Bioorganic & Medicinal Chemistry Letters 22 (2012) 2338–2342

Contents lists available at SciVerse ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

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

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ARTICLE INFO

Article history:
Received 6 October 2011
Accepted 13 January 2012
Available online 2 February 2012

Keywords:
GSNO
GSNOR
GSNOR inhibitor
Nitrosothiols
Nitric oxide
Asthma
N6022

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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doi:10.1016/j.bmcl.2012.01.047

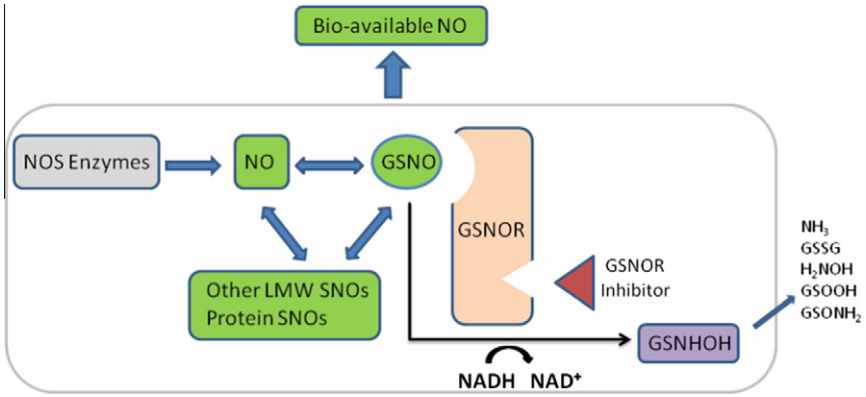


Figure 1. Role of GSNOR enzyme.

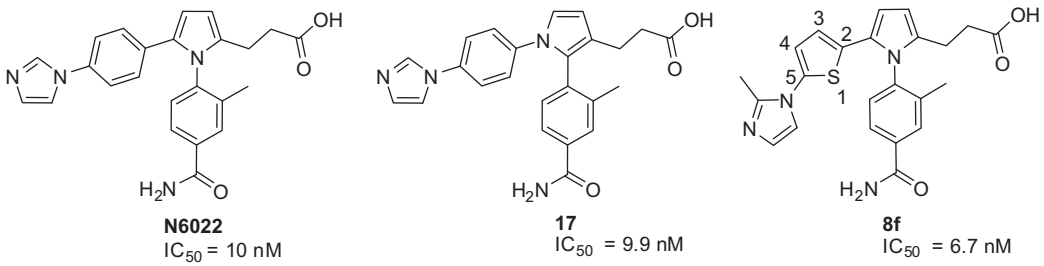
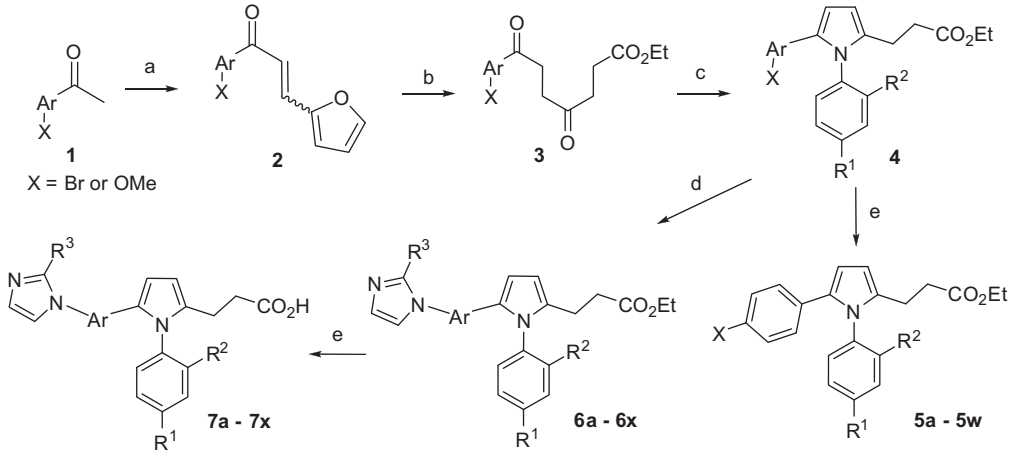


Figure 2. Potent GSNOR inhibitors (IC_{50} determined in plate format).



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).

Table 1
Structure–activity relationship of non-imidazole analogs

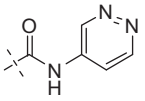
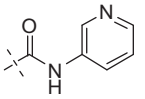
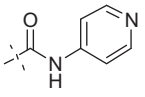
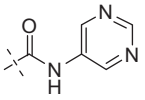
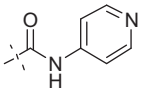
Compound	X	R^1	R^2	GSNOR IC_{50} (nM)
5a	4-OMe	OH	H	240
5b	4-OMe	OMe	H	1020
5c	4-OMe	Br	H	3250
5d	4-OMe	$CONH_2$	H	460
5e	4-OMe	COMe	H	960
5f	4-OMe	NHAc	H	3270
5g	4-OMe	CONHMe	H	4560
5h	4-OMe	CH_2CONH_2	H	Inactive
5i	4-OMe	$NHCONH_2$	H	4800
5j	4-OMe	OH	Me	1550
5k	4-OMe	Me	Me	8840
5l	4-OMe	OMe	Me	1800
5m	4-OMe	SO_2NH_2	Me	330
5n	4-OMe	$NHSO_2NH_2$	Me	710
5o	4-OMe	CONHMe	Me	2410
5p	4-OMe	$CONMe_2$	Me	1310
5q	4-OMe	$CONH(CH_2)_2OMe$	Me	730
5r	4-OMe	$CONH(CH_2)_2OH$	Me	660
5s	4-OMe		Me	640
5t	4-OMe		Me	440
5u	4-OMe		Me	170
5v	4-OMe		Me	3740
5w	4-Br		Me	61

Table 2
Structure–activity relationship of imidazole containing analogs

Compound	R^1	R^2	R^3	Ar	GSNOR IC_{50} (nM)
8f	$CONH_2$	Me	Me	2,5-Thienyl	17 (6.7*)
7a	SO_2NH_2	Me	Me	2,5-Thienyl	22
7b	$NHSO_2Me$	Me	Me	2,5-Thienyl	43
7c	$NHSO_2Me$	Me	Me	2,4-Thienyl	76
7d	$NHSO_2Me$	Me	Me	1,4-Phenyl	180
7e	NHCOMe	Me	Me	1,4-Phenyl	450
7f	OH	Me	H	1,4-Phenyl	180
7g	NH_2	Me	H	1,4-Phenyl	1410
7h	CH_2NH_2	Me	H	1,4-Phenyl	1590
7i	CO_2H	Me	H	1,4-Phenyl	110
7j	NHCOMe	Me	H	1,4-Phenyl	56
7k	$NHSO_2Me$	Me	H	1,4-Phenyl	29
7l	NHCOEt	Me	H	1,4-Phenyl	88
7m	$NHCOCH_2OMe$	Me	H	1,4-Phenyl	96
7n	OH	H	Me	1,4-Phenyl	54
7o	OH	H	Me	2,5-Thienyl	36
7p	OH	H	Me	2,4-Thienyl	96
7q	OH	H	Me	3,5-Thienyl	45
7r	SO_2NH_2	H	Me	2,5-Thienyl	23
7s	NHAc	H	Me	2,4-Thienyl	140
7t	$NHSO_2Me$	H	Me	2,4-Thienyl	360
7u	NHAc	H	Me	1,4-Phenyl	3890
7v	$NHSO_2Me$	H	Me	1,4-Phenyl	1790
7w	OH	H	H	2,5-Thienyl	32
7x	OH	H	H	1,4-Phenyl	39

* IC_{50} was determined in plate format.

Further SAR was explored with the imidazole series to achieve better enzyme inhibition activity (Table 2). In comparison to **8f** published earlier,[26] replacing the amide with sulfonamide **7a** and reverse sulfonamide **7b** maintained the GSNOR inhibitory activity. It is clear that exchanging the phenyl ring (**7d**, **7u** and **7v**) by thienyl (**7b, 7s** and **7t**) improved the GSNOR inhibition activity 4–10-fold. Within the phenyl series **7f**–**7m**, the reverse sulfonamide **7k** demonstrated the best activity, followed by the reverse amides **7j**, **7l** and **7m**. Compounds with basic functionalities such as amine (**7g**) and aminomethyl (**7h**) resulted in a substantial loss in GSNOR inhibitory activity. Within the methyl imidazole series **7n**–**7v**, 2,4-substituted thienyl analog **7p** is less active than the 2,5-disubstituted analog **7o**. In the phenol series **7n**–**7q**, **7w**, and **7x**, where R^1 = OH, des-methyl analog **7x** seems more active than the corresponding methyl analog **7f**, this was not observed in the other amide replacement compounds. Methyl imidazole **7n** is also less active than its des-methyl imidazole analog **7x**.

Selected GSNOR inhibitors were screened for potential off-target activity with a panel of 55 transmembrane and soluble receptors, ion channels, and monoamine transporters involved in maintaining homeostasis of critical organ systems. Typical binding assays were performed with a minimum of 6-control wells with/without vehicle for soluble compounds. Inhibition of 50% or greater was considered a positive response. Off-target effects were estimated from the percent inhibition of receptor radio-ligand binding in the presence of 10 μM of test compound. Compound **7b** and **7k** did not show any off-target activity in the Cerep receptor/ion channel panel, by contrast with **N6022** as reported earlier.[24]

Compounds **7a–7c**, **7k**, **7r**, and **7o** were also screened for cytotoxicity towards the A549 epithelial lung cell line. The IC_{50} values for all compounds tested were >150 μM.

Compounds **7o** and **7x** were selected for pharmacokinetic studies in mice. Oral bioavailability of these compounds was 3.9% and 6.8%, respectively, compared to 4.4% for **N6022** reported previously.[24] The plasma clearance (CL) after intravenous (IV) administration was 23.9 and 37.1 ml/min/kg for **7o** and **7x**, respectively, which is comparable to 37.7 ml/min/kg for **N6022**.[24]

Compound **7b** was tested in a 5-day mouse toxicity study with intravenous QD dosing at 1, 10, or 50 mg/kg. Surprisingly, despite a better off-target activity profile of this compound compared to **N6022**, the treatment of male CD-1 mice with **7b** for 5 days resulted in significant adverse effects. In particular, histological findings demonstrated toxicity to the liver, spleen, and thymus of treated animals. The NOAEL for **7b** from the study was determined to be 1 mg/kg/day.

The efficacy of GSNOR inhibitors was assessed in an animal model of asthma, a disease influenced by dysregulated GSNOR and altered function of NO, GSNO, and SNOs.[32] Asthma was induced by exposure of mice to OVA. **7b** was given as a single 1 mg/kg IV dose 24 h prior to challenge with aerosolized methacholine (MCh). Other groups of mice were treated with 3 inhaled doses of Combivent (5.2 mg/kg albuterol and 0.9 mg/kg ipratropium per dose at 48, 24, and 1 h prior to MCh) or a single iv administration of PBS vehicle as study controls. Efficacy was assessed by measuring attenuation of the MCh-induced bronchoconstriction using whole body plethysmography (Buxco) and attenuation of the eosinophil infiltration into the bronchoalveolar lavage fluid (BALF). Values are means ± SEM of 10 mice per group. Compound **7b** attenuated methacholine-induced bronchoconstriction (airway hyper-responsiveness) and eosinophil infiltration into the lungs following a single IV dose administered 24 h prior to the methacholine challenge. Significant efficacy was observed for compound **7b** at dose 1 mg/kg (Figs. 3 and 4).

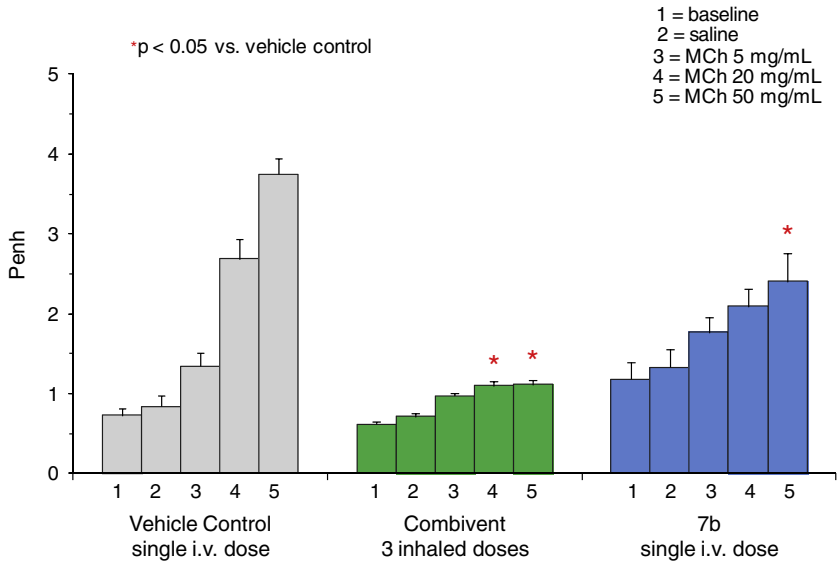


Figure 3. Bronchodilatory action in a mouse model of OVA-induced asthma.

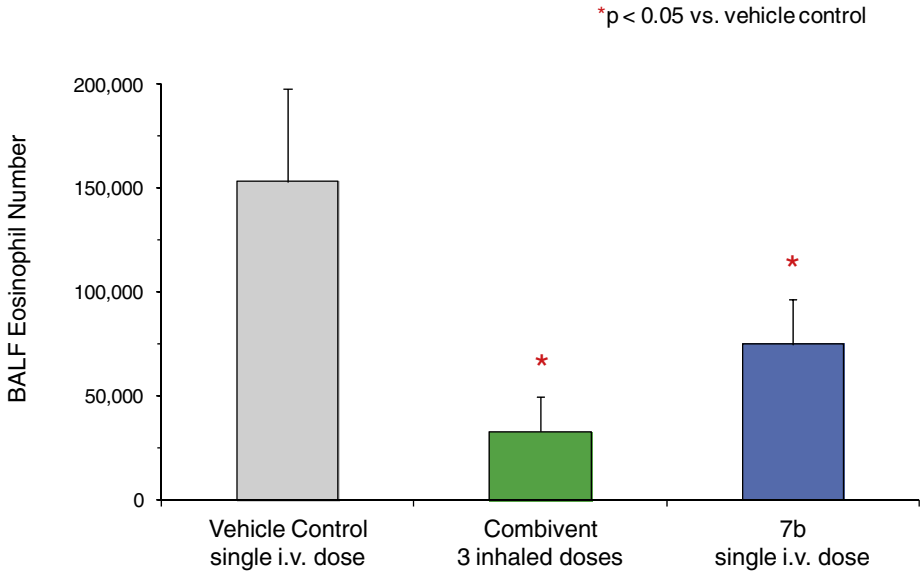


Figure 4. Anti-inflammatory action in a mouse model of OVA-induced asthma.

In conclusion, the carboxamide substituent on the pendant *N*-phenyl ring of pyrrole based GSNOR inhibitors can be replaced by a number of functional groups such as hydroxyl, sulfonamide, reverse amide, and reverse sulfonamide without losing significant GSNOR inhibition activity. The thienyl analogs are generally more potent than their phenyl counter parts. Compound **7b** demonstrated potent inhibitory activity, while having no off-target activities in the Cerep receptor/ion channel panel screening and a clean profile in cytotoxicity assay. In vivo efficacy was achieved with **7b** in the OVA induced asthma model in mice. Compound **7b** was well tolerated when administered IV in 5-day toxicity evaluations in mice up to 50 mg/kg. However, this compound had a less desirable safety profile with a NOAEL of 1 mg/kg as compared to **N6022** with a NOAEL of 30 mg/kg.

Acknowledgments

The authors are grateful to Dr. Jasbir Singh and his team at deCODE chemistry for the synthetic chemistry support. We also thank Dr. Zhaojun Zhang and his colleagues at Chempartner, Shanghai for the synthesis of some of the GSNOR inhibitors and Dr. Larry Ross at SRI for screening GSNOR inhibitors in the enzyme assay.

Supplementary data

Supplementary data (experimental details and characterization of selected compounds) associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2012.01.047.

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