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Fluorinated dihydroquinolines as potent *n*-NOS inhibitors

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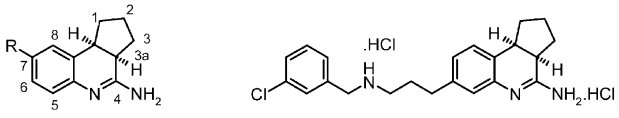
Dedicated to Professor Wolfgang Steglich on the occasion of his 70th birthday.

Abstract—Fluorinated dihydroquinolines showed reduced basicity of the amidine function. Their syntheses and potencies as neuronal nitric oxide synthase (*n*-NOS) inhibitors are reported.

Nitric oxide synthases (NOS) catalyze the formation of nitric oxide (NO) through conversion of arginine into citrulline. NO acts as a messenger in diverse tissues, however, elevated NO levels in the brain may be associated with neurotoxicity, especially in the wake of an ischemic event.[1] Therefore, inhibition of the neuronal isoform, *n*-NOS, may be regarded as a possible therapeutic strategy to treat neurodegenerative diseases.[2] Besides *n*-NOS, two further enzyme isoforms are known, inducible nitric oxide synthase (*i*-NOS), which is predominantly expressed in macrophages and enacts host defense in the immune system, and endothelial NOS (*e*-NOS) expressed in the lining of blood vessels and playing a pivotal role in vascular homeostasis. When designing *n*-NOS inhibitors, selectivity for *n*-NOS relative to *e*-NOS is of paramount importance in order to avoid adverse cardiovascular effects. In contrast, additional *i*-NOS inhibition appears to be advantageous, as increased *i*-NOS expression has also been observed during cerebral ischemia.[3]

Recently, we described dihydroquinoline **1a** and derivatives, for example **1b**, as novel *n*-NOS inhibitors (Fig. 1).[4] Introduction of an amine-containing side-chain gave particularly potent and selective inhibitors, for example **2**.[5] Since the compounds are targeted to the brain, passage through the blood brain barrier (BBB) is crucial. Brain penetration is linked to the basicity of agents, with too basic compounds prevailing as protonated species at physiological pH (7.4) and thus being unable to pass the BBB. According to the Henderson–Hasselbalch equation, for our lead **1a** with a pK_a value[6] of 9.7 only a small fraction of compound ($f_u = 0.5\%$) is unprotonated at pH 7.4.[7] In an effort to increase the amount of non-ionized species, fluorine was introduced at the benzene core and at the 3a-position to lower the pK_a of the amidine function.[8]



1a R = H
1b R = Cl

2

Figure 1.

The syntheses of analogues fluorinated at the benzene core have been described previously[4] and are given for compounds **8a–c** in Scheme 1. Boronic acids **4** were obtained through *ortho*-lithiation of *N*-pivaloyl fluoroanilines **3**. Suzuki coupling with triflate **5** and acidic hydrolysis gave quinolones **6**. Reduction to dihydroquinolones **7** was accomplished with magnesium, predominantly yielding the *cis*-stereoisomer. The sequence was completed by a two-step conversion of lactams **7** into the amidines **8**.

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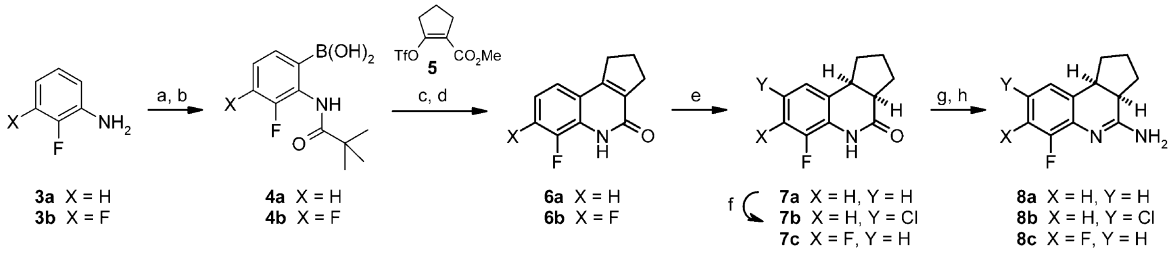
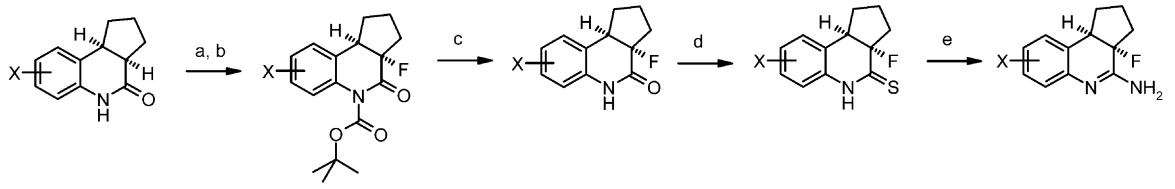


Scheme 1. (a) *t*-BuCOCl, Py; (b) *n*-BuLi, THF, $B(OMe)_3$; aq HCl; (c) **5**, $Pd(PPh_3)_4$, Na_2CO_3, DME–H_2O; (d) concd HCl; (e) Mg, MeOH; (f) NCS, DMF; (g) Lawesson's reagent, DME; (h) NH_3, MeOH.



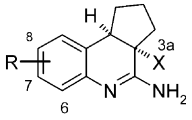
Scheme 2. (a) Boc_2O, DMAP, THF; (b) $(PhSO_2)_2NF$, KHMDS or $LiNEt_2$, THF, −78 °C; (c) CF_3CO_2H, CH_2Cl_2; (d) Lawesson's reagent, THF; (e) NH_3, MeOH.

Introduction of a fluoro-substituent at C-3a was realized through an electrophilic fluorination reaction (Scheme 2). Lactams **9** were N-protected with Boc_2O, deprotonated at C-3a, and subjected to *N*-fluorobenzenesulfonimide treatment. Removal of the protecting group and thionation gave thiolactams **12**, with the stereochemical assignment being confirmed by X-ray analysis of **12a**. Finally, ammonolysis delivered the amidines **13**.

First, we examined the basicity of analogues carrying a fluorine atom at the benzene ring in close proximity to the amidine. Literature data indicate that the basicity of fluoro anilines is most markedly influenced by fluoro substitution in close proximity, that is, at the *ortho*-position, to the amino group.[9] Indeed, as can be derived from Table 1, the pK_a is reduced by an order of magnitude when a 6-fluoro substituent is introduced (**8a**). A chloro substituent in *para*-position (cf. **8b**) leads to a further decrease, which is more pronounced when a second fluorine is added (cf. **8c**). Fluorination at C-3a was even more effective in decreasing the basicity: **13a** was less basic than difluoro analogue **8c**. Addition of a chlorine at C-8 (**13b**) again lowered the pK_a by further 0.3 units, and combination of 3a- and 6-fluorination resulted in compound **13c**, which is three orders of magnitude less basic than the original lead, **1a**, and remains predominantly unprotonated at physiological pH ($f_{u(7.4)} = 86\%$). In terms of potency, the core fluorinated analogues **8b** and **8c** are less potent inhibitors of *n*-NOS than **1a**, whereas **8a** is equipotent.[4] Equally, **13a** is as potent and selective as its non-fluorinated counterpart **1a**, which shows that reduced amidine basicity does not affect potency. However, substitution at the benzene core in the 3a-fluoro series is not as well tolerated as in the non-fluorinated series: The 8-chloro analogue **13b** is 2-fold less potent than **1b** and the potency of difluoro analogue **13c** drops 3-fold with respect to **1a** and **8a**.

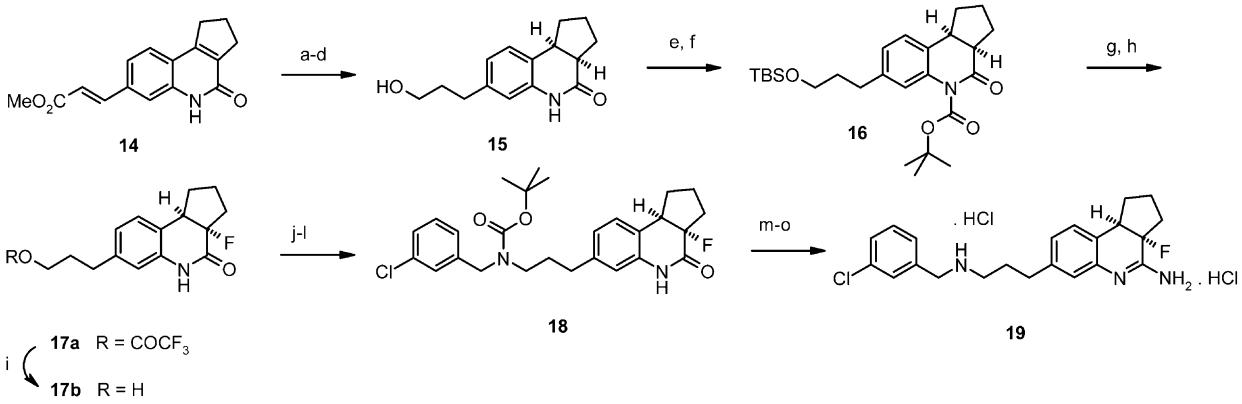
Based on these results, we set out to further explore the 3a-fluoro series by attaching aminoalkyl residues, that is chlorobenzylaminopropyl, methylaminoethyl, and methylaminomethyl, at the 7-position (cf. **2**). The 3a-fluoro analogue of **2**, **19**, was prepared as described in Scheme 3. Acrylester **14**[5] was transformed into propyl alcohol **15** and protected as *tert*-butyldimethylsilyl (TBS) ether. The lactam was *tert*-butoxy carbonylated and fluorinated to **16**. Removal of both the Boc and TBS protecting groups was accomplished with trifluoroacetic acid leading to trifluoroacetate **17a** and alcohol **17b**. The former was converted into the latter by treatment with

Table 1. Inhibition of NOS isoforms by 3a-fluorinated and non-fluorinated dihydroquinolines

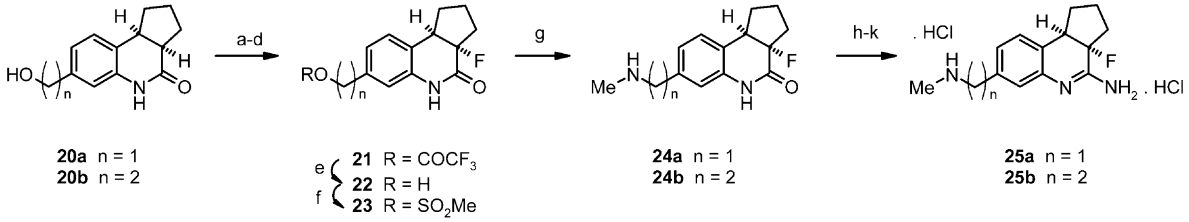


Compd	R	X	pK_a[b]	$f_{u(7.4)}$[c](%)	IC$_{50}$ (μM)[a]			Selectivity	
					n-NOS	*e*-NOS	*i*-NOS	*e*/*n*[d]	*i*/*n*[d]
1a	H	H	9.7	0.5	0.16	3.3	2.7	21	17
1b	8-Cl	H	9.4	1	0.14	6.2	5.7	44	41
8a	6-F	H	8.5	7	0.17	5.8	2.3	34	14
8b	8-Cl, 6-F	H	8.2	14	0.29	14	8.1	48	28
8c	6,7-F_2	H	8.0	20	0.68	20	5.1	29	8
13a	H	F	7.9	24	0.10	2.7	2.1	27	21
13b	8-Cl	F	7.6	39	0.29	14	8.1	48	28
13c	6-F	F	6.6	86	0.59	22	25	42	37

[a] NOS activity was determined with recombinant human enzyme based on the method given in ref 10.
[b] The pK_a of the corresponding acid was determined by photometric titration.[11]
[c] See ref 7.
[d] *e*/*n* represents IC$_{50}$(*e*-NOS)/IC$_{50}$(*n*-NOS) and *i*/*n* represents IC$_{50}$(*i*-NOS)/IC$_{50}$(*n*-NOS).

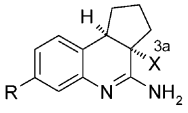


Scheme 3. (a) Mg, MeOH; (b) NaOH, EtOH–THF; H_2SO_4; (c) $ClCO_2Et$, Et_3N, THF; (d) $NaBH_4$, MeOH; (e) TBSCl, imidazole, DMF; (f) Boc_2O, DMAP, THF; (g) $(PhSO_2)_2NF$, KHMDS, THF; (h) CF_3CO_2H, CH_2Cl_2; (i) K_2CO_3, MeOH; (j) $(COCl)_2$, DMSO, Et_3N, CH_2Cl_2; (k) 3-$ClC_6H_4CH_2NH_2$, $NaBH(OAc)_3$, AcOH, $(CH_2Cl)_2$; (l) Boc_2O, cat DMAP, CH_2Cl_2; (m) Lawesson's reagent, THF; (n) NH_3, MeOH; (o) HCl, dioxane.



Scheme 4. (a) TBSCl, imidazole, DMF; (b) Boc_2O, DMAP, CH_2Cl_2; (c) $(PhSO_2)_2NF$, KHMDS, THF; (d) CF_3CO_2H, CH_2Cl_2; (e) K_2CO_3, MeOH; (f) $MeSO_2Cl$, Et_3N, CH_2Cl_2; (g) $MeNH_2$, MeOH; (h) Boc_2O, cat DMAP, CH_2Cl_2; (i) Lawesson's reagent, THF; (j) NH_3, MeOH; (k) HCl, dioxane.

Table 2. Inhibition of NOS isoforms by 3a-fluorinated 7-(aminoalkyl)dihydroquinolines



Compd	R	X	pK_a[b]	IC_{50} (μM)[a]			Selectivity	
				n-NOS	*e*-NOS	*i*-NOS	*e*/*n*[c]	*i*/*n*[c]
2	3-ClBnNHCH$_2$CH$_2$CH$_2$	H	9.9	0.042	9.4	0.58	224	14
19	3-ClBnNHCH$_2$CH$_2$CH$_2$	F	8.0	0.31	38	2.2	123	7
25a	MeNHCH$_2$CH$_2$	F	7.9	0.39	79	1.9	203	5
25b	MeNHCH$_2$	F	n.d.	0.66	66	1.4	100	2

[a] NOS activity was determined with recombinant human enzyme based on the method given in ref 10.
[b] The pK_a of the amidine was determined by photometric titration; n.d. means not determined.[11]
[c] *e*/*n* represents IC_{50}(*e*-NOS)/IC_{50}(*n*-NOS) and *i*/*n* represents IC_{50}(*i*-NOS)/IC_{50}(*n*-NOS).

potassium carbonate in methanol. Swern oxidation, reductive amination and Boc protection of the secondary amine yielded lactam **18** which was transformed into the final product **19** through the standard operations.[5]

Methylaminomethyl and methylaminoethyl analogues **25a** and **25b** were synthesized in a similar fashion to **19** (Scheme 4). The methylamino group was introduced through a nucleophilic displacement reaction of mesylates **23** with methylamine.

The biological data for the fluorinated 7-(aminoalkyl)-dihydroquinolines are given in Table 2 compared to the non-fluorinated analogue **2**. The data confirm the trend that substitution at the benzene core of 3a-fluorodihydroquinolines is critical; a drop in potency for all fluorinated derivatives is observed, though an interesting selectivity profile with >100-fold selectivity against *e*-NOS and a decent inhibitory potency against *i*-NOS is maintained. The 3a-fluoro analogue **19** loses out 8-fold compared to **2** and is threefold less potent than the unsubstituted **13a**. Truncation of the side chain as in **25a** and **25b** does not improve potency, but for **25a** an enhanced selectivity versus *e*-NOS is observed.

In summary, fluorination at 3a proves to be an efficient means of reducing the basicity of dihydroquinolines and maintaining potent *n*-NOS inhibition. Substitution at the benzene ring in the 3a-fluoro series is not as broadly tolerated as in the non-fluorinated series. In particular,

the introduction of amine-containing side chains, while not improving potency, gives highly selective *n*-NOS inhibitors.

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