd, $J = 13.4$, 7.4, 2H, H-4/6), 3.81 (dd, $J = 13.5$, 4.5, 2H, H-4/6), 4.45 (m, 1H, H-5), 7.69 (m, 2H, Naphth-H), 7.92 (t, $J = 8.0$, 1H, H-5′), 8.04/8.08/8.10/8.16 (4 × m, 4H, Naphth-H), 8.23 (ddd, $J = 7.8$, 1.5, 0.9, 1H, H-6′), 8.56 (ddd, $J = 8.1$, 2.1, 0.9, 1H, H-4′), 8.65 (t, $J = 2,1$, 1H, H-42), 8.87 (br s, 1H, H-1″), 10.62 (br s, 2H, NH). ^{13}C NMR δ: 34.9 (+), 41.3 (−), 122.9 (+), 123.7 (+), 127.1 (+), 127.4 (+), 127.7 (+) ,128.6 (+), 129.1 (+), 129.6 (+), 130.0, 130.7 (+), 130.8 (+), 132.2, 132.3, 134.1 (+), 135.3, 147.6, 156.9, 198.0. MS *m/z*: 359 (M^+, 1), 204 (100), 174 (66), 155 (40), 127 (77), 119 (20), 92 (13), 77 (20), 56 (96), 44 (29). HR–ESI(+)-MS: Calcd for $[C_{21}H_{17}N_3O_3 + H]^+$ 360.1343, found 360.1340; calcd for $[^{13}C^{12}C_{20}H_{17}N_3O_3 + H]^+$ 361.1377, found 361.1375; calcd for $[(C_{21}H_{17}N_3O_3)_2 + H]^+$ 719.2613, found 719.2607.

2-(4′-Carbamoylphenyl)-5-(2″-naphthoyl)-1,4,5,6-tetrahydropyrimidine hydrochloride (4hc). 255 mg (1.0 mmol) of **2h** and 200 mg (1.0 mmol) of **3c** afforded 140 mg (0.32 mmol, 32%) of **4hc** as white solid, mp 279°C (dec.). ^1H NMR δ: 3.49/3.71 (2 × m, 2 × 2H, H, H-4/6), 3.96 (m, 1H, H-5), 7.39 (br s, 1H, $CONH_2$), 7.62 (m, 2H, ArH), 7.87 (m, 4H, ArH), 7.89–8.06 (m, 4H, 3 × ArH, $CONH_2$), 8.16 (d, $J = 7.7$, 1H, ArH), 8.81 (s, 1H, ArH), NH not observed. ^{13}C NMR δ: 37.9 (+), 40.3 (−), 126.0 (+), 126.9 (+), 127.1 (+),127.6 (+),128.4 (+), 128.7 (+), 129.7 (+), 130.4 (+), 132.3, 133.1, 135.0, 135.2, 137.7, 152.6, 167.3, 200.5. MS *m/z*: 358 (M^+, 1), 202 (100), 184 (16), 165 (9), 155 (23), 147 (13), 127 (38), 77 (10), 56 (62), 44 (37). HR–ESI(+)-MS: Calcd for $[C_{22}H_{19}N_3O_2 + H]^+$ 358.1550, found 358.1546; calcd for $[^{13}C^{12}C_{21}H_{19}N_3O_2 + H]^+$ 359.1584, found 359.1579; calcd for $[(C_{22}H_{19}N_3O_3)_2 + H]^+$ 715.3027, found 715.3014.

5-(3″,4″-Dimethoxybenzoyl)-2-(3′-nitrophenyl)-1,4,5,6-tetrahydropyrimidine hydrochloride (4ib). 265 mg (0.92 mmol) of **2i** and 171 mg (1.03 mmol) of **3b** afforded 163 mg (0.40 mmol, 44%) of **4ib** as white solid, mp 260°C, dec. ^1H NMR δ: 3.61/3.79 (2 × m, 2 × 2H, 2 × H4/6) 3.84/3.88 (2 × s, 2 × 3H, 2 × OMe), 4.31 (m, 1H, H-5), 7.15 (d, $J = 8.6$, 1H, H-5″), 7.53 (d, $J = 2.0$, 1H, H-2″), 7.83 (dd, $J = 8.5$, 2.0, 1H, H-6″), 7.94 (dd, $J = 8.1$, 8.0, 1H, H-5′), 8.22 (ddd, $J = 7.9$, 1.6, 1.0, 1H, H-6′), 8.56 (ddd, $J = 8.4$, 2.2, 1.0, 1H, H-4′), 8.63 (t, $J = 2.0$, 1H, H-2′), 10.74 (br s, 2H, NH). ^{13}C NMR δ: 34.1 (−), 41.2 (+), 55.7 (+), 55.9 (+), 110.7 (+), 111.1 (+), 123.0, (+), 123.5 (+), 127.6 (+), 127.7, 129.5, 130.8 (+), 134.2 (+), 147.5, 148.9, 153.8, 157.1, 196.3. MS *m/z*: 369 (M^+, 1), 204 (100), 174 (12), 165 (19), 158 (5), 149 (4), 137 (6), 103 (5), 77 (9), 56 (42). HR–ESI(+)-MS: Calcd for $[C_{19}H_{19}N_3O_5 + H]^+$ 370.1397, found 370.1395; calcd for $[^{13}C^{12}C_{18}H_{19}N_3O_5 + H]^+$ 371.1431, found 371.1429; calcd for $[(C_{19}H_{19}N_3O_5)_2 + H]^+$ 739.2722, found 739.2728.

2-(4′-Carbamoylphenyl)-5-(3″,4″-dimethoxybenzoyl)-1,4,5,6-tetrahydropyrimidine hydrochloride (4ic). 285 mg (1.00 mmol) of **2i** and 206 mg (1.03 mmol) of **3c** afforded 163 mg (0.40 mmol, 40%) of **4ic** as white solid, mp 265°C (dec.). ^1H NMR δ: 3.60 (dd, $J = 13.5$, 6.7, 2H, H-4/6), 3.78 (dd, $J = 13.4$, 4.7, 2H, H-4/6), 3.84/3.88 (2 × s, 2 × 3H, 2 × OMe), 4.30 (m, 1H, H-5), 7.14 (d, $J = 8.6$, 1H, H-5″), 7.52 (d, $J = 2.0$, 1H, H-2″), 7.61/8.20 (2 × br s, 2 × 1H, $CONH_2$), 7.81 (dd, $J = 8.1$, 2.0, 1H, H-6″), 7.82 (d, $J = 8.4$, 2H, H-3′/5′), 8.09 (d, $J = 8.4$, 2H, H-3′/5′), 10.35 (br s, 2H, NH). ^{13}C NMR δ: 34.3 (+), 41.0 (−), 55.7 (+), 55.9 (+), 110.7 (+),111.2 (+),123.6 (+), 127.7, 127.9 (2 overlapping signals, +), 130.1, 138.2, 148.9, 153.7, 157.8, 166.6, 196.4. MS *m/z*: 367 (M+, 1), 204 (100), 174 (12), 165 (19), 158 (5), 149 (4), 137 (6), 103 (5), 77 (9), 56 (42). HR–ESI(+)-MS: Calcd for $[C_{20}H_{21}N_3O_4 + H]^+$ 368.1605, found 368.1601; calcd for $[^{13}C^{12}C_{19}H_{21}N_3O_4 + H]^+$ 369.1639, found 369.1637; calcd for $[(C_{20}H_{21}N_3O_4)_2 + H]^+$ 735.3137, found 735.3139.

2-(4′-Carbamoylphenyl)-5-(3″,4″-dichlorobenzoyl)-1,4,5,6-tetrahydropyrimidine hydrochloride (4kc). 170 mg (0.57 mmol) of **2k** and 161 mg (0.80 mmol) of **3c** afforded 201 mg (0.49 mmol, 62%) of **4kc** as white solid, mp 226°C. *Anal*., %: calcd for $C_{18}H_{16}N_3O_2Cl_3$ C 52.39, H 3.91, N 10.18; found C 52.28, H 4.01, N 10.06. ^1H NMR δ: 3.60 (dd, $J = 13.7$, 4.7, 2H, H-4/6), 3.80 (dd, $J = 13.5$, 4.6, 2H, H-4/6), 4.33 (m, 1H, H-5), 7.63/8.22 (2 × br s, 2 × 1H, $CONH_2$), 7.83 (d, $J = 8.4$, 2H, H-2′/6′), 7.92 (d, $J = 8.4$, 1H, H-5″), 8.03 (dd, $J = 8.4$, 2.0, 2H, H-6″), 8.08 (d, $J = 8.4$, 2H, H-3′/5′), 8.33 (d, $J = 2.0$, 1H, H-2″), 10.45 (br s, 2H, NH). ^{13}C NMR δ: 35.0 (+), 40.6 (–), 127.8 (+), 127.9 (+), 128.5 (+), 130.1, 130.5 (+), 131.3 (+), 132.1, 135.2, 136.7, 138.3, 158.1, 166.5, 197.3. MS *m/z*: 375 (M^+, 1), 202 (100), 188 (11), 173 (16), 164 (50), 148 (79), 120 (26), 103 (49), 56 (67), 44 (48). HR–ESI(+)-MS: Calcd for $[C_{18}H_{15}N_3O_2^{35}Cl_2 + H]^+$ 376.0614, found 376.0613; calcd for $[C_{18}H_{15}N_3O_2^{35}Cl^{37}Cl + H]^+$ 378.0585, found 378.0583; calcd for $[C_{18}H_{15}N_3O_2^{35}Cl_2 + C_{18}H_{15}N_3O_2^{35}Cl^{37}Cl + H]^+$ 753.1126, found 753.1138.

5-(4″-Biphenyloyl)-2-phenyl-1,4,5,6-tetrahydropyrimidine hydrochloride (4la). 302 mg (1.0 mmol) of **2l** and 156 mg (1.0 mmol) of **3a** × **H_2O** afforded 112 mg (0.30 mmol, 30%) of **4la** as white solid, mp = 283°C (dec.). ^{1}H NMR δ: 3.64 (dd, J = 13.5/6.6, 2H, H-4/6), 3.82 (dd, J = 13.5/4.6, 2H, H-4/6), 4.36 (m, 1H, H-5), 7.43–7.57 (m, 3H, H-4′/3‴/5‴), 7.63 (t, J = 7.8, 2H, H-3′/5′), 7.71–7.82 (m, 5H, ArH), 7.91 (d, J = 8.3, 2H, H-3″/5″), 8.17 (d, J = 8.3, 2H, H-2″/6″), 10.43 (br s, 2H, NH). 10.06 (br s, 2H, NH). ^{13}C NMR δ: 34.9 (+), 40.8 (–), 127.0 (+), 127.1 (+), 127.6 (+), 128.0, 128.5 (+), 129.0 (+), 129.1 (+), 129.3 (+), 133.3 (+), 133.6, 138.6, 145.2, 158.8, 197.5. MS *m/z*: 340 (M^+, 1), 310 (4), 181 (16), 159 (100), 152 (14), 145 (7), 117 (4), 104 (12), 77 (10), 56 (27). HR–ESI(+)-MS: Calcd for $[C_{23}H_{20}N_2O + H]^+$ 341.1648, found 341.1646; calcd for $[^{13}C^{12}C_{22}H_{20}N_2O + H]^+$ 342.1682, found 342.1679; calcd for $[(C_{23}H_{20}N_2O)_2 + H]^+$ 681.3224, found 681.3222.

5-(4″-Biphenyloyl)-2-(3′-nitrophenyl)-1,4,5,6-tetrahydropyrimidine hydrochloride (4lb). 300 mg (1.0 mmol) of **2l** and 166 mg (1.0 mmol) of **3b** afforded 224 mg (0.53 mmol, 53%) of **4lb** as pale yellow solid, mp >280°C. ^{1}H NMR δ: 3.64 (dd, J = 13.3, 7.2, 2H, H-4/6), 3.85 (dd, J = 13.6, 4.6, 2H, H-4/6), 4.37 (m, 1H, H-5), 7.42–7.55 (m, 3H, H-4″/H-3‴/5‴), 7.79 (d, J = 7.3, 2H, H-2‴/6‴), 7.90–7.97 (m, 3H, H-5′/3″/5″), 8.18 (d, J = 8.4, 2H, H-2″/6″), 8.23 (d, J = 7.9, 1H, H-6′), 8.55 (d, J = 8.4, 1H, H-4′), 8.65 (br m, 1H, H-2′), 10.77 (br s, 2H, NH). ^{13}C NMR δ: 34.9 (+), 41.0 (–), 123.0 (+), 127.0 (+), 127.1 (+), 127.4 (+), 128.5 (+), 129.0 (+), 129.3 (+), 129.7, 130.7 (+), 133.7, 134.2 (+), 138.6, 145.2, 147.5, 156.9, 197.6.MS *m/z*: 385 (M^+, <1), 351 (7), 204 (100), 181 (29), 174 (22), 152 (31), 103 (8), 76 (9), 56 (66), 44 (34). HR–ESI(+)-MS: Calcd for $[C_{23}H_{19}N_3O_3 + H]^+$ 386.1499, found 386.1494; calcd for $[^{13}C^{12}C_{22}H_{19}N_3O_3 + H]^+$ 387.1533, found 387.1533; calcd for $[(C_{23}H_{19}N_3O_3)_2 + H]^+$ 771.2926, found 771.2917.

5-(4″-Bromobenzoyl)-2-(3′-nitrophenyl)-1,4,5,6-tetrahydropyrimidine hydrochloride (4mb). 610 mg (2.0 mmol) of **2m** and 332 mg (2.0 mmol) of **3b** afforded 395 mg (0.93 mmol, 47%) of **4mb** as yellowish solid, mp 230°C. ^{1}H NMR δ: 3.60 (dd, J = 13.6, 7.0, 2H, H-4/6), 3.81 (dd, J = 13.6, 4.6, 2H, H-4/6), 4.30 (m, 1H, H-5), 7.83 (d, J = 8.6, 2H, H-3″/5″), 7.93 (t, J = 8.0, 1H, H-5′), , 8.02 (d, J = 8.6, 2H, H-2″/′6″), 8.23 (d, J = 8.4, 1H, H-6′), 8.55 (dd, J = 8.3, 2.3, 1H, H-4′), 8.61 (t, J = 2.0, 1H, H-2′), 10.81 (br s, 2H, NH). ^{13}C NMR δ: 34.8 (+), 40.8 (–), 123.0 (+), 127.6 (+), 128.1, 129.4, 130.5 (+), 130.7 (+), 132.0 (+), 133.9, 134.3 (+), 147.5, 157.1, 197.2. MS *m/z*: 387/385 (M^+, 1), 204 (100), 185/183 (12), 174 (12), 157/155 (9), 131 (8), 119 (5), 103 (18), 76 (18), 56 (55). HR–ESI(+)-MS: Calcd for $[C_{17}H_{14}N_3O_3^{79}Br + H]^+$ 388.0291, found 388.0290; calcd for $[C_{17}H_{14}N_3O_3^{81}Br + H]^+$ 390.0271, found 390.0268; calcd for $[^{13}C^{12}C_{13}H_{14}N_3O_3^{81}Br + H]^+$ 391.0305, found 391.0304.

2-(4′-Carbamoylphenyl)-5-(4″-bromobenzoyl)-1,4,5,6-tetrahydropyrimidine hydrochloride (4mc). 610 mg (2.00 mmol) of **2a** and 400 mg (2.00 mmol) of **3c** afforded 364 mg (0.86 mmol, 42%) of **4mc** as white solid, mp 272°C (dec.). ^{1}H NMR δ: 3.60 (dd, J = 13.6, 6.6, 2H, H-4/6), 3.79 (dd, J = 13.4, 4.5, 2H, H-4/6), 4.29 (m, 1H, H-5), 7.63/8.24 (2 × br s, 2 × 1H, $CONH_2$), 7.82 (d, J = 8.4, 2H, H-3″/5″), 7.85 (d, J = 8.3, 2H, H-2′/6′), 8.03 (d, J = 8.4, 2H, H-2″/′6″), 8.08 (2 × d, J = 8.3, 2 × 2H, H-3′/5′), 10.50 (br s, 2H, NH). ^{13}C NMR δ: 34.9 (+), 40.7 (–), 127.7 (+), 127.9 (+), 128.1, 130.2, 132.0 (+), 133.9, 138.3, 158.1, 166.5, 197.3. MS *m/z*: 387/385 (M^+, 1), 202 (100), 185/183 (15), 164 (24), 148 (34), 130 (13), 103 (13), 76 (31), 56 (66), 44 (34). HR–ESI(+)-MS: Calcd for $[C_{18}H_{16}N_3O_2^{79}Br + H]^+$ 386.0499, found 386.0498; calcd for $[C_{18}H_{16}N_3O_2^{81}Br + H]^+$ 388.0478, found 388.0478; calcd for $[C_{18}H_{16}N_3O_2^{79}Br + C_{18}H_{16}N_3O_2^{81}Br + H]^+$ 773.0904, found 773.0916.

2-Phenyl-5-(3″,4″,5″-trimethoxybenzoyl)-1,4,5,6-tetrahydropyrimidine hydrochloride (4na). 631 mg (2.0 mmol) of **2n** and 313 mg (2.0 mmol) of **3a** × **H_2O** afforded 232 mg (0.60 mmol, 30%) of **4na** as white solid, mp 278°C. ^{1}H NMR δ: 3.59 (dd, J = 13.6, 6.7, 2H, H-4/6), 3.77/3.88 (2 × s, 3H/6H, 3 × OMe), 3.79 (m, 2H, H-4/6), 4.41 (m, 1H, H-5), 7.37 (s, 2H, H-2″/6″), 7.63 (m, 2H, H-3′/H-5′), 7.74 (m, 1H, H-4′), 7.81 (d, J = 7.3, 2H, H-2′/6′), 10.41 (br s, 2H, NH). ^{13}C NMR δ: 34.4 (+), 40.9 (–), 56.3 (+), 60.2 (+), 106.3 (+), 127.7, (+), 127.9, 129.0 (+), 130.1, 133.3 (+), 142.6, 153.0, 158.6, 197.0. MS *m/z*: 354 (M^+, 1), 195 (6), 179 (2), 168 (2), 159 (100), 145 (5), 117 (3), 104 (9), 77 (7), 56 (23). HR–ESI(+)-MS: Calcd for $[C_{20}H_{22}N_2O_4 + H]^+$ 355.1652, found 355.1650; calcd for $[^{13}C^{12}C_{19}H_{22}N_2O_4 + H]^+$ 356.1686, found 356.1685; calcd for $[(C_{20}H_{22}N_2O_4)_2 + H]^+$ 709.3232, found 709.3232.

2-(3′-Nitrophenyl)-5-(3″,4″,5″-trimethoxybenzoyl)-1,4,5,6-tetrahydropyrimidine hydrochloride (4nb). 631 mg (2.0 mmol) of **2m** and 332 mg (2.0 mmol) of **3b** afforded 275 mg (0.63 mmol, 32%) of **4nb** as slightly yellowish solid, mp >269°C (dec.). ^{1}H NMR δ: 3.60 (dd, J = 13.5, 6.7, 2H, H-4/6), 3.78/3.88 (2 × s, 3H/6H, 3 × OMe), 3.81 (dd, J = 13.5, 4.2, 2H, H-4/6), 4.42 (m, 1H, H-5), 7.39 (s, 2H, H-2″/6″), 7.94 (t, J = 8.0, 1H, H-5′), 8.29 (d, J = 8.0, 1H, H-6′), 8.55 (dd, J = 8.3, 2.2, 1H, H-4′), 8.67 (m, 1H, H-2′), 10.94 (br s, 2H, NH). ^{13}C NMR δ: 34.1 (+), 41.0 (–), 56.3 (+), 60.2 (+), 106.4 (+), 123.1 (+), 127.6, (+), 129.3, 130.1, 130.7 (+), 134.4 (+), 142.7, 147.5, 153.0, 157.0, 197.0. MS *m/z*: 399 (M^+, 1), 204 (100), 195 (11), 174 (21), 153 (7), 149 (4), 119 (4), 103 (6), 71 (5), 56 (22). HR–ESI(+)-MS: Calcd for $[C_{20}H_{21}N_3O_6 + H]^+$ 400.1503, found 400.1500; calcd for $[^{13}C^{12}C_{19}H_{21}N_3O_6 + H]^+$ 401.1537, found 401.1534; calcd for $[(C_{20}H_{22}N_2O_4)_2 + H]^+$ 799.2933, found 799.2936.

2-(4′-Carbamoylphenyl)-5-(3″,4″,5″-trimethoxybenzoyl)-1,4,5,6-tetrahydropyrimidine hydrochloride (4nc). 631 mg (2.0 mmol) of **2n** and 399 mg (2.0 mmol) of **3c** afforded 230 mg (0.52 mmol, 26%) of **4nc** as white solid, mp 263°C (dec.). ^{1}H NMR δ: 3.60 (dd, J = 13.8, 6.9, 2H, H-4/6), 3.78/3.88 (2 × s, 3H/6H, 3 × OMe), 3.79 (m, 2H, H-4/6), 4.41 (m, 1H, H-5), 7.38 (s, 2H, H-2″/6″), 7.64/8.24 (2 × br s, 2 × 1H, $CONH_2$), 7.87 (d, J = 8.4, 2H, H-2′/6′), 8.09 (d, J = 8.4, 2H, H-3′/5′), 10.52 (br s, 2H, NH). ^{13}C NMR δ: 34.3 (+), 41.0 (–), 56.3 (+), 60.2 (+), 106.3 (+), 127.7 (+), 127.9 (+), 130.1, 136.5, 138.3, 142.7, 153.0, 158.2, 167.2, 197.0. MS *m/z*: 397 (M^+, 1), 202 (14), 164 (84), 148 (100), 120 (30), 103 (48), 76 (18), 65 (23), 51 (24), 44 (54). HR–ESI(+)-MS: Calcd for $[C_{21}H_{23}N_3O_5 + H]^+$ 398.1710, found 398.1708; calcd for $[^{13}C^{12}C_{20}H_{23}N_3O_5 + H]^+$ 399.1744, found 399.1742; calcd for $[(C_{21}H_{23}N_3O_5)_2 + H]^+$ 795.3348, found 795.3356.

Biological testing. L-Arginine, L-NNA, dithiothreitol, hemoglobin, superoxide dismutase (SOD), catalase, human iNOS, and porcine brain CaM were purchased from Sigma.

(6R)-5,6,7,8-Tetrahydrobiopterin was purchased from Alexis (Coger, France) and NADPH from Boehringer. Recombinant nNOS was isolated and purified from the yeast *Saccharomyces cerevisiae* transformed with a plasmid containing rat brain nNOS [75]. Full length recombinant murin iNOS and bovine eNOS were expressed in *Escherichia coli* and purified in the absence of H_4B and L-arginine as described previously [76,77]. They were estimated to be more than 95% pure by SDS–PAGE electrophoresis.

Nitrite determination (Griess assay). The formation of nitrite (stable end-product of NO) was followed by the reaction with sulphanilamide and 1-naphthylethylenediamine hydrochloride in hydrochloric acid. The absorption of the resulting azo dye was measured at 543 nm on a Beckman DU7500 UV/vis spectrophotometer (Beckman Coulter, Fullerton, USA). Because NADPH can affect low values by reducing the diazonium cation as well as the azo dye, it was necessary to remove NADPH from the reaction using an additional incubation with lactate dehydrogenase and sodium pyruvate [73].

Buffers contained 50 mM triethanolamine (iNOS and eNOS, pH = 7.5; nNOS, pH = 7.0) and 50 mM triethanolamine, 1 mM CHAPS, 10 mM 2-mercaptoethanol, and 0.5 mM EDTA. Typical assay mixtures contained 0.5 mM Ca^{++} (nNOS and eNOS), 1 mM Mg^{++} (iNOS), 5 μM flavine adenine dinucleotide, 5 μM flavine mononucleotide, 0.5 mM NADPH, 10 μM H4B, 10 μg/mL CaM, 0.5 mM (nNOS), or 1 mM L-arginine (iNOS and eNOS) in a total volume of 80 μL. These mixtures were incubated at 37°C (nNOS 30 min, iNOS, and eNOS 20 min) after the addition of proteins. Then a solution of 1.6 mM pyruvic acid, sodium salt, and 20 U/mL L-lactic dehydrogenase (20 μl) was added. These preparations were incubated for 20 min at 37°C, and the reactions were stopped by the addition of 50 μL ice cold acetonitrile and centrifugation (15,000 rpm, 4°C). Then 12 μL each of a solution of 5.8 mM naphthylethylendiamine dihydrochloride and 52 mM sulfanilamide in 1 M HCl were added to 120 μL of the supernatants. Absorbances at 543 nm were measured after 5 min at 20°C. All data were referred to 100% control experiments containing 1% DMSO but without inhibitor. All experimental data are means of four determinations.

Hemoglobin assay. The rates of NO synthesis were determined at 37°C on an Uvikon 941 spectrophotometer. Usual incubation mixtures were performed in 1-cm path length cuvettes (total volume 150 μL) containing 50 mM Hepes buffer (pH 7.4) with 0.1 M KCl supplemented with DTT (5 mM), oxyhemoglobin (10–20 μM), 1000 U/mL each of SOD and catalase, 10 μM each of H_4B and L-arg, $CaCl_2$ (1 mM), CaM (10 μg/mL), NADPH (1 mM), and inhibitors. Compounds were added to the incubation mixtures as 1.5 μL solutions in DMSO. The mixtures were preincubated for 2 min at 37°C prior to initiation of the reaction by the addition of 2–5 μL aliquots of NOS to the sample cuvettes. The NO-mediated conversion of oxyhemoglobin to methemoglobin was monitored by repetitive scanning between 380 and 480 nm every 0.2 min and quantitated using an extinction coefficient of $77\ mM^{-1}.cm^{-1}$ between peak at 401 nm and valley at 420 nm [72]. The production of NO was linear over all the entire reaction time for each isoform. Control incubations contained similar amounts (1%) of DMSO without inhibitor. All values are expressed relative to the DMSO control.

Effects of THP on NADPH consumption by nNOS. The initial rates of NADPH oxidation by nNOS were quantitated spectrophotometrically at 340 nm by using an extinction coefficient of $6.2\ mM^{-1} \times cm^{-1}$. Cuvettes contained 150 μL of 50 mM Hepes, pH 7.4, 5 mM DTT, 100 μM $CaCl_2$, 200 μM NADPH, 10 μM H_4B, 100 μM L-arginine, and the tested agents. After preincubating the cofactor mixture and the inhibitor for 2 min at 37°C, the nNOS sample (final concentration 15–50 nM) was added, and the absorption at 340 nm was followed for 3 min. Control incubations were run in the presence of 1% DMSO, in the absence of NADPH, or in the presence of 1 mM L-NNA, a known inhibitor of NADPH consumption by NOS [74].

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