Evaluation of analogues of furan-amidines as inhibitors of NQO2

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ABSTRACT

Inhibitors of the enzyme NQO2 (NRH: quinone oxidoreductase 2) are of potential use in cancer chemotherapy and malaria. We have previously reported that non-symmetrical furan amidines are potent inhibitors of NQO2 and here novel analogues are evaluated. The furan ring has been changed to other heterocycles (imidazole, *N*-methylimidazole, oxazole, thiophene) and the amidine group has been replaced with imidate, reversed amidine, *N*-arylamide and amidoxime to probe NQO2 activity, improve solubility and decrease basicity of the lead furan amidine. All compounds were fully characterised spectroscopically and the structure of the unexpected product *N*-hydroxy-4-(5-methyl-4-phenylfuran-2-yl)benzamidine was established by X-ray crystallography. The analogues were evaluated for inhibition of NQO2, which showed lower activity than the lead furan amidine. The observed structure-activity relationship for the furan-amidine series with NQO2 was rationalized by preliminary molecular docking and binding mode analysis. In addition, the oxazole-amidine analogue inhibited the growth of *Plasmodium falciparum* with an IC_{50} value of 0.3 μM.

NRH: quinone oxidoreductase 2 (NQO2) is a cytosolic flavoprotein enzyme [1] widely distributed in human heart, brain, lung, liver and skeletal muscle [2]. NQO2 is a potential target for cancer chemotherapy as its inhibition has therapeutic and/or preventative potential. In our laboratory, non-symmetrical furan-amidine **1** (Figure 1) and *para*-substituted analogues were identified as novel lead inhibitors of NQO2 with both anti-cancer and anti-malarial activities [3]. Here, further modifications to these non-symmetrical furan-amidines have been evaluated. Some of the non-symmetrical furan-amidines [3] showed poor water solubility, therefore the furan ring of **1** was replaced by more water-soluble isosteric heterocycles, including imidazole and oxazole. The lead NQO2 furan inhibitor possesses the highly basic amidine group, which will potentially decrease its passive diffusion and oral bioavailability [4, 5]. Here, analogues of the non-symmetrical furan-amidine **1** were synthesized in which the amidine group was isosterically replaced with less basic groups: imidate, *N*-aryl amidine (reversed amidine), *N*-aryl amide and amidoxime groups. From the initial virtual screening study, one of the first reported potent NQO2 inhibitors was the symmetrical 3,4-dimethyl-substituted furan-amidine **2** (Figure 1) with an IC_{50} of 50 nM [6]. Given the structurally similarity of compounds **1**

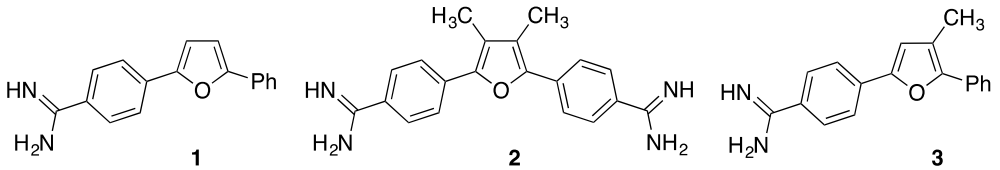

Figure 1. Structures of the non-symmetrical furan-amidine **1,** the symmetrical 3,4-dimethylfuran-amidine **2** and the proposed 4-methylfuran-amidine **3**.

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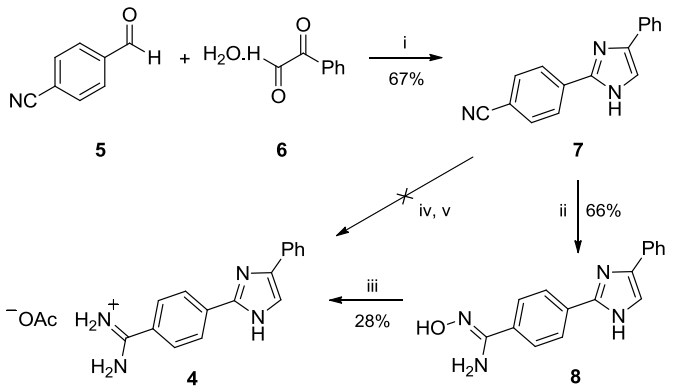
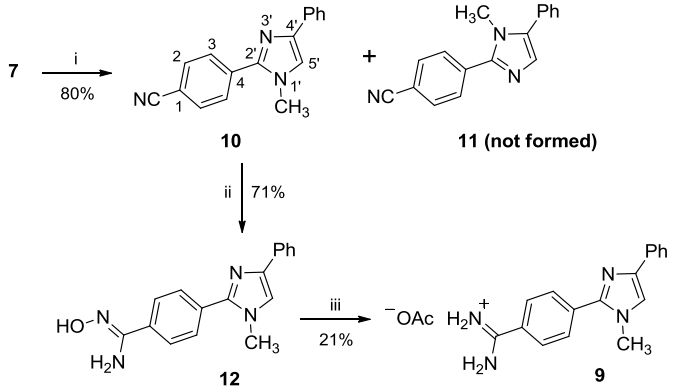


Scheme 1. Synthesis of 4-(4-phenyl-1*H*-imidazol-2-yl)benzamidine acetate **4**; Reagents and conditions: (i) NH_4OAc, MeOH, rt. (ii) $NH_2OH.HCl$, *t*-BuOK, dry DMSO, 0 °C - rt; (iii) HCO_2NH_4, Pd/C, AcOH, reflux; (iv) $HCl_{(g)}$, abs. EtOH, $CHCl_3$, 0 °C- rt; (v) NH_4OAc, Abs. EtOH, rt, 12 hr.



Scheme 2. Synthetic pathway for 4-(1-methyl-4-phenyl-1*H*-imidazol-2-yl)benzamidine **9**; Reagents and conditions: (i) CH_3I, KOH, acetone, rt. (ii) $NH_2OH.HCl$, *t*-BuOK, dry DMSO, 0 °C - rt; (iii) HCO_2NH_4, Pd/C, AcOH, reflux.

and **2**, it is of interest to assess the activity of non-symmetrical 4-methylfuran-amidine analogue **3** (Figure 1) as an NQO2 inhibitor, the synthesis of which is attempted in this study.

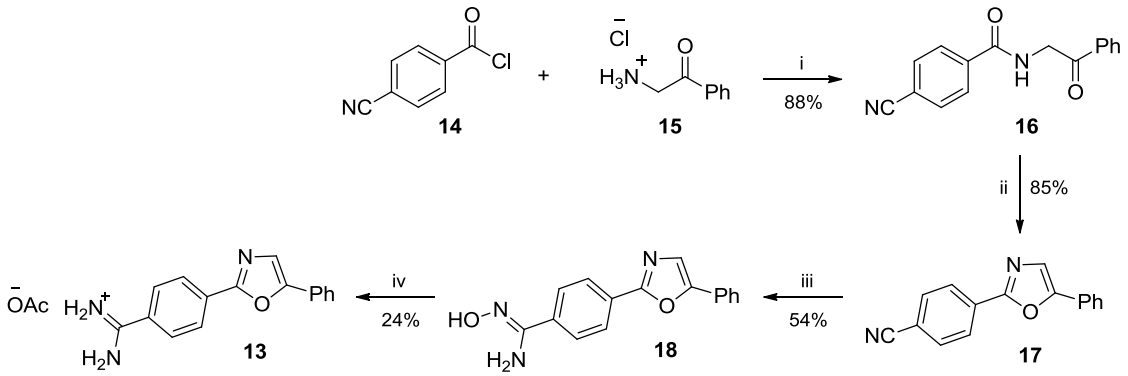
In order to enhance the aqueous solubility of furan amidine **1** (clogS -1.81, 4.0 mg/ml [7]), the furan ring was first replaced with an imidazole group to give **4** (clogS -1.27, 13.9 mg/ml [7]). The synthesis of imidazole-amidine **4** is shown in Scheme 1. 4-(4-Phenyl-1*H*-imidazol-2-yl)benzonitrile **7** was synthesized by the reaction of 4-cyanobenzaldehyde **5** with phenylglyoxal monohydrate **6** in the presence of ammonium acetate (Scheme 1) [8]. Attempts to convert the nitrile **7** directly into amidine **4** using the Pinner synthesis (Scheme 1, steps iv and v) failed because of the basicity of the nitrogen of the imidazole ring (pK_a 6.9), causing precipitation of **7** as the hydrochloride salt. Therefore the aryl nitrile **7** was reacted with hydroxylamine to give the amidoxime intermediate **8** [5], which was reduced to the amidine **4** using ammonium formate [9] (Scheme 1) [10].

The *N*-methylimidazole analogue **9** was synthesized from the reaction of nitrile **7** with methyl iodide (Scheme 2) giving the possibility of the formation of two regioisomers **10** or **11**. The NOESY spectrum confirmed the formation of the least hindered regioisomer **10** (see Figure S1) which showed a long-range interaction between the *N*-methyl protons and H-5'. 4-(1-Methyl-4-phenyl-1*H*-imidazol-2-yl)benzamidine **9** was synthesized from **10** through the formation of amidoxime **12** (Scheme 2).

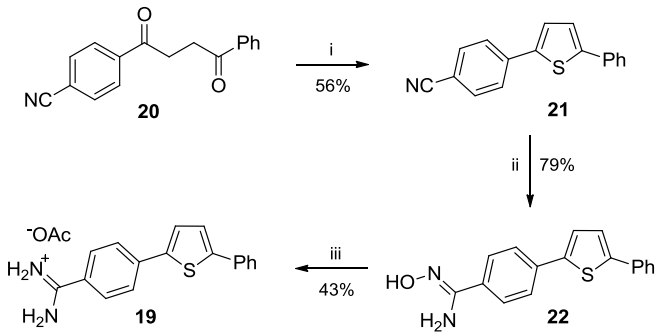
The oxazole-amidine **13** (clogS -1.30, 13.3 mg/ml [7]) was synthesized as shown in Scheme 3. The key precursor 4-cyano-*N*-(2-oxo-2-phenylethyl)benzamide **16** was prepared from the coupling between 4-cyanobenzoyl chloride **14** and 2-amino-1-phenylethanone hydrochloride **15**, in the presence of sodium bicarbonate [11]. In the presence of acetic anhydride/conc. sulfuric acid, the benzamide **16** readily cyclised to give 4-(5-phenyloxazol-2-yl)benzonitrile **17** [12, 13], which was converted to the oxazole-amidine **13** through the formation of the amidoxime intermediate **18** (Scheme 3).

The thiophene-amidine **19** (clogS -2.25, 1.57 mg/ml [7]) was also synthesised (Scheme 4) as a more lipophilic isostere of the furan-amidine **1** (clogS -1.81, 4.03 mg/ml [7]). The synthesis of **19** first required the Paal-Knorr synthesis of 2,5-diarylthiophene **21** from the reaction between the 1,4-diketone **20** [3] and Lawesson's reagent. The conversion of the nitrile group of **21** to the amidine **19** was *via* the amidoxime intermediate **22**. Reduction of the amidoxime **22** to the amidine **19** was attempted by heating at reflux in acetic acid in the presence of ammonium formate and Pd. Only starting material **22** was recovered, which was attributed to poisoning of the Pd catalyst by the thiophene. The reduction of **22** to amidine **19** was therefore achieved using triethylsilane as hydrogen donor in the presence of palladium (II) chloride catalyst (Scheme 4) [14].

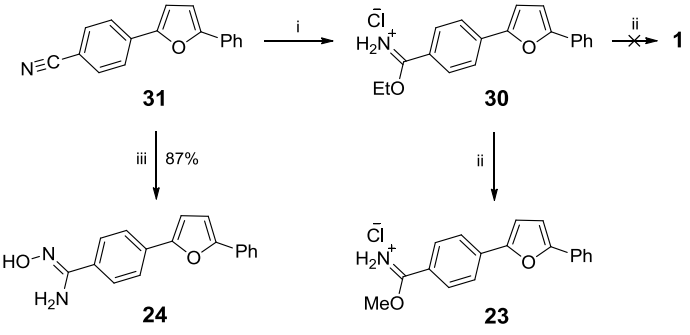
To address the high basicity of the amidine group, several less basic isosteres of **1** were synthesized in which the amidine group was replaced with methyl imidate **23**, amidoxime **24**, *N*-aryl amidines (reversed amidines) **25-26** and *N*-aryl amide **27-29**. pK_a and clogS are given in Table 1 and clogP and solubilities (mg/ml) are given in SI for the key compounds, with the non-amidine analogues being less basic, potentially enhancing passive permeability. The syntheses of these analogues are illustrated in Schemes 5 and 6. It was anticipated that heating of ethyl benzimidate hydrochloride **30** (prepared by reaction of nitrile **31** with ethanol) [3] at reflux with ammonium chloride methanol/water would give the furan-amidine **1**, however the isolated product was the methyl imidate **23** [15] (Scheme 5)**.** The methyl imidate group is a much less basic isostere (pKa 6.2) [15] than the highly basic amidine group (pKa 11.8) [16].



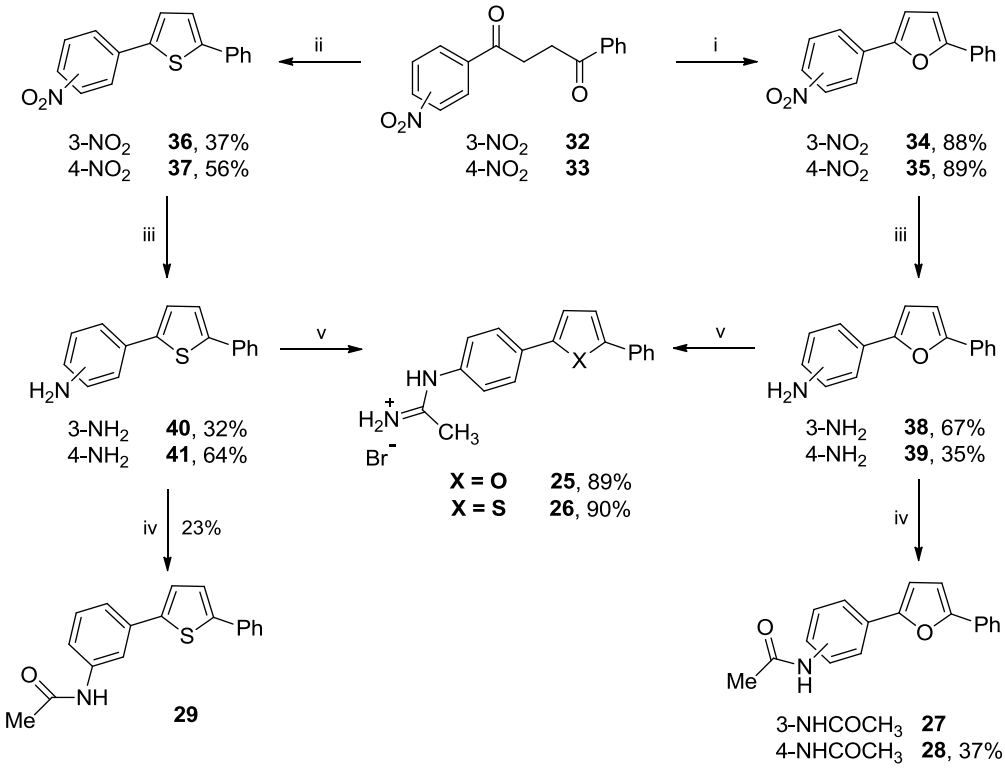
Scheme 3. Synthesis of 4-(5-phenyloxazol-2-yl)benzamidine acetate **13**; Reagents and conditions: (i) $NaHCO_3$, DCM, 0 °C – rt; (ii) Ac_2O, conc. H_2SO_4, rt; (iii) $NH_2OH.HCl$, *t*-BuOK, dry DMSO, 0 °C - rt; (iv) $HCOONH_4$, Pd/C, AcOH, reflux.



Scheme 4. Synthesis of the non-symmetrical 4-(5-phenylthiophen-2-yl)benzamidine acetate **19**; Reagents and conditions: (i) 60 °C, Lawesson's reagent; (ii) $NH_2OH.HCl$, t-BuOK, dry DMSO, 0 °C - rt; (iii) $(Et)_3SiH$, $PdCl_2$, AcOH, Ac_2O, rt - reflux.



Scheme 5. Syntheses of methyl 4-(5-phenylfuran-2-yl)benzimidate hydrochloride **23** and *N*-hydroxy-4-(5-phenylfuran-2-yl)benzamidine **24** from nitrile **31**; Reagents and conditions: i-EtOH, ii- NH_4Cl, MeOH/ H_2O, reflux.; iii- NH_2OH. HCl, t-BuOK, dry DMSO, 0 °C - rt.

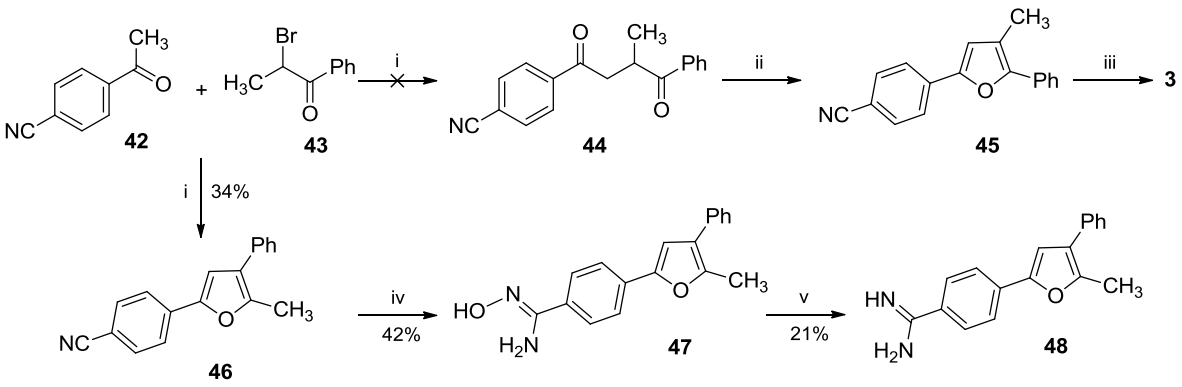


Scheme 6. Synthesis of *N*-aryl amidines (reversed amidines) **25**-**26** and *N*-aryl amides **27**-**29**; Reagents and conditions: i- $HCl_{(g)}$, $CHCl_3$, 0 °C – rt; (ii) Lawesson's reagent, THF, 55 °C; (iii) $NaBH_4$, $CuSO_4$, EtOH, 0 °C – rt; (iv) AcCl, dry CH_3CN, rt; (v) S-2-naphthylmethyl thioacetimidate hydrobromide, AcOH, $CHCl_3$, rt.

An isosteric analogue of the asymmetric furan-amidine **1** with an amidoxime group **24** was synthesized as a less basic isostere (pKa 5-6) for the furan amidine [17]. In addition, the amidoxime group is a known prodrug for the amidine group and can enhance oral bioavailability of amidine-containing drugs [4, 5] which is activated through reduction of the amidoxime group by human liver microsomes [18]. N-Hydroxy-4-(5-phenylfuran-2-yl)benzamidine **24** was synthesized by the reaction of nitrile **31** with hydroxylamine (Scheme 5).

The first step in the syntheses of the reverse amidine and amide analogues **25**-**29** was the preparation of the key 1,4-diketone intermediates **32** and **33** [3] (Scheme 6). The cyclization of the 1,4-diketones **32**, **33** into furans **34**, **35** and thiophenes **36, 37** were catalysed by dry hydrogen chloride gas and Lawesson's reagent, respectively. The nitro-groups in the intermediates **34**-**37** were reduced to amines **38**-**41** using sodium borohydride in the presence of catalytic copper sulfate [19]. The reduction of the nitro-groups into amines was confirmed by upfield shift of the protons on the aromatic ring: The peaks of the H-2', H-4', H-5' and H-6' protons of **34** were shifted up-field from 8.57, 8.12, 7.59 and 8.05 ppm to 6.87, 6.52, 7.08 and 6.94 ppm in **38**, respectively (Figure S2). The *N*-aryl amidines **25** and **26** were synthesized from the reaction of the amines **39** and **41**, respectively, with S-2-naphthylmethyl thioacetimidate hydrobromide (Scheme 6) [20-22]. The furan *N*-aryl amides **27** and **28** and *N*-(3-(5-phenylthiophen-2-yl)phenyl)acetamide **29** were synthesized from the reaction of acetyl chloride with amines **38, 39** and **40**, respectively (Scheme 6).

The synthesis of the 3-methylfuran-amidine analogue **3** was attempted as shown in Scheme 7, however coupling of 4-cyanophenyl methyl ketone **42** and α-bromomethyl phenyl ketone **43** failed to give the diaryl mono-methyl 1,4-diketone **44**. Diketone **44** would have cyclised to give furan **45**, a precursor for amidine **3**. Instead, the condensation of **42** and **43** led to the formation of 5-methyl-2,4-diarylfuran nitrile **46**. The structure was confirmed by X-ray crystallography of its amidoxime derivative **47**, the ORTEP diagram of which is shown in Figure 2, annotated with the numbering scheme adopted. Further



Scheme 7. Synthetic pathway for the preparation of furan-amidine **48**; Reagents and conditions: (i) EtMgBr, Et_2NH, dry THF, 0 °C – rt; (ii) $HCl_{(g)}$, abs. EtOH, $CHCl_3$, 0 °C- rt; (iii) NH_4OAc, Abs. EtOH, rt; (iv) NH_2OH. HCl, *t*-BuOK, dry DMSO, 0 °C – rt.; (v) NH_4 formate, Pd/C.

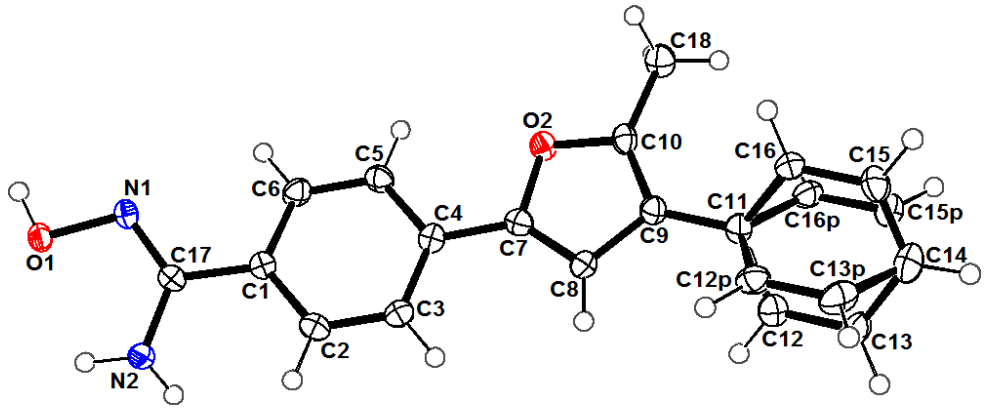
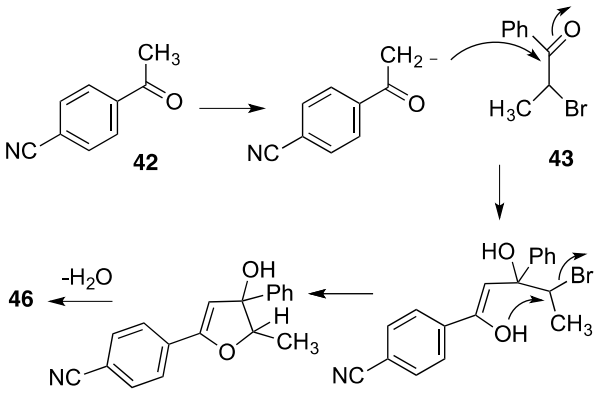


Figure 2. ORTEP diagram for the amidoxime derivative **47**.



Scheme 8. Proposed mechanism for the formation of furan nitrile **46**.

detailed crystallographic discussion of compound **47** can be found in Supplementary Material. A proposed mechanism for the formation of nitrile **46** is shown in Scheme 8. The amidoxime **47** was then converted to the amidine **48** for evaluation as an NQO2 inhibitor.

The ability of the synthesized compounds to inhibit the enzymatic activity of NQO2 was determined by a spectrophotometric method that monitored the decolouration of the blue redox dye 2,6-dichlorophenolindophenol (DCPIP) (pH 7.4). The rate of decolouration of DCPIP is indicative of NQO2 activity [3].

The isosteric replacement of furan ring **1** into imidazole **4**, *N*-methylimidazole **9**, oxazole **13** and thiophene **19** led to an increase of the IC_{50} values when compared with **1** (Table 1).

The isosteric replacement of amidine group **1** by methyl imidate **23**, amidoxime **24,** *N*-aryl amidines (reversed amidines) **25-26** and *para*-*N*-aryl amide **28** all led to the loss of NQO2 inhibitory activity (Table 1). The *meta*-*N*-aryl amides **27** and **29** showed moderate NQO2 inhibitory activity with IC_{50} values of approximately 1 and 2 μM, respectively. It should be noted that as the assay is performed at physiological pH, the amidine group is protonated. On the other hand, imidate **23** will be present as both the protonated and neutral forms since the pK_a of the conjugate acid is estimated to be 6.7.

In order to provide insight into the observed SAR, the synthesized NQO2 inhibitors were then computationally docked into the NQO2 active site using the X-ray crystal structure of human NQO2 with bound FAD (PDB code 1QR2; resolution of 2.1 Å) [23]. This was performed *via* the GOLD 5.1 docking software (CCDC, Cambridge, UK) with the ChemScore scoring function [24]. The ten top-scoring poses were saved and those with a steric clash term exceeding 6 kJ/mol were omitted.

Our prior *in silico* prediction of the NQO2-bound poses of a set of asymmetric furan-amidine compounds, including compound **1**,[3] found that π–π stacking interactions between ligands and NQO2 were a common feature, formed by the ligand aromatic rings with the isoalloxazine ring of FAD in particular, but also with other aromatic residues such as Phe178' and Phe126'. Secondly, the bound ligand poses were found to fall into three basic types [3], depending on the hydrogen bonding interaction of their amidine group with rather distinct regions of the active site: with Gln122 (pose I), with Asn161' (pose II) or with Thr71 (pose III).

As for this previous work, the binding geometries of asymmetric furan-, imidazole-, N-methylimidazole- and thiophene-amidines predicted here fit well in the deep NQO2 pocket and form a range of π–π stacking interactions. Furan-amidine **1,** the experimentally most potent NQO2 inhibitor, is

Table 1. cLogS, pKa, IC_{50} (± SE) for the inhibition of NQO2 and IC_{50} (± SE) for the inhibition of growth of *Plasmodium falciparum* by the non-symmetrical analogues of the lead furan-amidine **1**.

Compound	cLogS[a]	pK_a[a,b]	IC_{50} NQO2 (μM) ± SE	IC_{50} *Pf* (μM) ± SE
1	-1.81	11.1	0.068 ± 0.009	1.5 ± 0.002
4	-1.27	11.2	1.111 ± 0.005	5.3 ± 0.001
9	-0.75	11.2	Inactive[c]	NT[d]
13	-1.30	10.8	Inactive[c]	0.31 ± 0.001
19	-2.25	11.3	0.773 ± 0.004	18.9 ± 0.002
23	-5.64	6.7	Inactive[c]	NT[d]
24	-5.02	8.7	Inactive[c]	NT[d]
25	-3.42	9.5	Inactive[c]	0.77 ± 0.002
26	-3.87	9.6	Inactive[c]	0.97 ± 0.001
27	-5.34	-	1.14 (n=1)	NT[d]
28	-5.34	-	Inactive[c]	NT[d]
29	-5.90	-	2.00 (n=1)	NT[d]
48	-1.98	11.1	Inactive[c]	NT[d]
Resveratrol	-3.09	-	0.913 ± 0.023	NT[d]
DB75 [3,26]	0.00	11.42	0.035±0.0008	0.0005±0.0002

[a]Calculated using ChemAxon's Chemicalize program [8].

[b]pK_a of the conjugate acid of the strongest basic group.

[c]Inactive at 10 μM.

[d]NT – not tested.

predicted to adopt only pose I (magenta, Figure 3), forming a strong hydrogen bond between the amidine group and Gln122 sidechain and nearby sidechain of Glu193. For imidazole-amidine compound **4** (gold, Figure 3) and N-methylimidazole-amidine **9** (cyan, Figure 3), pose I structures are preferred and superimpose well with **1**. The N-methyl group of compound **9** points into the active site, slightly displacing the ligand outwards from the bracing Trp105 (Figure S8). For thiophene-amidine **19**, pose I (Figure 3) and pose III orientations (Figure S9) are predicted. For the latter, the amidine group hydrogen bonds to the sidechain OH of Thr71 and the backbone carbonyl of Asp117, although this pose possesses a relatively high active site clash energy, of 5.2 kJ/mol, as compared with pose I (1.9 kJ/mol). Therefore despite predicting a preference for pose I for compounds **1**, **4**, **9** and **19**, these results are unlikely to explain the observed differences in their inhibitory activity. Nevertheless, subtle changes in electrostatic and steric interactions associated with the O to S substitution may be at play in determining the overall difference in experimental potency for compounds **1** and **19**.

The malaria parasite *Plasmodium falciparum* has an enzyme that has a similar activity to NQO2, PfNDH2 [25], therefore some of the analogues were also tested against *Plasmodium* (Table 1). The oxazole-amidine **13** (IC_{50} 0.3 μM) was more active than the furan **1**, imidazole **4** and thiophene **19** analogues. The reverse amidine analogues **25** and **26,** which were not active as NQO2 inhibitors, both showed sub-micromolar IC_{50} activities in the *Plasmodium* parasite assay, indicating the intrinsic difference between the actual targets in human and in parasites.

In conclusion, novel heterocyclic derivatives (e.g. imidazole, oxazole and thiophene) with a range of side chains (e.g. imidate, N-aryl amide, amidoxime) were designed to enhance the drug-like properties (improved aqueous solubility and decreased basicity) of the lead furan-amidine **1**. Most of the synthesized analogues showed decreased or loss of activity as NQO2 inhibitors, when compared with **1**, however these results provide an insight into the SAR. The inactive amidoxime **24** is of interest as a potential pro-drug for the non-symmetric furan-amidine **1**. The oxazole-amidine **13** is the most active in the preliminary *Plasmodium falciparum* screen, showing a different SAR, promising therapeutic index against human cells and with mechanism of action studies on-going.

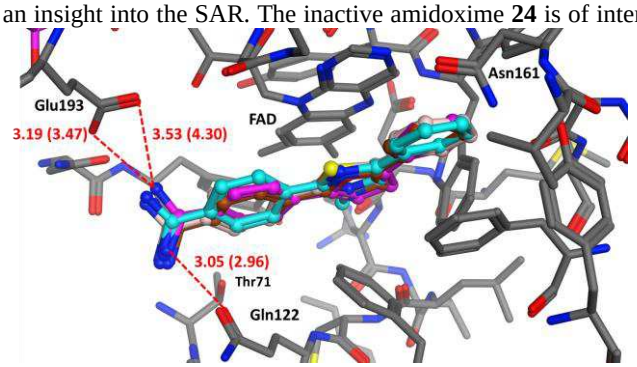


Figure 3. (A) Docked pose I of compounds **1** (magenta), **4** (gold), **9** (cyan) and **19** (pink) in NQO2 active site for. Distances in Å; in parentheses for distances involving **19**.

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Supplementary Material

Supplementary data related to this article can be found, in the online version, at…

References

1. Liao S, Williams-Ashman HG. Enzymatic oxidation of some non-phosphorylated derivatives of dihydronicotinamide. *Biochem. Biophys. Res. Co.* 1961;4:208-213.
2. Jaiswal AK. Human NAD(P)H: quinone oxidoreductase 2, gene structure, activity and tissue expression. *J. Biol. Chem.* 1994;269:14502-14508.
3. Alnabulsi S, Santina E, Russo I, Hussein B, Kadirvel M, Chadwick A, Bichenkova EV, Bryce RA, Nolan K, Demonacos C, Stratford IJ, Freeman S. Non-symmetrical furan-amidines as novel leads for the treatment of cancer and malaria. *Eur. J. Med. Chem.* 2016;111:33-45.
4. Weller T, Alig L, Beresini M, Blackburn B, Bunting S, Hadvary P, Muller HM, Knopp D, Levet-Trafit B, Lipari MT, Modi NB, Muller M, Refino CJ, Schmitt M, Schonholzer P, Weiss S, Steiner B. Orally active fibrinogen receptor antagonists. 2. Amidoximes as prodrugs of amidines. *J. Med. Chem.* 1996;39:3139-3147.
5. Ansede JH, Anbazhagan M, Brun R, Easterbrook JD, Hall JE, Boykin DW. O-Alkoxyamidine prodrugs of furamidine: in vitro transport and microsomal metabolism as indicators of in vivo efficacy in a mouse model of Trypanosoma brucei rhodesiense infection. *J. Med. Chem.* 2004;47:4335-4338.
6. Nolan KA, Dunstan MS, Caraher MC, Scott KA, Leys D, Stratford IJ. In silico screening reveals structurally diverse, nanomolar inhibitors of NQO2 that are functionally active in cells and can modulate NF-kB signaling. *Mol. Cancer Ther.* 2012;11:194-203.

7. Chem Axon, calculation of LogP, pKa and LogS www.chemaxon.com
8. Zuliani V, Cocconcelli G, Fantini M, Ghiron C, Rivara M. A practical synthesis of 2,4(5)-diarylimidazoles from simple building blocks. *J. Org. Chem.* 2007;72:4551-4553.
9. Anbazhagan M, Boykin DW, Stephens CE. Direct conversion of amidoximes to amidines via transfer hydrogenation. *Synthesis*. 2003;16:2467-2469.
10. Brieger G, Nestrick TJ. Catalytic Transfer Hydrogenation. *Chem. Rev.* 1974;74:567-580.
11. Lakner FJ, Parker MA, Rogovoy B, Khvat A, Ivachtchenko A, Synthesis of novel trisubstituted imidazolines. *Synthesis*. 2009;12:1987-1990.
12. Robinson R. A new synthesis of oxazole derivatives. *J. Chem. Soc.* 1909;95:2167-2174.
13. Wasserman HH, Vinick FJ. The mechanism of the Robinson-Gabriel synthesis of oxazoles. *J. Org. Chem.* 1973;38:2407-2408.
14. Mahajan US, Godinde RR, Mandhare PN. Preparation of amidines from amidoximes via transfer hydrogenation. *Synthetic Commun.* 2011;41:2195-2199.
15. Dabak K. Synthesis and protection of some amidines. *Turk. J. Chem.* 2002;26:547-550.
16. Aly AA, Nour-El-Din AM. Functionality of amidines and amidrazones. *ARKIVOC*. 2008;1:153-194.
17. Pearse GA, Pflaum RT, Interaction of metal ions with amidoximes. *J. Am. Chem. Soc.* 1959;81:6505-6508.
18. Clement B, Jung F. N-hydroxylation of the antiprotozoal drug pentamidine catalyzed by rabbit liver cytochrome P-450 2C3 or human liver microsomes, microsomal retroreduction and further oxidative transformation of the formed amidoximes. *Drug Metab. Dispos.* 1994;22:486-497.
19. Yoo S, Lee S. Reduction of organic compounds with sodium borohydride-copper (II) sulfate system. *Synlett.* 1990;7:419-420.
20. Shearer BG, Oplinger JA, Lee S. S-2-Naphthylmethyl thioacetimidate hydrobromide: a new odorless reagent for the mild synthesis of substituted acetamidines. *Tetrahedron Lett.* 1997;38:179-182.
21. Schnur RC. A generalized and proton-catalyzed synthesis of amidines from thioimidates. *J. Org. Chem.* 1979;44:3726-3727.
22. Stephens CE, Tanious F, Kim S, Wilson WD, Schell WA, Perfect JR, Franzblau SG, Boykin DW. Diguanidino and "reversed" diamidino 2,5-diarylfurans as antimicrobial agents. *J. Med. Chem.* 2001;44:1741-1748.
23. Foster CE, Bianchet MA, Talalay P, Zhao Q, Amzel, LM. Crystal Structure of Human Quinone Reductase Type 2, a Metalloflavoprotein. *Biochemistry*. 1999;38:9881-9886.
24. Eldridge MD, Murray CW, Auton TR, Paolini GV, Mee RP. Empirical scoring functions: I. The development of a fast empirical scoring function to estimate the binding affinity of ligands in receptor complexes. *J. Comput. Aided Mol. Des.* 1997;11:425-445.
25. Nixon GL, Pidathala C, Shone AE, Antoine T, Fisher N, O'Neill PM, Ward SA, Biagini GA. Targeting the mitochondrial electron transport chain of Plasmodium falciparum: new strategies towards the development of improved antimalarials for the elimination era. *Future Med. Chem.* 2013;5:1573-1591.
26. A.E. Purfield, R.R. Tidwell, S.R. Meshnick, The diamidine DB75 targets the nucleus of Plasmodium falciparum, *Malar. J.* 2009; 8.