Bioorganic & Medicinal Chemistry Letters xxx (2018) xxx–xxx

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

# Evaluation of analogues of furan-amidines as inhibitors of NQO2

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ARTICLE INFO

*Article history:*
Received 23 January 2018
Revised 8 March 2018
Accepted 10 March 2018
Available online xxxx

*Keywords:*
NQO2 inhibitors
Furan-amidines
Isosteres
Anti-cancer
Malaria
SAR

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** (Fig. 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** (Fig. 1) with an $IC_{50}$ of 50 nM.[6] Given the structurally similarity of compounds **1** and **2**, it is of interest to assess the activity of non-symmetrical 4-methylfuran-amidine analogue **3** (Fig. 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

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https://doi.org/10.1016/j.bmcl.2018.03.025
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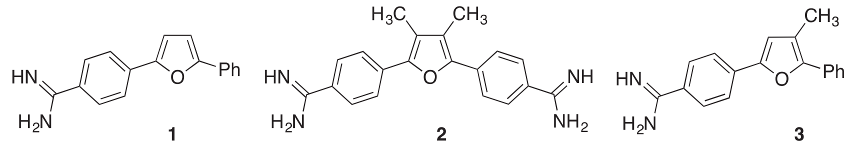
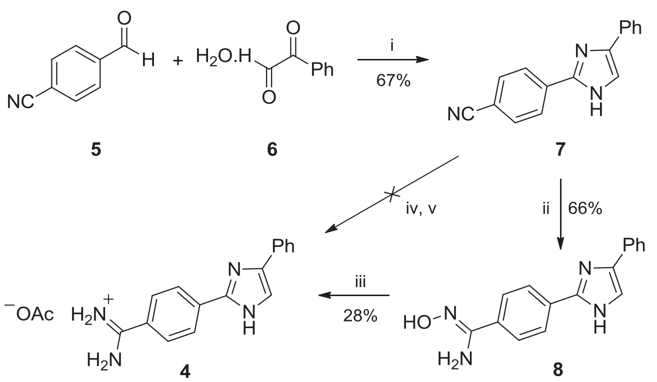
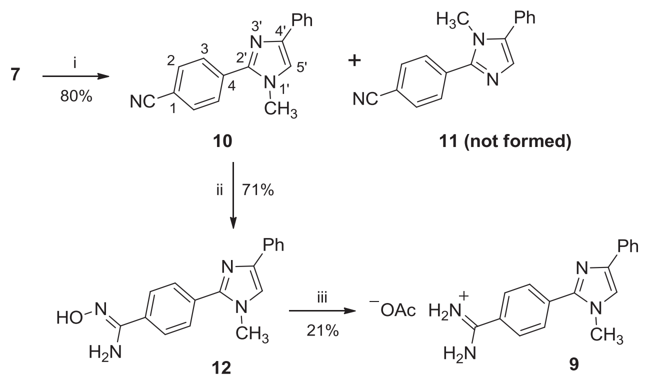


**Fig. 1.** Structures of the non-symmetrical furan-amidine **1**, the symmetrical 3,4-dimethylfuran-amidine **2** and the proposed 4-methylfuran-amidine **3**.



**Scheme 1.** Synthesis of 4-(4-phenyl-1*H*-imidazol-2-yl)benzamidine acetate **4**; Reagents and conditions: (i) $NH_4OAc$, MeOH, rt. (ii) $NH_2OH{\cdot}HCl$, *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 h.



**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) $NH_2OH{\cdot}HCl$, *t*-BuOK, dry DMSO, 0 °C – rt; (iii) $HCO_2NH_4$, Pd/C, AcOH, reflux.

possibility of the formation of two regioisomers **10** or **11**. The NOESY spectrum confirmed the formation of the least hindered regioisomer **10** (see Fig. 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[9]) was also synthesized (Scheme 4) as a more lipophilic isostere of the furan-amidine **1** (clogS −1.81, 4.03 mg/ml[9]). 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]

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 (Fig. 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