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Dihydroquinolines as Novel n-NOS Inhibitors

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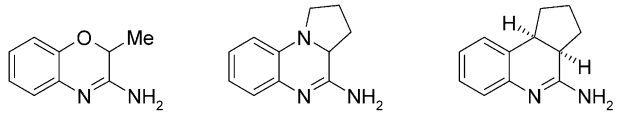
Abstract—Dihydroquinolines have been synthesized and have been shown to be potent n-NOS inhibitors. Selectivity versus e-NOS was increased to approximately 100-fold through appropriate substitution at the benzene ring.

Excessive brain levels of nitric oxide (NO) have been linked to tissue injury in the wake of a cerebral ischemic event and other neurodegenerative processes.[1] Since NO is formed in central and peripheral nerves through transformation of arginine into citrulline by constitutive neuronal nitric oxide synthase (n-NOS),[2] suppression of NO production with an n-NOS inhibitor appears as a promising neuroprotective treatment for a variety of disease states, notably stroke. Two further isoforms of nitric oxide synthase are known, one constitutively expressed in the endothelial lining of blood vessels (e-NOS) and another inducible form found in cells of the immune system (i-NOS). Due to the blood pressure modulating properties of endothelial NO, it is of paramount importance to identify a selective n-NOS inhibitor having minimal interaction with e-NOS.[3]

Our compound design started with 3-aminobenzoxazine **1** (Fig. 1) which emerged as a hit from high throughput screening.[4] In an effort to explore the steric demand of the oxazine moiety, among several structural variations, the lactate fragment was replaced by the rigid framework of proline leading to dihydroquinoxaline **2**[5] as prototype. However, this compound proved to be sensitive towards air oxidation, though it still exhibited an $IC_{50} = 3.3$ μM for n-NOS. Thus, further optimization seemed worthwhile and we moved from the oxidation-prone dihydroquinoxaline core[6] on to the less labile dihydroquinoline[7] template. Fortunately, dihydroquinoline **3** proved to be far superior to **2** both in terms of stability and potency, which was increased 30-fold. The syntheses and structure–activity relationship (SAR) in the dihydroquinoline series are described in this paper.



1 2 3

Figure 1.

The compounds were synthesized via two different routes as outlined in Schemes 1 and 2. The first path involved a conrotatory[8] Nazarov cyclization[9] of a phenyl cycloalkenyl ketone as a key step establishing the *cis*-stereochemistry at the stereogenic centers which eventually would become C-3a and C-9b in the final product.[10] The synthesis was accomplished with a reaction sequence including a Beckmann rearrangement,[11] a thionation process,[12] and an ammonolysis step.[13]

The second route (Scheme 2) relied on a dissolving metal reduction of a quinolone,[14] which provided predominantly the *trans*-dihydroquinolone, followed by the same endgame as described above. Generally, the quinolones were accessible through enamine addition to isocyanates followed by sulfuric acid-mediated cyclization.[15] However, in the case of electron-deficient phenyl isocyanates (e.g., R = F, CF_3) this process failed to deliver quinolones. Fortunately, an alternate sequence[16] proved to be successful comprising Suzuki coupling of *N*-pivaloylanilide-derived[17] boronic acids with triflates **II**

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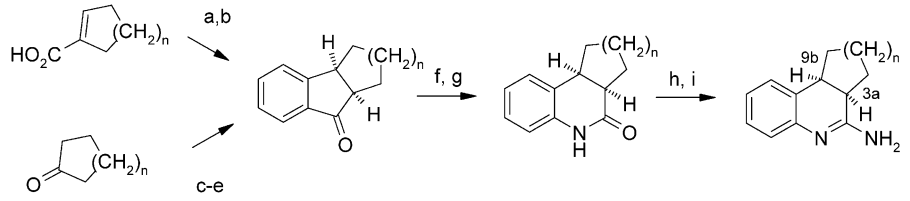
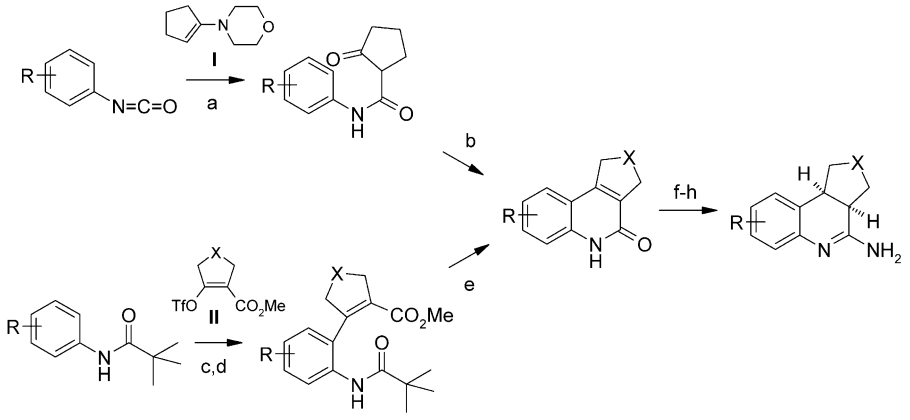
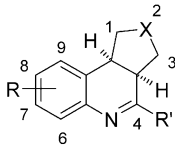


Scheme 1. (a) $SOCl_2$, reflux; (b) PhH, $AlCl_3$; (c) Me_3SiCN, *n*-BuLi, THF; (d) PhMgCl, THF; aq H_2SO_4; (e) concd H_2SO_4; (f) H_2NOH $x^1/_2H_2SO_4$, THF–EtOH–H_2O; (g) PPA, 120 °C; (h) Lawesson's reagent, DME; (i) NH_3, MeOH.



Scheme 2. (a) **I**, $CHCl_3$; (b) concd H_2SO_4, 100 °C; (c) *n*-BuLi, THF; $B(OMe)_3$; aq HCl; (d) **II**, $Pd(PPh_3)_4$, Na_2CO_3, DME-H_2O; (e) concd HCl, reflux; (f) Mg, MeOH; (g) Lawesson's reagent, DME; (h) NH_3, MeOH.

Table 1. Inhibition of NOS isoforms by dihydroquinolines



Compd	X	R	R′	IC_{50} (μM)[a]			Selectivity	
				n-NOS	e-NOS	i-NOS	e/n[b]	i/n[b]
3	CH_2	H	NH_2	0.16	3.3	2.7	21	17
4	$(CH_2)_2$	H	NH_2	6.80	—	—	—	—
5	$(CH_2)_3$	H	NH_2	100	—	—	—	—
6	O	H	NH_2	0.13	0.96	4.1	7	32
7	CH_2	H	NHMe	>100	—	—	—	—
8	CH_2	H	NHOH	>100	—	—	—	—
9[c]	CH_2	H	H[c]	>200	—	—	—	—
10	CH_2	8-Me	NH_2	0.50	21	12	42	24
11	CH_2	8-F	NH_2	0.11	2.3	1.7	21	15
12	CH_2	8-Cl	NH_2	0.14	6.2	5.7	44	41
13	CH_2	8-Br	NH_2	0.31	17	14	55	45
14	CH_2	8-CF_3	NH_2	1.6	57	89	36	56
15	CH_2	8-NO_2	NH_2	0.25	34	15	136	60
16	CH_2	8-CN	NH_2	0.64	54	32	84	50
17	CH_2	8-OMe	NH_2	0.64	24	13	38	20
18	CH_2	7-Me	NH_2	0.19	6.2	1.1	33	6
19	CH_2	7-F	NH_2	0.13	2.3	1.1	18	8
20	CH_2	7-NO_2	NH_2	0.31	12	2.6	39	8
21	CH_2	7-OMe	NH_2	0.24	4.1	1.4	17	6
22	CH_2	6-F	NH_2	0.17	5.8	2.3	34	14
23	CH_2	6,7-F_2	NH_2	0.68	20	5.1	29	8
24	CH_2	6-F, 8-Cl	NH_2	0.73	72	35	99	48
25	CH_2	6,8-Cl_2	NH_2	4.1	>200	30	—	7
26	CH_2	6,7-F_2, 8-Cl	NH_2	2.1	>200	78	—	37
Standards:								
L-NAME				1.6	1.5	8	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 at least three times with recombinant human enzyme according to ref 20.
[b]e/n means IC_{50}(e-NOS)/ IC_{50}(n-NOS) and i/n means IC_{50}(i-NOS)/ IC_{50}(n-NOS).
[c]This compound contains an endocyclic CH_2–NH group instead of the CH=N given in the generic structural formula on top of the table (i.e., 2,3,3a,4,5,9b-hexahydro-1*H*-cyclopenta[*c*]quinoline (cf. ref 21)).

followed by depivaloylation and cyclization. Further introduction of substituents into the benzene ring is feasible at the dihydroquinolone stage through classical aromatic substitution reactions.[18]

We started to explore the SAR of the dihydroquinolines by determining the optimal ring size of the annulated ring. As is apparent from Table 1 (**3**–**5**) both cyclohexane and cycloheptane annulation led to a dramatic loss in potency. The cyclopentane could be replaced by a tetrahydrofuran ring at the expense of diminished selectivity versus e-NOS (**6**). Substitution at or removal of the 4-amino group was detrimental for activity (**7**–**9**). Modification of the benzene substitution pattern allowed us to improve the selectivity versus e-NOS. Whereas substitution at C-7 was broadly accepted irrespective of the electronic nature of the substituent and had only minor effects on the selectivity against e-NOS (**18**–**21**), introduction of residues at position 8 had a more severe impact (**10**–**17**). The 8-chloro derivative **12** proved to be more potent than both the methyl or methoxy analogue **10** and **17**. In the 8-halogen series, increasing the size of the substituent correlated with a moderate loss in potency (**11**–**14**) with the maximum selectivity against e-NOS found for the bromo derivative **13**. A more than 100-fold selectivity and a fair potency was observed for the 8-nitro derivative **15**, while the high selectivity of 8-cyanoquinoline **16** was compromised by a drop in potency. The 6-fluoro derivative **22** showed a moderately improved selectivity compared to **3**. Combination with a chloro substituent into 8-chloro-6-fluoroquinoline **24** led to an increased selectivity but a 5-fold loss in potency, a profile comparable to that of 8-cyanoquinoline **16**. Further di- and trisubstitution resulted in poorly active n-NOS inhibitors (**23**, **25**, and **26**). Taken together, compounds displaying reasonable potency and fair selectivity were 8-chloro-, 8-bromo-, 8-nitro-, and 6-fluoroquinoline (**12**, **13**, **15**, **22**); these seem to be clearly superior to arginine-derived standards[19] especially in terms of selectivity against e-NOS.

In summary, novel, potent, and selective dihydroquinoline-based n-NOS inhibitors have been identified, and two synthetic routes have been described. The SAR reported herein sets the stage for further medicinal chemistry optimization and for an extensive pharmacological characterization.

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