

Structure – Activity Relationships 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, cardiovascular, and gastrointestinal 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 analogues of **N6022** focusing on carboxamide modifications. We have identified potent and novel GSNOR inhibitors that demonstrate efficacy in an ovalbumin (OVA) induced asthma model in mice.

Introduction

S-nitrosoglutathione reductase, an enzyme that catalyzes the reduction of S-nitrosoglutathione¹⁻² has been recognized as a potential therapeutic target for the treatment of broad range of diseases due to the important role that GSNO plays in the biological system (Figure 1).³⁻⁶ We recently reported the discovery of **N6022**,⁷ 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 compounds **17**⁸ and **8f**⁹ as shown in Figure 2. In this poster, we discuss the synthesis and structure activity relationship of the pyrrole based GSNOR inhibitors mainly focusing on the replacement/modification of the carboxamide, in an attempt to further understand the SAR and improve enzyme inhibitory potency and ADME properties.

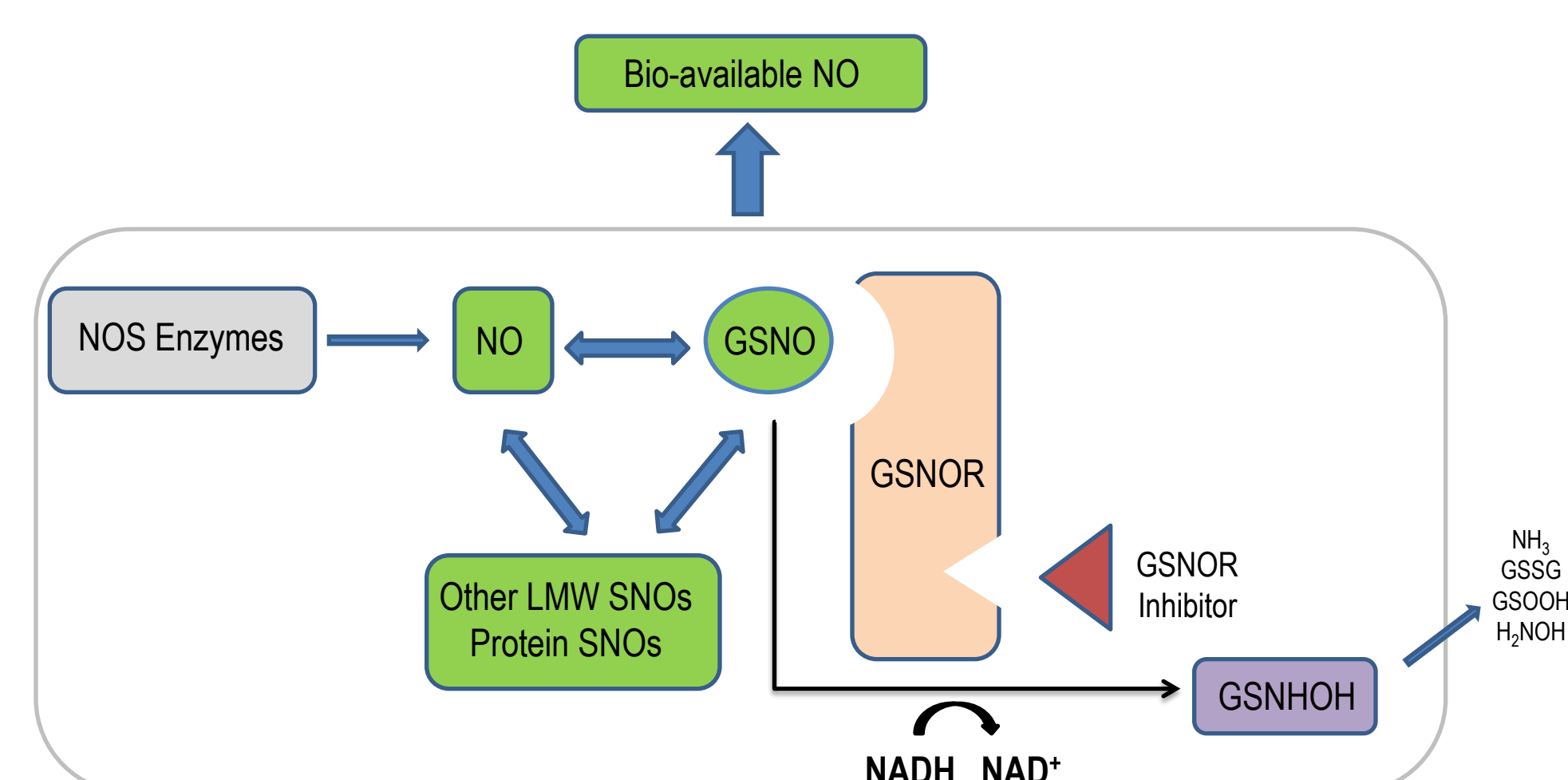


Figure 1. Role of GSNOR enzyme

Figure 2. Potent GSNOR inhibitors (IC₅₀ determined in plate format)

Synthesis of GSNOR inhibitors

Scheme 1. Synthesis of GSNOR inhibitors

Reagents and conditions: (a) furan-2-carbaldehyde/NaOMe/MeOH, room temperature, overnight. (b) HBr/EtOH, reflux. (c) aniline/pTsOH/EtOH, reflux, overnight. (d) imidazole/L-proline/CuI/K₂CO₃/DMSO (e) LiOH.

The GSNOR inhibitors were synthesized according to Scheme 1. The synthesis started from either commercially available ketones or the ketones prepared according to literature procedures.⁹ In Scheme 1, condensation of ketones **1** and 2-furaldehyde provided intermediates **2** in good yield. Furan ring opening of intermediates **2** by HBr in ethanol under reflux conditions provided diketones **3**. Pyrrole formation was achieved by condensation of the diketones **3** with anilines under acidic conditions to afford compounds **4**. 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** using L-proline as a catalyst in the presence of copper iodide (I) and potassium carbonate in DMSO followed by hydrolysis of esters **6a-6x** in aqueous lithium hydroxide.

Structure activity relationship of GSNOR inhibitors

Table 1 SAR of non-imidazole analogs

cmpd	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 ₂ CONH 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 ₂ NH ₂	Me	330
5n	4-OMe	NHSO ₂ NH ₂	Me	710
5o	4-OMe	CONHMe	Me	2410
5p	4-OMe	CONMe ₂	Me	1310
5q	4-OMe	CONH(CH ₂) ₂ OMe	Me	730
5r	4-OMe	CONH(CH ₂) ₂ OH	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

- Within the des-methyl series **5a-5i**, where R² = H, the hydroxyl analog **5a** is the most potent inhibitor followed by the amide analog **5d**.
- Within the methyl series **5j-5v**, where R² = Me, 4-pyridylamide **5u** offers best activity followed by sulfonamide **5m** and 3-pyridyl amide **5t**.
- When the methoxy group of **5u** was replaced with bromo **5w**, double digit nM IC₅₀ was achieved.

Table 2 SAR of imidazole containing analogs

cmpd	R ¹	R ²	R ³	Ar	GSNOR IC ₅₀ (nM)
8f	CONH ₂	Me	Me	2,5-thienyl	17 (6.7*)
7a	SO ₂ NH ₂	Me	Me	2,5-thienyl	22
7b (N6625)	NHSO ₂ Me	Me	Me	2,5-thienyl	43
7c	NHSO ₂ Me	Me	Me	2,4-thienyl	76
7d	NHSO ₂ Me	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 ₂ NH ₂	Me	H	1,4-phenyl	1590
7i	CO ₂ H	Me	H	1,4-phenyl	110
7j	NHCOMe	Me	H	1,4-phenyl	56
7k	NHSO ₂ Me	Me	H	1,4-phenyl	29
7l	NHCOEt	Me	H	1,4-phenyl	88
7m	NHCOCH ₂ OMe	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 ₂ NH ₂	H	Me	2,5-thienyl	23
7s	NHSO ₂ Me	H	Me	2,4-thienyl	360
7t	NHAc	H	Me	2,4-thienyl	140
7u	NHAc	H	Me	1,4-phenyl	3890
7v	NHSO ₂ Me	H	Me	1,4-phenyl	1790
7w	OH	H	H	2,5-thienyl	32
7x	OH	H	H	1,4-phenyl	39

*IC₅₀ determined in plate format

- In comparison to **8f**,⁹ the sulfonamide **7a** and reverse sulfonamide **7b** maintained the GSNOR inhibitory activity.
- Thienyl analogs **7b**, **7s** and **7t** are more potent than phenyl counterparts **7d**, **7v** and **7u** respectively.
- In the phenyl series **7f-7m**, reverse sulfonamide **7k** demonstrated best activity, followed by reverse amides **7j**, **7l** and **7m**.
- Within the methyl imidazole series **7n-7v**, 2,4-substituted thienyl analog **7p** is less active than the 2,5-disubstituted thienyl compound **7o**.
- In the phenol series, where R¹ = OH, des-methyl analog **7x** seems more active than methyl analog **7f**, this was not observed in the other amide replacement compounds. Methyl imidazole **7n** is also less active than des-methyl imidazole **7x**, observed similarly before.⁹

In vivo activities of GSNOR inhibitors

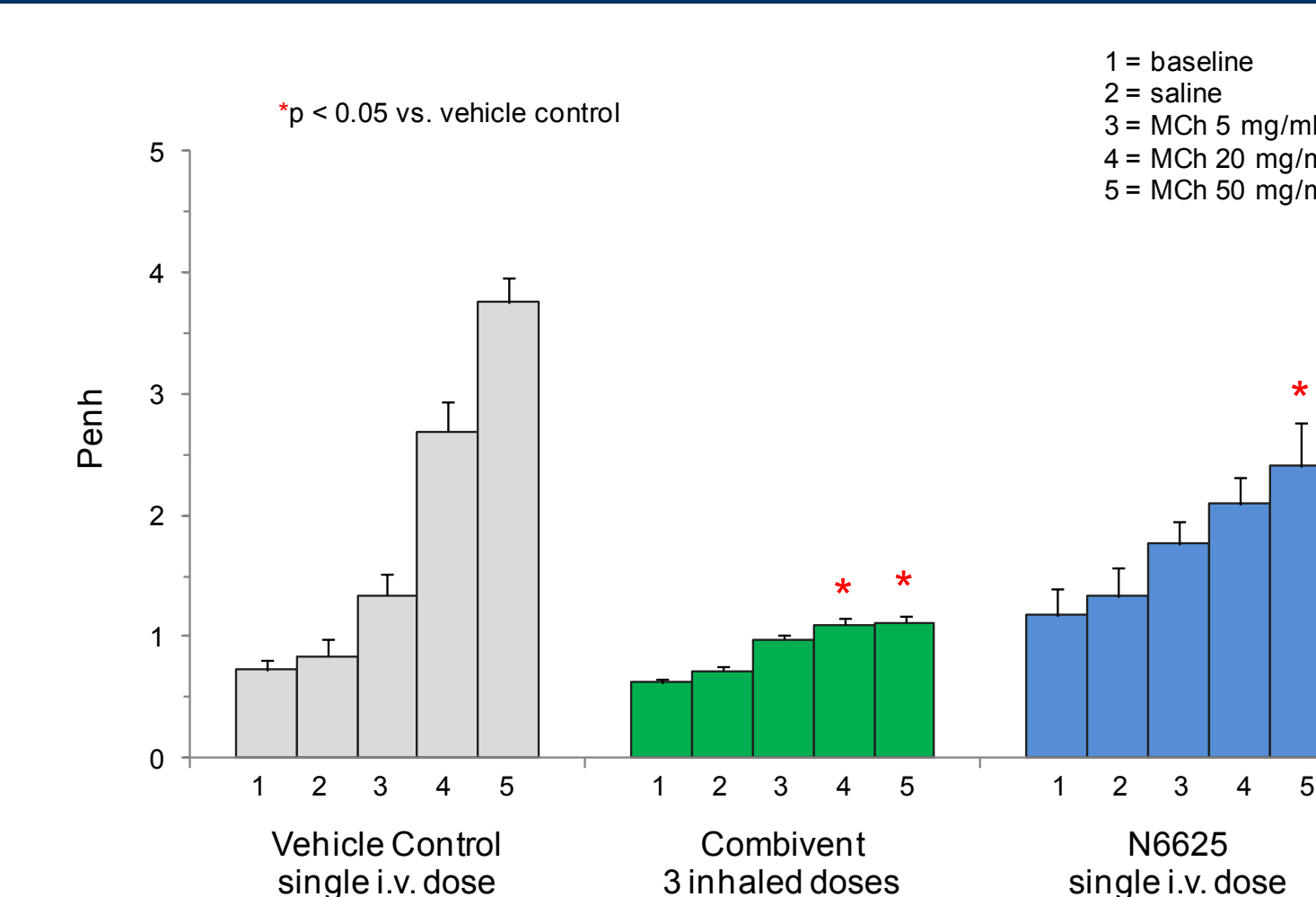


Figure 3 Bronchodilatory action in a mouse model of OVA-induced asthma

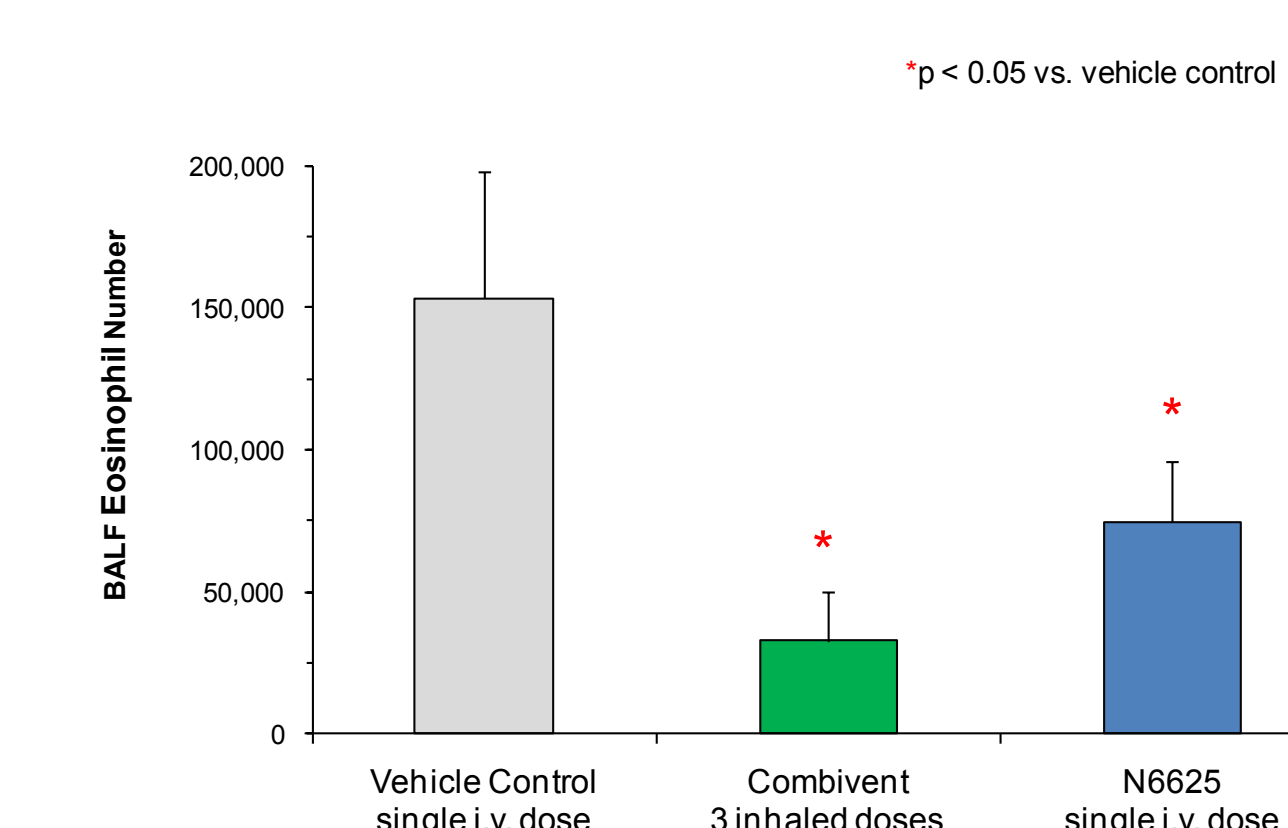


Figure 4 Anti-inflammatory action in a mouse model of OVA-induced asthma

Asthma was induced by exposure of mice to OVA. **N6625** was given as a single 1 mg/kg i.v. 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 h, 24 h, and 1 h prior to MCh) or a single i.v. 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.

Conclusions

The carboxamide can be replaced by a number of functional groups such as hydroxyl, sulfonamide, reverse amide, and sulfonamide without losing significant GSNOR inhibition activity. The thienyl analogs are generally more potent than the phenyl counter parts. Compound **N6625** demonstrated potent inhibitory activity, while having no off-target activities in the Cerep receptor/ion channel panel screening. *In vivo* efficacy was achieved with **N6625** in the OVA induced asthma model in mice. Compound **N6625** 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.

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