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1,2-DIAMINOBENZIMIDAZOLES : SELECTIVE INHIBITORS OF NITRIC OXIDE SYNTHASE DERIVED FROM AMINOGUANIDINE

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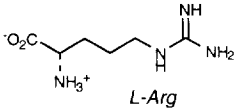
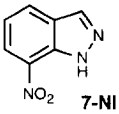
Abstract. The synthesis of a series of novel 1,2-diaminobenzimidazoles is described. While the parent compound **4a** is a weak, modestly selective inhibitor of the induced isoform of nitric oxide synthase (both mouse and human), a small structural change led to compound **5**, a highly selective inhibitor of neuronal enzyme.

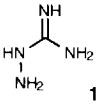
Introduction. The diatomic, short-lived inorganic molecule nitric oxide (·NO) has recently been revealed as a novel biological messenger and immunological regulator.[1] It is synthesised *in vivo* from L-arginine by nitric oxide synthases (NOS). Three isoforms of NOS have been identified positively. A constitutive form (type I) is found in neuronal tissue and is thought to be a retrograde messenger involved in long-term potentiation.[2] An inducible form (type II) occurs in various immune cells. Cytokine-dependent expression of this isoform leads to release of ·NO in larger, cytotoxic quantities. A second constitutive form (type III) is present in the endothelium and maintains vascular tone via ·NO-mediated smooth muscle relaxation. Isoform selectivity is a critical issue in inhibitor design, since inhibition of endothelial enzyme could lead to undesirable cardiovascular effects. A potent and selective inhibitor of the induced enzyme would be expected to have broad clinical utility as overexpression of this isoform may potentiate a number of inflammatory conditions or lead to excessive smooth muscle relaxation and, hence, hypotension which is the hallmark of septic shock,[3] while inhibitors of the neuronal isoform may be useful in stroke[4] and addiction.[5] Most inhibitors described to date are close analogues of L-arginine and show little or no selectivity. However, 7-nitroindazole (**7-NI**) displays some selectivity for the neuronal enzyme,[6] while aminoguanidine (**1**)[7] is a weak selective inhibitor of the murine induced isoform. Potent isothioureas with some selectivity for neuronal and induced vs. endothelial enzyme have also been described.[8]



L-Arg



7-NI



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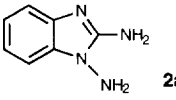
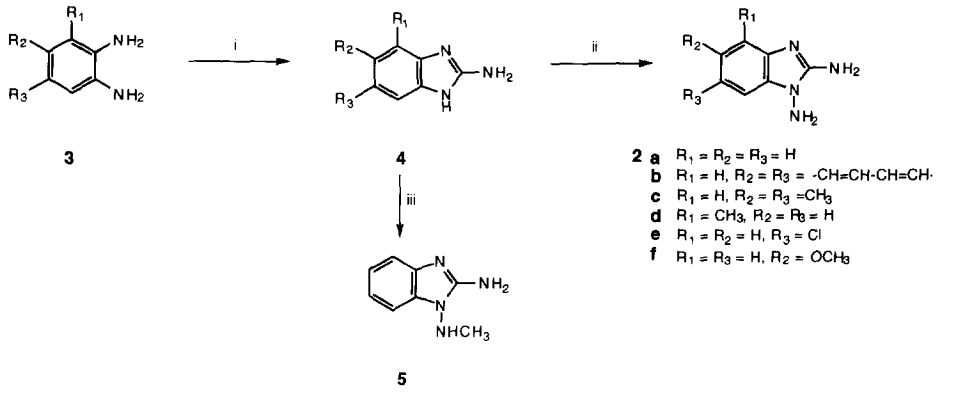


2a

We felt that incorporation of aminoguanidine into a more rigid structure might lead to novel, selective inhibitors of the induced isoform without amino acid functionality. 1,2-Diaminobenzimidazole **2a** fits this requirement by effectively constraining two of the α-nitrogens across the *ortho* positions of a phenyl ring. Indeed it proved to be a weak NOS inhibitor (Table 1). A limited number of compounds with substitution on the benzene ring and the two amino groups was therefore investigated. We describe herein the outcome of these studies which, surprisingly, led to a compound with high selectivity for the *neuronal* enzyme.

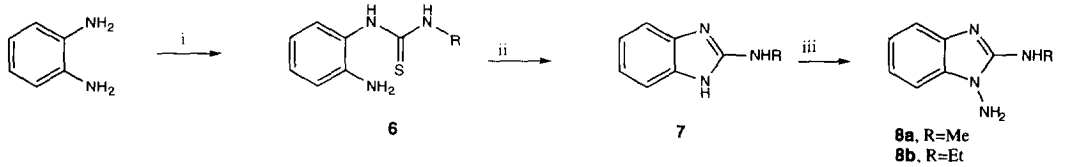
Chemistry. Diaminobenzimidazoles unsubstituted on nitrogen were prepared using the methods developed by Joullie.[9] Thus, 2-aminobenzimidazoles **4a-f**, which were synthesised by reaction of commercially available 1,2-diaminobenzenes **3** with cyanogen bromide, could be aminated under basic conditions with N-hydroxylamine-O-sulphonic acid.



Scheme 1. *Reagents and conditions:* **i,** a) BrCN b) $(CO_2H)_2$ (54-79%); **ii,** H_2NOSO_3H , KOH aq. (11-62%) (**2d** 62%, as 3:1 mixture with 7-Me isomer; **2e** 46%, as 97:3 mixture with 5-Cl isomer; **2f** 55%, as 1:1 mixture with 6-OMe isomer); **iii,** $MeHNOSO_3H$ (5 eq.), KOH aq; reflux (12%).

In the case of unsymmetrical 2-aminobenzimidazoles **4d-f**, two possible regioisomers can result, depending on which of the ring nitrogens is aminated. In fact, we observed some selectivity in certain cases. Thus, 2-amino-4-methylbenzimidazole **4d** gave a 3:1 ratio favouring **2d**, the product of amination at the less sterically crowded N, while 2-amino-5-chlorobenzimidazole **4e** gave a 93:7 crude ratio of isomers favouring **2e**. The source of the regioselectivity in the latter case is less obvious but is presumably electronic in origin. (It is known also that regioselectivity in benzimidazole N-substitution is influenced by reaction conditions.[10]) In each case the major isomer could be isolated readily by recrystallisation. In contrast, the 5-methoxy compound **4f** gave a 1:1 mixture of regioisomers. The structures of **2d** and **2e** were confirmed by ^1H NMR n.O.e. measurements.[11]

The 2-amino-1-(methylamino)benzimidazole **5** was synthesised by a novel methylamination of benzimidazole **4a** using N-methylhydroxylamine-O-sulphonic acid, prepared from N-methylhydroxylamine and chlorosulphonic acid.[12] This amination was considerably more sluggish than that with the unsubstituted hydroxylamine-O-sulphonic acid. The 1-amino-2-(alkylamino)benzimidazoles **8** can be prepared by cyclisation of the thiourea **6** to **7**, via the intermediate isothiouronium salt generated with methyl iodide, followed by amination (Scheme 2).



Scheme 2. *Reagents and conditions:* **i**, RNCS, Et_2O (78%); **ii**, MeI, EtOH (78%); **iii**, H_2NOSO_3H, aq. KOH (16%).

Biology. Compounds[13] were assayed for their ability to inhibit the conversion of [^{3}H]-L-arginine to [^{3}H]-L-citrulline catalysed by: i. neuronal NOS from rat cerebellum (Type I); ii. inducible NOS from mouse macrophage J774 or human DLD-1 cells (Type II); iii. endothelial NOS from human umbilical vein endothelial cells (Type III) using minor modifications[14] of methods previously described.[15] The initial arginine concentration used (3 μM) was chosen as being equal to or lower than measured values of K_m (arginine), which varied between 3 and 15 μM depending on isoform. Assays were performed at 22°C for 10 mins. (Type I and III) and 1hr. (Type II). Results are given in Table 1 (mean of at least two determinations. Highest and lowest obtained values shown where appropriate).

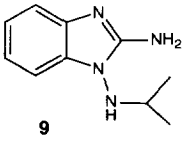
Table 1 *Inhibition of NOS isoforms by 1,2-diaminobenzimidazoles.*§

IC_{50} (μM)

Compound	Type I	Type II (human)	Type II (mouse)	Type III
1(aminoguanidine)	na	54 (27 - 80)	9 (4 - 20)	na
2a	77	25 (18 - 32)	31 (28 - 32)	na
5	5.8 (3.8 - 8.2)	na	na	na
7-NI	2 (1.1 - 5.0)	24 (21 - 28)	20 (19 - 22)	36

§ na = No significant inhibition of the isoform below 100μM. Compounds **2b-e**, **8a-b**, **9** and **10** did not inhibit any of the enzymes significantly at concentrations below 100 μM, the effective solubility limit for most compounds in the assay media.

Results. 1,2-diaminobenzimidazole (**2a**) proved to be a weak, modestly selective inhibitor of induced NOS. Unlike aminoguanidine **1**, however, it is equally effective against human as against mouse enzyme.[16] It has been shown to be competitive with L-arginine for mouse induced enzyme.[17] Most compounds derived from **2a** by substitution in the benzo (**2b-e**) or imidazo (**8a-b**) ring show no significant affinity for any of the isoforms, which may indicate a very severe steric constraint in these regions of the active site of NOS. A surprising exception is the 1-methylamino compound **5** which, though essentially inactive against the induced and endothelial enzymes, has an IC_{50} of ~6μM for the neuronal isoform. It is at least as selective as 7-nitroindazole (**7-NI**), a widely used selective inhibitor of neuronal NOS. The origin of this reversal of selectivity is obscure, but may reflect a small hydrophobic pocket in type I NOS which is absent in the other forms. Compound **10**, in which the 1-amino N atom of **5** has been replaced by a methylene unit, was essentially inactive, suggesting a vital role also for this nitrogen in binding. Furthermore, replacement of the methyl group of **5** by the larger isopropyl substituent (**9**) again destroyed activity, suggesting a further stringent size requirement at this position.



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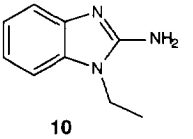

10

These results demonstrate that it is indeed possible to inhibit selectively both the neuronal and induced isoforms of NOS and that inhibitors which interact with the L-arginine binding site can be derived from compounds other than L-arginine. The lack of endothelial activity is particularly encouraging. It does appear however that the active site has rather stringent structural requirements. Although its properties have yet to be fully evaluated *in*

vivo, we anticipate that compound **5** may be of considerable use in the elucidation of the various biological roles of the neuronal enzyme.

Acknowledgements

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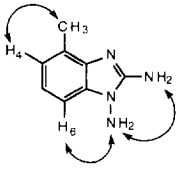
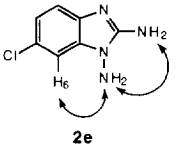


2d



2e

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