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# Heteroaromatic-aminomethyl quinolones: Potent and selective iNOS inhibitors

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ABSTRACT

The overproduction of nitric oxide during the biological response to inflammation by the nitric oxide synthase (NOS) enzymes have been implicated in the pathology of many diseases. By removal of the amide core from uHTS-derived quinolone **4**, a new series highly potent heteroaromatic-aminomethyl quinolone iNOS inhibitors **8** were identified. SAR studies led to identification of piperazine **22** and pyrimidine **32**, both of which reduced plasma nitrates following oral dosing in a mouse lipopolysaccharide challenge assay.



Nitric oxide synthase's (NOS) are three closely related heme-based oxygenases which include endothelial (eNOS), neuronal (nNOS) and inducible (iNOS) NOS isoforms. They are responsible for the biosynthesis of nitric oxide (NO) by the catalytic oxidation of the natural amino acid L-arginine to L-citrulline.[1] Endothelial NOS (eNOS) and neuronal NOS (nNOS) are constitutively expressed and play important roles in blood pressure regulation and neurotransmission, respectively. The third isoform, inducible NOS (iNOS), is synthesized in response to exposure to inflammatory and immunologic stimuli and has been implicated in the pathophysiology of a range of human diseases including septic shock, asthma, inflammatory bowel disease, arthritis and neuropathic pain.[2,3]

There has been substantial activity in the pharmaceutical industry to identify selective iNOS inhibitors (Fig. 1), with selectivity over eNOS being particularly important as inhibition can result in an elevation of blood pressure. Early iNOS inhibitors such as GW271450 (**1**) share structural similarity with L-arginine, leading to concerns with interference with physiological processes dependent on arginine transport and metabolism.[4] Small molecule inhibitors, structurally distinct from arginine but that still incorporate a guanidine motif have also been identified. Amongst these is **2** (AR-C102222), a substrate competitive inhibitor which showed good selectivity for iNOS and was efficacious in a rat adjuvant-induced arthritis model.[5] Inhibitors of iNOS dimerization have also been identified such as **3** (BBS-4), a potent and selective imidazole-based inhibitor that coordinates to the heme iron in the active site of the enzyme.

Previously we described the discovery of a quinolinone based iNOS dimerization inhibitor **4**, identified by optimization of a hit from an ultra high-throughput screen.[6] Although a potent and selective iNOS inhibitor, **4** suffered from high clearance, limiting the duration of its in vivo efficacy. We thus sought to evolve the quinolinone amide series to identify potent iNOS inhibitors with a suitable drug property profile for development. Towards this

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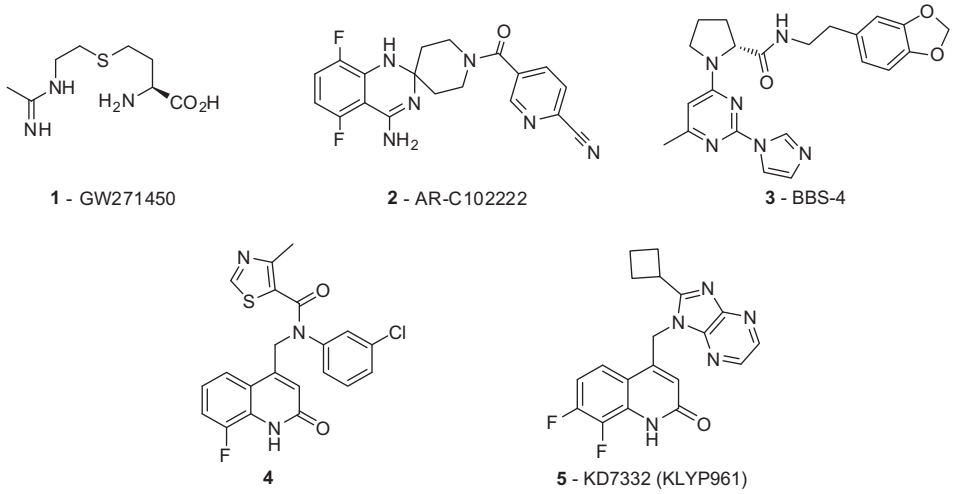
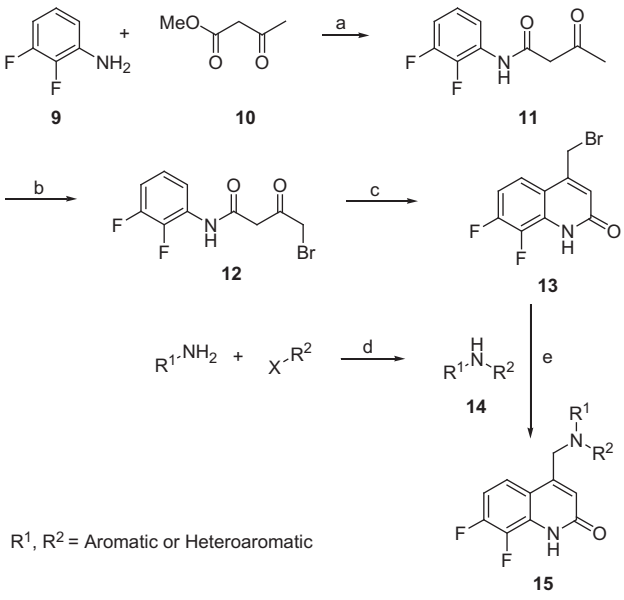



**Figure 1.** NOS inhibitors.

end we removed the amide functionality of **6** by performing connection 'A' or 'B' (Fig. 2), generating the 'benzimidazole series' (represented by **7**) and the 'heteroaromatic-aminomethyl quinolone series' (represented by **8**).[7] Previously we described the optimization of the benzimidazole series to the development candidate **5** (**KD7332**, **KLYP961**);[8] this Letter describes the profile of the 'heteroaromatic-aminomethyl quinolones'.

The desired heteroaromatic-aminomethyl quinolones were synthesized as described in Scheme 1. Amide bond formation between aniline **9** and β-keto ester **10** provided the coupled product **11**, which was then selectively brominated using bromine/acetic acid followed by acetone to afford the desired terminal monobromide **12**.[9] The bromide **12**, then underwent an acid catalyzed intramolecular cyclization/condensation to construct the quinolone core **13**. Finally, the resulting benzylic bromide **13** was reacted with a variety of heteroaromatic-aminomethyl synthons **14**, synthesized from coupling of substituted anilines with heteroaryl halides (via palladium catalyzed amination or via $SN_{Ar}$) in the presence of sodium hydride. Using this general scheme the heteroaromatic-aminomethyl quinolones **15** were prepared. The isoquinoline and isoquinoline N-oxide illustrated in Figure 3 were in turn prepared using the methodology presented previously.[6]




**Scheme 1.** Reagents and conditions: (a) Xylene, pyridine reflux, 3–18 h; (b) (i) $Br_2$, AcOH, 25–50 °C, 5–18 h; (ii) acetone, 25 °C, 2 h; (c) $H_2SO_4$, 40 °C, 2–18 h; (d) $Pd_2(dba)_3$, BINAP, NaO*t*Bu, toluene, microwave, 150 °C, 15 min or NMP, microwave, 200 °C, 15 min; (e) NaH, DMF, 25 °C, 1–5 h.

The inhibitory activity against human iNOS of the compounds was measured as described previously using transiently transfected HEK-293 cells, using 2,3-diaminonaphthalene to measure NO production via fluorescence.[6] To determine NOS selectivity, human eNOS and nNOS inhibitory activity was also determined using an analogous assay.

Encouragingly, the prototypical compound of the 'heteroaromatic-aminomethyl quinolone series', compound **17** (Table 1) showed excellent human iNOS activity ($IC_{50}$ = 0.031 μM) and selectivity over eNOS (>1000×), while showing slight selectivity over nNOS (fivefold). The importance of having a nitrogen in the ring of **17** (that is believed to mimic the amide of **6**—Fig. 2) was clearly demonstrated as the phenyl derivative **16** lost considerable

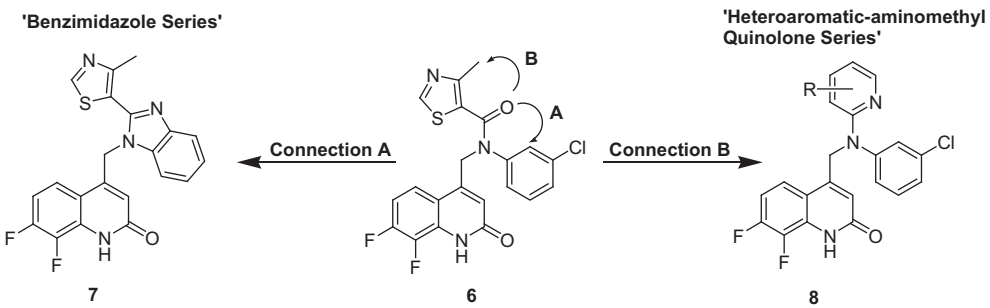



**Figure 2.** Restricting conformations of the amide of **6** leads to two new series.

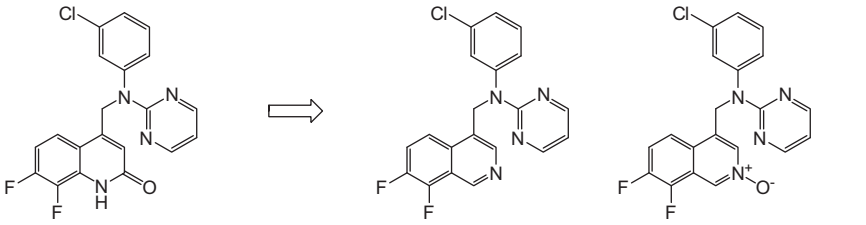
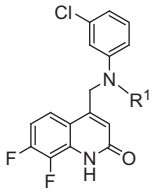



**Figure 3.** Isoquinoline N-oxide replacement of the quinolone moiety.

**Table 1**
Nature of the heteroaromatic ring effects iNOS potency



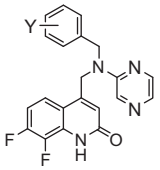
| Compound | $R^1$ | $IC_{50}$[a] (μM) | | | Selectivity eNOS/iNOS | Selectivity nNOS/iNOS |
|---|---|---|---|---|---|---|
| | | iNOS | eNOS | nNOS | | |
| **16** |  | 1.5 | >30[b] | ND[c] | >20 | NA[d] |
| **17** |  | 0.031 | >30[b] | 0.17 | >1000 | 5 |
| **18** |  | 0.077 | 14 | 0.37 | 180 | 5 |
| **19** |  | 0.13 | 23 | 0.24 | 170 | 2 |
| **20** |  | 0.043 | 21 | 0.36 | 500 | 8 |
| **21** |  | 0.014 | 13 | 0.15 | 1000 | 11 |
| **22** |  | 0.005 | 1.5 | 0.012 | 300 | 2 |
| **23** |  | 0.013 | 5.5 | 0.069 | 425 | 5 |
| **24** |  | 0.21 | 79 | 1.6 | 375 | 8 |

[a] Cell-based NOS assay using transiently transfected HEK293 cells; NO measured using 2,3-diaminonapthalene (DAN).
[b] <50% inhibition at 30 μM.
[c] Not determined.
[d] Not applicable.

potency (hiNOS $IC_{50}$ = 1.5 μM). For optimal iNOS potency, the position of the nitrogen atom was also important with the 3-pyridyl (**18**) and 4-pyridyl (**19**) giving a drop off in potency. Fixing the 2-pyridyl in place and then scanning a second nitrogen around the ring (**20–23**) produced additional potent heteroaromatic compounds including pyrimidine **21** (hiNOS $IC_{50}$ = 0.014 μM) and piperidine **22** (hiNOS $IC_{50}$ = 0.005 μM) with the good selectivity over eNOS maintained (300 to 1000-fold). Further piperidine **22** showed good potency against mouse iNOS ($IC_{50}$ = 0.008 μM), making it an attractive compound for profiling in vivo (vide infra), as well as for further SAR studies.

Previous SAR had illustrated that a 3-chlorophenyl ring was a preferred substituent in the amide series (i.e., **6**)[6] and that a benzylic substituent was not well tolerated (data not shown). In contrast to amide **6**, a benzyl-substituent on amino-piperazine **22** was well tolerated as illustrated by **25** (Table 2), which maintained hiNOS potency ($IC_{50}$ = 0.013 μM) and selectivity (eNOS/iNOS = 500). Further, substitution around the benzylic ring was tolerated with small, lipophilic substituents at the *ortho*-position preferred as demonstrated by **26**, **29** and **30** which maintained hiNOS potency ($IC_{50}$ <0.015 μM) and selectivity (eNOS/iNOS >500). *meta*-Chloro (**27**) or *para*-chloro (**28**) both suffered from a drop off in potency, as did 2-pyridyl **31**. Despite maintaining good levels of in vitro potency, derivatives of this type were not pursued due to short liver microsomal half-life's ($t_{1/2}$ <20 min).[10]

**Table 2**
Benzylic-substituted aminopyrazines are well tolerated



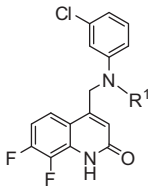
| Compound | Y | $IC_{50}$[a] (μM) | | | Selectivity eNOS/iNOS | Selectivity nNOS/iNOS |
|---|---|---|---|---|---|---|
| | | iNOS | eNOS | nNOS | | |
| **25** | H | 0.013 | 6.4 | 0.1 | 500 | 8 |
| **26** | 2-Cl | 0.012 | 12 | 0.06 | 1000 | 5 |
| **27** | 3-Cl | 0.22 | 8.3 | 0.33 | 40 | 2 |
| **28** | 4-Cl | 0.077 | 7.1 | 0.12 | 90 | 2 |
| **29** | 2-F | 0.012 | 11 | 0.064 | 900 | 5 |
| **30** | 2-Me | 0.010 | 13 | 0.046 | 1300 | 5 |
| **31** | [2-Pyridyl] | 0.078 | 11 | 0.44 | 140 | 6 |

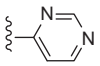
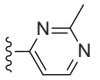
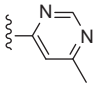
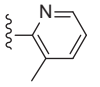
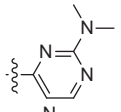
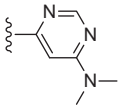
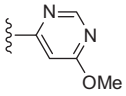
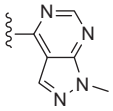
[a] Cell-based NOS assay using transiently transfected HEK293 cells; NO measured using 2,3-diaminonapthalene (DAN).

Further SAR work examining substitution on the heteroaromatic ring was carried out as exemplified in Table 3 using pyrimidine **21** as a base compound. It was found that methyl substitution at the 3- or 5-positions of pyrimidine **21** were preferred—compare **32** and **33–34** (pyrimidine version was not prepared). Importantly, **32** also had a reasonable mouse liver microsome half-life of 90 min, substantially longer than many of the other derivatives in the heteroaromatic-aminomethyl quinolone series, which lead to its profiling in vivo (vide infra). More polar groups however were only tolerated at the 3-position as illustrated by **36** ($IC_{50}$ = 0.028 μM) and **37** ($IC_{50}$ = 0.061 μM) compared to **35** ($IC_{50}$ = 1.3 μM). Finally, high potency compounds, that approximately fill the same space as the thiazole-carbonyl portion of **6** could be obtained with fused systems such as pyrimidinopyrazole **38** ($IC_{50}$ = 0.011 μM). However, despite its high human iNOS potency and good selectivity, **38** suffered from a short liver microsome half life of <30 min.

Previously we showed that the key quinolone moiety maybe replaced with an isoquinoline group in the amide series.[6] This replacement can also be carried out in the heteroaromatic-aminomethyl quinolone series as illustrated in Figure 3, where isoquinoline N-oxide **40** is equipotent to its quinolone analogue **23**, also maintaining excellent selectivity over eNOS (>1000-fold).

**Table 3**
Effect of substitution on the heteroaromatic ring




| Compound | $R^1$ | $IC_{50}$[a] (μM) | | | Selectivity eNOS/iNOS | Selectivity nNOS/iNOS |
|---|---|---|---|---|---|---|
| | | iNOS | eNOS | nNOS | | |
| **21** |  | 0.014 | 13.3 | 0.15 | 1000 | 11 |
| **32** |  | 0.033 | 7.5 | 0.213 | 230 | 7 |
| **33** |  | 0.053 | >30[b] | 0.160 | >500 | 3 |
| **34** |  | 0.35 | >30[b] | ND[c] | >85 | NA[d] |
| **35** |  | 1.3 | >30[b] | ND[c] | >20 | NA[d] |
| **36** |  | 0.028 | >30[b] | 0.500 | >1000 | 20 |
| **37** |  | 0.061 | >30[b] | 0.13 | >500 | 2 |
| **38** |  | 0.011 | 8.3 | 0.062 | 730 | 3 |

[a] Cell-based NOS assay using transiently transfected HEK293 cells; NO measured using 2,3-diaminonapthalene (DAN).
[b] <50% inhibition at 30 μM.
[c] Not determined.
[d] Not applicable.

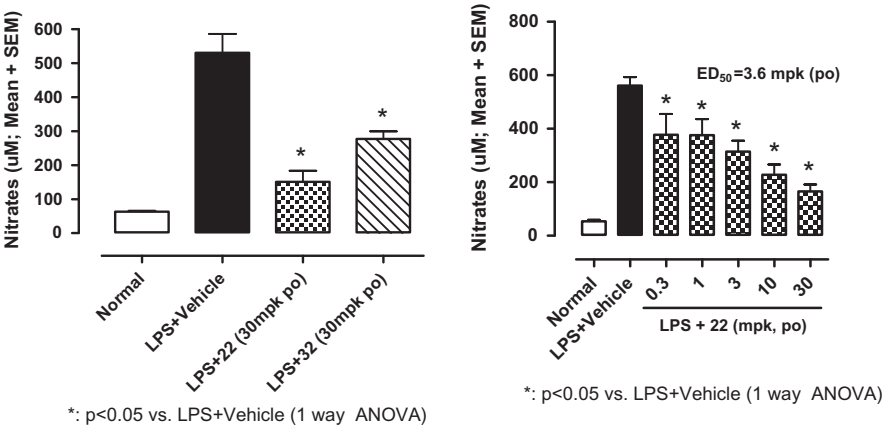



**Figure 4.** Performance of **32** and **22** following oral dosing in the mouse LPS challenge assay.

**Table 4**
Mouse iNOS $IC_{50}$ and pharmacokinetics of **22** and **5**

| Compound | hiNOS $EC_{50}$ (μM) | miNOS $EC_{50}$ (μM) | Clp (mL/min/kg) | $t_{1/2}$ (h) | Vdss (L/kg) | po Cmax (μg/mL) | po AUC (μg h/mL) | %*F* |
|---|---|---|---|---|---|---|---|---|
| **22** | 0.005 | 0.005 | 34 | 2.1 | 1.7 | 0.045 | 0.12 | 3 |
| **5** | 0.091 | 0.5 | 4.5 | 2.1 | 0.4 | 15 | 25 | 62 |

Isoquinoline **39** was also active against hiNOS, albeit 10-fold less potent.

Due to their encouraging in vitro profiles, piperazine **22** and pyrimidine **32** were profiled in a lipopolysaccharide (LPS) challenge assay in mice. This assay which measures nitrate levels (via NO levels—a biomarker reflective of activity in mechanism), was used extensively in the program to confirm that increases in in vitro iNOS potency translated to increases in in vivo activity, but also to get a rapid read on whether a compound had exposure following oral dosing. As can be seen in Figure 4, at 30 mg/kg, both **22** and **32** gave a statistical reduction in plasma nitrate levels of 80% and 55%, respectively. Due to its superior performance, a dose–response from 0.3 to 30 mg/kg was carried out with **22**, giving an $ED_{50}$ of 3.6 mpk (po).

Although **22** performs well in the LPS assay, benzimidazole **5** gave superior performance ($ED_{50}$ = 1 mpk po) in this assay. This is despite the fact that **5** was significantly less potent against mouse iNOS than **22** (miNOS = 0.5 μM vs 0.005 μM, respectively). This relative performance can be readily understood by examining the mouse pharmacokinetics of **22** (Table 4), which is characterized by limited exposure following oral dosing (%*F* = 3; AUC (10 mpk po) = 0.12 μg h/mL), in contrast to **5** (%*F* = 62; AUC (10 mpk po) = 25 μg h/mL). Despite the excellent in vitro potency and selectivity over eNOS of the heteroaromatic-aminomethyl quinolone series, we were unable to identify compounds with pharmacokinetic parameters comparable to the benzimidazole series and so this series was discontinued.

In summary, by removing the amide bond in the original screening hit, we were able to identify a series of highly potent heteroaromatic-aminomethyl quinolone iNOS inhibitors that displayed excellent selectivity over endothelial NOS (>300-fold) and moderate/low selectivity over neuronal NOS (2 to 20-fold). A heteroaryl ring containing a nitrogen at the 2-position was found to be optimal for potency, with piperazine **22** and pyrimidine **32** demonstrating a statistical reduction in plasma nitrate levels following oral dosing in a mouse LPS challenge assay. Despite in vivo activity, the series was discontinued in favor of the benzimidazole series due to inferior pharmacokinetics.

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10. Elimination rate under combined Phase 1 oxidative and Phase 2 glucuronide conjugation conditions (mouse)—for experimental protocol, see Supplementary data for Ref. 8.