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Structure–activity relationship of pyrrole based *S*-nitrosoglutathione reductase inhibitors: Carboxamide modification

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ABSTRACT

The enzyme *S*-nitrosoglutathione reductase (GSNOR) is a member of the alcohol dehydrogenase family (ADH) that regulates the levels of *S*-nitrosothiols (SNOs) through catabolism of *S*-nitrosoglutathione (GSNO). GSNO and SNOs are implicated in the pathogenesis of many diseases including those in respiratory, gastrointestinal, and cardiovascular systems. The pyrrole based **N6022** was recently identified as a potent, selective, reversible, and efficacious GSNOR inhibitor which is currently in clinical development for acute asthma. We describe here the synthesis and structure–activity relationships (SAR) of novel pyrrole based analogs of **N6022** focusing on carboxamide modifications on the pendant *N*-phenyl moiety. We have identified potent and novel GSNOR inhibitors that demonstrate efficacy in an ovalbumin (OVA) induced asthma model in mice.

Nitric oxide (NO) is synthesized from L-arginine by nitric oxide synthases (NOS).[1,2] *S*-nitrosoglutathione (GSNO), an adduct of NO and glutathione, exists in equilibrium with other low molecular weight and protein-bound *S*-nitrosothiols (SNOs). GSNO and SNOs serve as more stable reservoirs for bioavailable NO, in comparison to NO itself. *S*-nitrosoglutathione reductase (GSNOR, also known as alcohol dehydrogenase 3) catalyzes the reduction of GSNO[3,4] to the unstable intermediate *S*-(*N*-hydroxyamino)glutathione which spontaneously rearranges to glutathione sulfinamide or reacts with glutathione (GSH) to form glutathione disulfide and hydroxylamine.[4–8] At low pH, the glutathione sulfinamide is readily hydrolyzed to sulfinic acid and ammonia.[4] Therefore GSNOR indirectly controls intracellular levels of SNOs and thus, NO (Fig. 1).[9–16]

GSNOR knockout mice have been shown to have increased lung SNOs and were protected from airway hyperresponsiveness after methacholine or allergen challenge, suggesting that GSNOR is a crucial modulator of airway tone.[3,17] Given such findings, GSNOR has been recognized as a potential therapeutic target for the treatment of a broad range of diseases due to the important role that GSNO plays in the biological systems.[18–23] We recently reported the discovery of **N6022**,[24] a potent GSNOR inhibitor that is in clinical development for the treatment of acute asthma. Following this communication, we also disclosed the structure–activity relationship of the pyrrole based GSNOR inhibitors related to **N6022** including the identification of pyrrole regioisomer **17**[25] and potent GSNOR inhibitor **8f**[26] with reduced CYP inhibition, as shown in Figure 2. In this Letter, we discuss the synthesis and structure–activity relationship of the pyrrole based GSNOR inhibitors mainly focusing on the replacement and modification of the carboxamide, in an attempt to further understand the structure–activity relationship and improve enzyme inhibitory potency and ADME properties.

The general synthetic route of GSNOR inhibitors is outlined in Scheme 1. The synthesis started from either commercially available ketones or the ketones prepared according to the procedures described in the Supplementary data. In Scheme 1, condensation of ketones **1** and 2-furanaldehyde provided intermediate **2** in good yield.[27] Furan ring opening of intermediates **2** by hydrogen bromide in ethanol under reflux conditions provided diketones **3**.[28] Pyrrole formation was achieved by condensation of the diketones **3** with anilines under acidic conditions to afford compounds **4**.[29] The synthesis of compounds **5a–5w**, where the X is bromo or methoxy, was accomplished by hydrolysis of compounds **4** in aqueous lithium hydroxide. Compounds **7a –7x** were synthesized using substituted imidazoles as starting materials to couple with intermediates **4** (X = Br) using L-proline as a catalyst in the presence of copper iodide (I) and potassium carbonate in dimethylsulfoxide

Abbreviations: GSNOR, *S*-nitrosoglutathione reductase; GSNO, *S*-nitrosoglutathione; NO, nitric oxide; SNOs, nitrosothiols.

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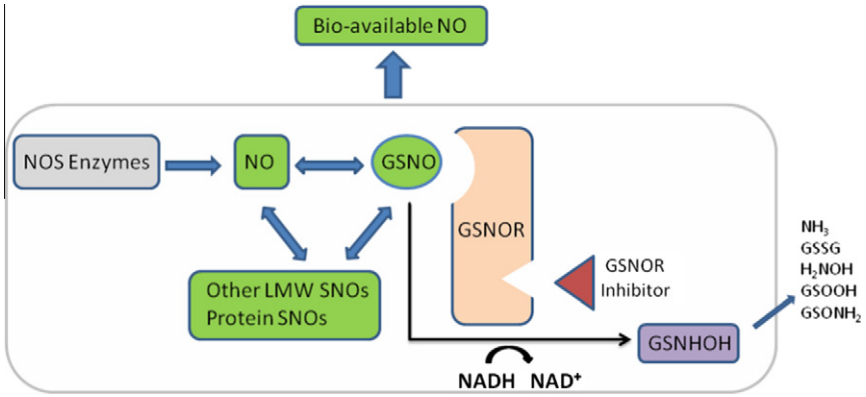


Figure 1. Role of GSNOR enzyme.

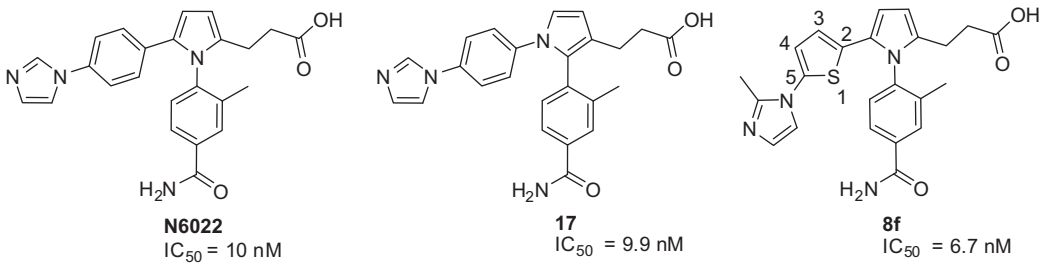
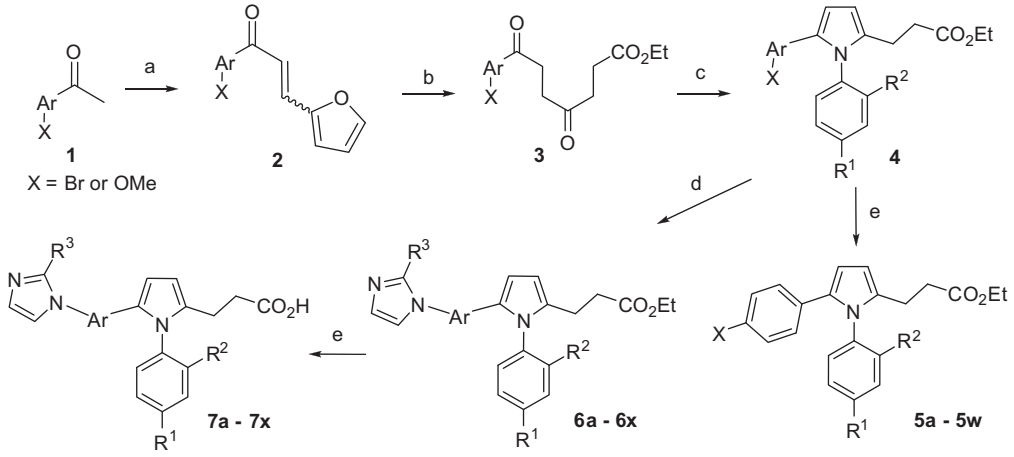


Figure 2. Potent GSNOR inhibitors (IC_{50} determined in plate format).



Scheme 1. Synthetic route of GSNOR inhibitors. Reagents and conditions: (a) furan-2-carbaldehyde/NaOMe/MeOH, room temperature, overnight; (b) HBr/EtOH, reflux, 8 h; (c) aniline/pTsOH/EtOH, reflux, overnight; (d) imidazole/L-proline/CuI/K_2CO_3/DMSO; (e) LiOH.

followed by hydrolysis of the ester in aqueous lithium hydroxide.[30,31] The synthesis of key compounds is described in the Supplementary data and the other compounds were prepared in the similar manners as detailed in our earlier publications.[24–26]

To examine the SAR of the amide replacement, we kept the rest of the molecule the same, X = OMe, and R^2 = H or Me (Table 1) except compound **5w**, where X is bromo. Within the des-methyl series **5a–5i**, where R^2 = H, the hydroxyl analog **5a** is the most potent inhibitor followed by the amide analog **5d.** Methylation of the hydroxyl analog **5a** (**5b**) resulted in a 4–5-fold loss in GSNOR inhibition activity. Replacing the hydroxyl group with bromide **5c** also diminished the binding affinity to the enzyme. The reversed amide **5f** lost 10-fold GSNOR inhibitory activity. Spacing the amide from the phenyl ring with either methylene **5h** or NH (urea) **5i** caused >10-fold loss in the GSNOR inhibitory activity. More extensive SAR was explored with the methyl series, where R^2 = Me. Sulfonamide **5m** achieved the best activity with IC_{50} = 330 nM followed by the sulfonyl diamide **5n**. Interestingly, the hydroxyl analog **5j** was not as potent as the des-methyl comparator **5a** and O-methylation also resulted in only a minor loss in activity. Substituted amide analogs **5o** and **5p** were much less active than the primary amide reported earlier (X = OMe, R^1 = $CONH_2$, R^2 = Me, IC_{50} = 210 nM).[24] However, introducing a methoxyethyl group **5q** or hydroxyethyl group **5r** recovered some of the loss in GSNOR inhibition. Furthermore, we prepared the heterocyclic amides **5s–5v** in an attempt to pick up more binding to the enzyme. The 4-pyridyl amide **5u** demonstrated an IC_{50} of 170 nM, which is the best within the series. The bromo analog of **5u** achieved double digit nanomolar IC_{50} (61 nM).