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Dihydroquinolines with Amine-Containing Side Chains as Potent n-NOS Inhibitors

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Abstract—Dihydroquinolines with aminoalkyl side chains have been synthesized and have been shown to be potent n-NOS inhibitors. A marked selectivity versus e-NOS of up to approximately 300-fold was observed, whereas i-NOS was moderately inhibited.

Elevated brain levels of nitric oxide (NO) are thought to cause neuronal damage in the wake of an ischemic cerebral incident.[1] NO is formed in central and peripheral neurons through transformation of arginine into citrulline by constitutive neuronal nitric oxide synthase (n-NOS),[2] but inducible nitric oxide synthase (i-NOS), though predominantly expressed in macrophages and enacting host defense in the immune system, may also be expressed in neurons under pathophysiological conditions.[3] A third NOS isoform (e-NOS) is located in the endothelial lining of blood vessels where it acts to modulate vascular tone and inhibit platelet aggregation.

A neuroprotective approach to the treatment of degenerative disease conditions has been targeted at the suppression of NO production with an n-NOS inhibitor. Due to the vascular effects of endothelial NO, it is of paramount importance to identify an n-NOS inhibitor having minimal interaction with e-NOS.[4] In contrast, selectivity for i-NOS seems to be less important. Since i-NOS activity is also linked to neurodegenerative processes,[3] a dual acting n-NOS/i-NOS inhibitor might even show added benefit.

Recently, we described dihydroquinolines of type **1** as novel n-NOS inhibitors (Chart 1).[5] In order to further increase potency and selectivity, we took into consideration data published in the n-NOS field by groups at Astra[6] and Glaxo,[7] who reported on highly potent and selective amidine-based n-NOS inhibitors containing a basic amine side-chain as depicted in Chart 1. For dipeptide-based n-NOS inhibitors, Silverman et al.[8] have shown that isoform selectivity is greatly enhanced by adding amine-containing side chains. We set out to improve our dihydroquinoline lead by drawing on these observations. Since our early SAR[5] indicated that a large substituent was poorly accommodated at C-8, we chose to attach aminoalkyl residues at the C-7 position.[9] Five derivatives were synthesized: (1) to examine the importance of the distance between aromatic ring and basic center (cf. **2a**, **2b**, **2d**); (2) to assess a potential synergy with an 8-chloro substituent, identified as favorable in our original SAR (cf. **2c**); (3) and to

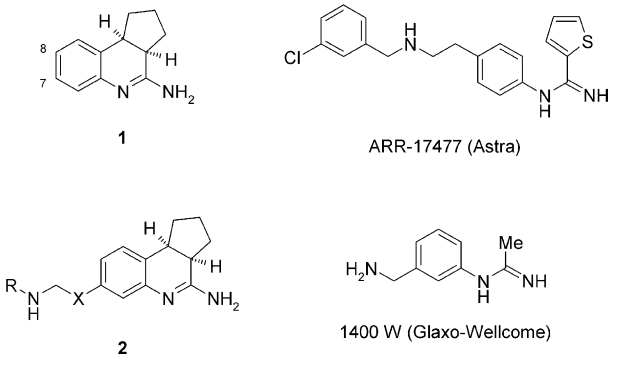

Chart 1.

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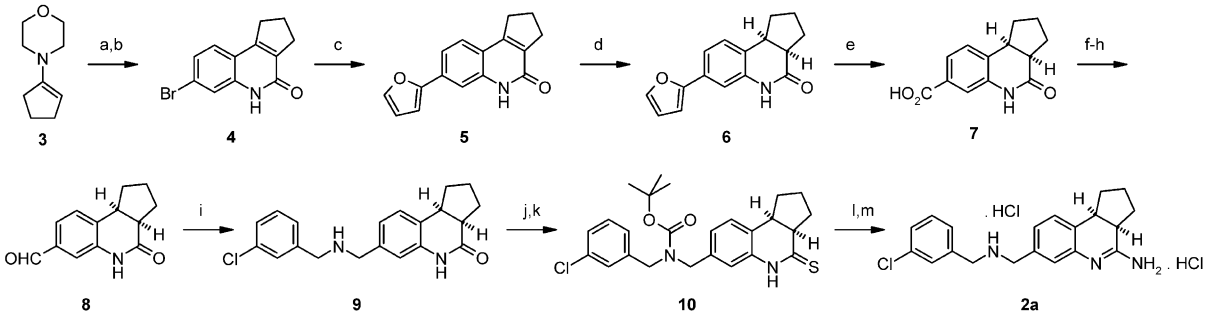
study the impact of a simpler, less lipophilic group, such as the 2-(methylamino)ethoxy residue (cf. **2e**). The syntheses and biological activity of aminoalkyl-dihydroquinolines **2a–e** are reported herein.

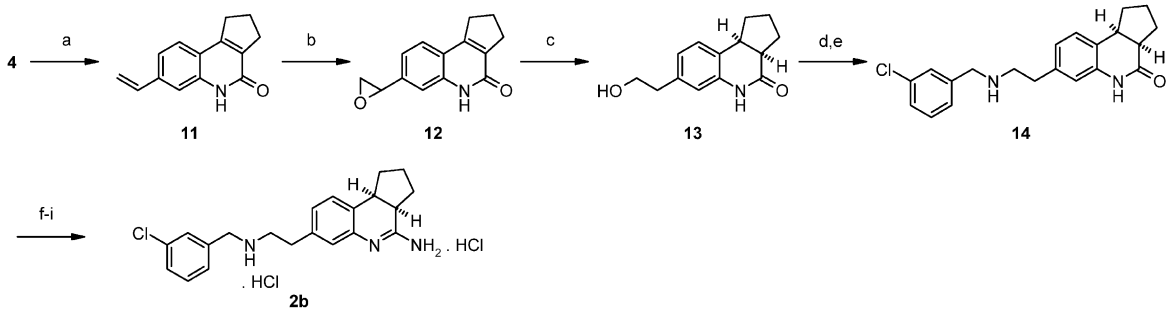
In general, the syntheses have been accomplished in three phases: (1) synthesis of the dihydroquinolone core, (2) construction of the aminoalkyl side chain, and (3) transformation of the quinolone into the amidine. The dihydroquinolone synthesis followed the general scheme given in ref 5. Starting with 4-bromo isocyanate and enamine **3** according to the Käppler route[10] led to quinolone **4** carrying a 7-bromo substituent which was a suitable handle for either Stille or Heck chemistry (Scheme 1). Coupling with furylstannane[11] introduced a latent carboxylate group[12] into **5** which was unmasked at the stage of dihydroquinoline **6** through Ru(VIII) oxidation[13] delivering **7**. Two-step conversion via reduction to the alcohol[14] and Griffith–Ley oxidation[15] to aldehyde **8** set the stage for reductive amination with 3-chlorobenzylamine.[16] The secondary amine functionality in **9** was protected as urethane, and the lactam group was transformed into the amidine group after thionation[17] and ammonolysis as described earlier.[5] Finally, the *tert*-butoxycarbonyl group was cleaved with hydrochloric acid yielding compound **2a**.

For the ethyl homologue **2b** the sequence had to be modified (Scheme 2). Stille coupling with vinyl-stannane[18] and oxidation with *m*CPBA gave epoxide **12**. Upon treatment with magnesium both double bond and epoxide reduction took place leading to 7-(2-hydroxy-ethyl)dihydroquinoline **13**, which could be oxidized to the highly sensitive homobenzaldehyde with Dess–Martin's periodinane (DMP)[19] only. The crude aldehyde was subjected to the reductive amination protocol and converted into **2b** as described for **2a**.

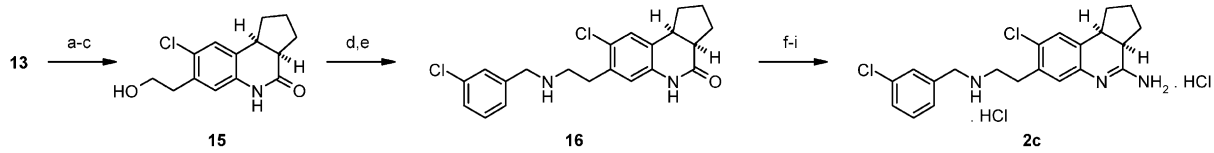
Homobenzylalcohol **13** was chlorinated at C-8 with *N*-chlorosuccinimide in DMF after protection as an acetate (Scheme 3).[20] Due to the 8-chloro substituent, oxidation to the homobenzaldehyde was less complicated than in the deschloro case and could be achieved under classical Swern oxidation conditions.[21] Finally, **2c** was obtained at the end of the usual reaction sequence.



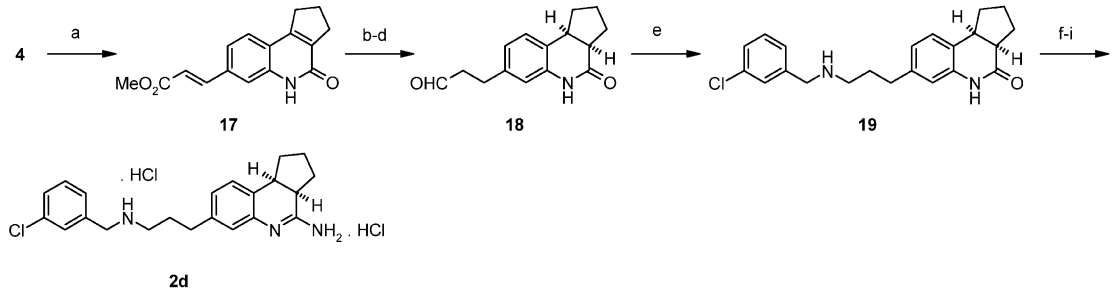
Scheme 1. (a) 4-BrC_6H_4NCO, $CHCl_3$; (b) concd H_2SO_4, 100 °C; (c) (2-furyl)$SnBu_3$, $Pd(PPh_3)_4$, PhMe, 110 °C; (d) Mg, MeOH; (e) cat RuO_2, $NaIO_4$, MeCN–CCl_4–EtOAc–H_2O; (f) $ClCO_2Et$, Et_3N, THF; (g) $NaBH_4$, MeOH; (h) $N(C_3H_7)_4RuO_4$, CH_2Cl_2–MeCN; (i) 3-$ClC_6H_4CH_2NH_2$, $NaBH(OAc)_3$, AcOH, $(CH_2Cl)_2$; (j) Boc_2O, cat DMAP, CH_2Cl_2; (k) Lawesson's reagent, THF; (l) NH_3, MeOH; (m) HCl, dioxane.



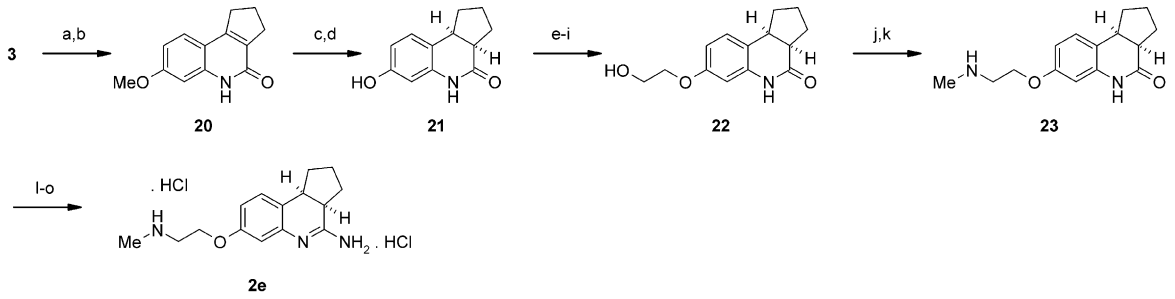
Scheme 2. (a) CH_2=$CHSnBu_3$, $Pd(PPh_3)_4$, PhMe, 110 °C; (b) *m*CPBA, CH_2Cl_2; (c) Mg, MeOH; (d) DMP, CH_2Cl_2; (e) 3-$ClC_6H_4CH_2NH_2$, $NaBH(OAc)_3$, AcOH, $(CH_2Cl)_2$; (f) Boc_2O, cat DMAP, CH_2Cl_2; (g) Lawesson's reagent, THF; (h) NH_3, MeOH; (i) HCl, dioxane.



Scheme 3. (a) Ac_2O, py; (b) NCS, DMF; (c) K_2CO_3, MeOH; (d) $(COCl)_2$, DMSO, Et_3N, CH_2Cl_2; (e) 3-$ClC_6H_4CH_2NH_2$, $NaBH(OAc)_3$, AcOH, $(CH_2Cl)_2$; (f) Boc_2O, cat DMAP, CH_2Cl_2; (g) Lawesson's reagent, THF; (h) NH_3, MeOH; (i) HCl, dioxane.



Scheme 4. (a) CH_2=$CHCO_2Me$, $Pd(PPh_3)_4$, Et_3N, DMF; (b) H_2, Pd/C, EtOAc; (c) Mg, MeOH; (d) DIBAH, PhMe; (e) 3-$ClC_6H_4CH_2NH_2$, $NaBH(OAc)_3$, AcOH, $(CH_2Cl)_2$; (f) Boc_2O, cat DMAP, CH_2Cl_2; (g) Lawesson's reagent, THF; (h) NH_3, MeOH; (i) HCl, dioxane.



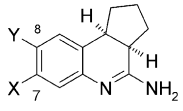
Scheme 5. (a) 3-$MeOC_6H_4NCO$, $CHCl_3$; (b) concd H_2SO_4, 100 °C; (c) Mg, MeOH; (d) BBr_3, CH_2Cl_2; (e) KOH, MeOH; (f) $BrCH_2CO_2Et$, DMF; (g) NaOH, THF–EtOH–H_2O; (h) $ClCO_2Et$, Et_3N, THF; (i) $NaBH_4$, MeOH; (j) $MeSO_2Cl$, Et_3N, THF; (k) $MeNH_2$, MeOH; (l) Boc_2O, cat DMAP, THF; (m) Lawesson's reagent, THF; (n) NH_3, MeOH; (o) HCl, dioxane.

For the synthesis of aminopropyldihydroquinoline **2d**, use was made of the Heck reaction[22] of methyl acrylate with 7-bromoquinolone **4** (Scheme 4). A reduction sequence comprising a hydrogenolysis, a dissolving metal reduction, and a hydride reduction gave the propionaldehyde **18** which was transformed into **2d** through the standard route.

The synthesis of 7-[2-(methylamino)ethoxy]dihydroquinoline **2e** started with 7-methoxyquinolone **20**, which was first reduced to the dihydroquinoline and then demethylated to **21** (Scheme 5). The phenol **21** was converted into its potassium salt and alkylated with ethyl bromoacetate. The ester was stepwise reduced to the alcohol through the mixed anhydride yielding **22**. Mesylation and nucleophilic displacement with methylamine completed the synthesis of the side chain (**23**). The synthesis of **2e** was accomplished from here following the standard process outlined before.

As expected, the basic side chain had a positive impact on both potency and selectivity as can be seen from Table 1.

Table 1. Inhibition of NOS isoforms by 7-(aminoalkyl)dihydroquinolines



Compd	X	Y	IC_{50} (μM)[a]			Selectivity	
			n-NOS	e-NOS	i-NOS	e/n[b]	i/n[b]
1	H	H	0.16	3.3	2.7	21	17
2a	3-$ClC_6H_4CH_2NHCH_2$	H	0.14	9.6	0.95	69	7
2b	3-$ClC_6H_4CH_2NHCH_2CH_2$	H	0.048	6.7	0.53	140	11
2c	3-$ClC_6H_4CH_2NHCH_2CH_2$	Cl	0.17	8.7	1.6	51	9
2d	3-$ClC_6H_4CH_2NHCH_2CH_2CH_2$	H	0.042	9.4	0.58	224	14
2e	$MeNHCH_2CH_2O$	H	0.17	52	3.9	325	27
24	H	Cl	0.14	6.2	5.7	44	41
ARR-17477[c]			0.035	3.5	5.0	100	143
Standards[23]							
L-NAME			1.6	1.5	8.0	1	5
L-NNA			0.08	0.32	5.5	4	69
L-NMMA			0.89	0.54	1.0	0.6	1

[a]NOS activity was determined with recombinant human enzyme according to ref 24.
[b]e/n represents IC_{50}(e-NOS)/IC_{50}(n-NOS) and i/n represents IC_{50}(i-NOS)/IC_{50}(n-NOS).
[c]Cited according to Salerno, et al.; see ref 2.

Compounds **2b** and **2d** are the best in this series: They were 3–4-fold more potent and 6–10 times more selective versus eNOS than the unsubstituted lead **1**. For the distance between the aromatic moiety and the benzylamino group an ethyl or propyl spacer as in **2b** and **2d** were clearly superior to a simple methyl spacer (**2a**) with respect to both potency and selectivity. The 8-chloro motif which had a favorable impact on selectivity towards e-NOS in the original series[5] (cf. **24**) proved to be detrimental with **2c** being 3–4-fold less potent and 2–3 times less selective versus e-NOS than the deschloro derivative **2b**. (Methylamino)ethyl ether **2e** was equipotent to **2c** and lead **1**, however, it is the most selective inhibitor described herein. The most potent compounds, **2b** and **2d**, exhibited a similar potency and selectivity towards e-NOS as ARR-17477 based on literature values.[2] However, they differed significantly in their selectivity towards i-NOS in that **2b** and **2d** inhibited i-NOS with submicromolar IC_{50}.

In summary, highly potent n-NOS inhibitors which display more than 100-fold selectivity against the e-NOS isoform were identified in the dihydroquinoline series. In addition, the most active compounds displayed submicromolar i-NOS inhibition, which might be advantageous in the treatment of stroke.

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