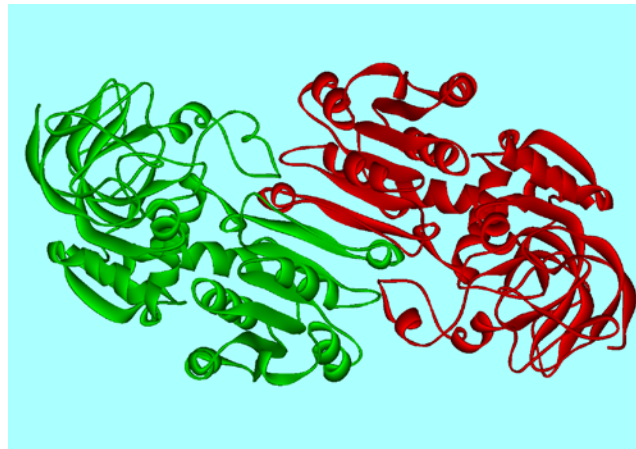


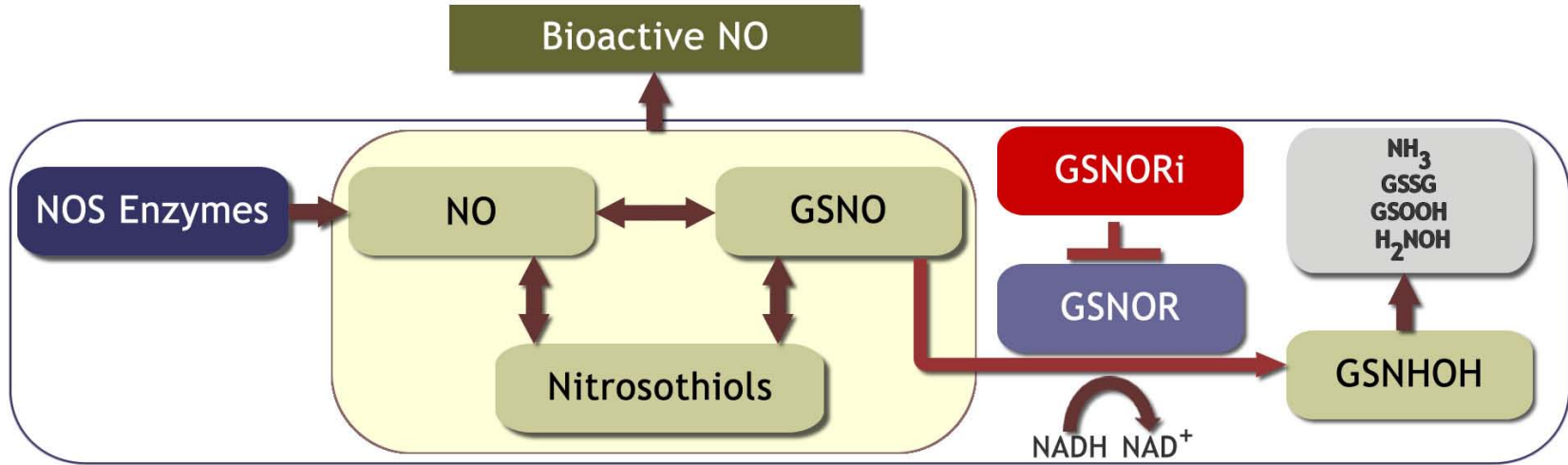
# Discovery of S-nitrosogluthatione reductase inhibitors as potential agents to treat asthma, COPD and IBD

N30 Pharmaceuticals LLC, Boulder, Colorado

Xicheng Sun, PhD



# GSNO – Bioactive NO



**S-nitrosothiols (SNO's):** Stabilize bioactive nitric oxide (NO) and promote its critical role in signaling throughout the body.

**S-nitrosoglutathione (GSNO):** The most abundant SNO and widely considered the primary source of bioactive nitric oxide in the body

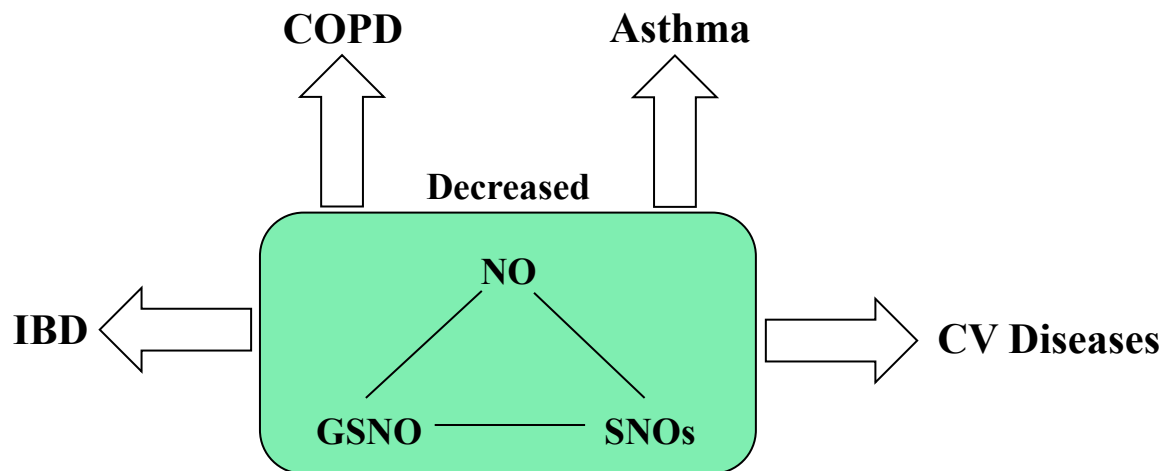
**GSNO reductase (GSNOR):** GSNOR regulates SNO levels and NO availability through GSNO catabolism

# GSNOR KO Mice – Target Validation

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- Healthy, reproduce, and grow similarly to normal animals
- They have **increased circulating nitric oxide**
- **They are resistant to provoked asthma**
- Increased **capillary density** in the micro-circulation
- Smooth muscle relaxation and increased airflow
- Basal blood pressures same as wild type animals
- Increased  $\beta$ -receptor density
- Decreased  $\beta$ -receptor tachyphylaxis

# Biological Rationale of Preserving Endogenous GSNO



1. Some of the most intractable diseases, including asthma, COPD, IBD and CV disease, have **decreased bio-available NO** as central to their pathophysiology
2. GSNOR inhibition, by decreasing the rate of normal or aberrant GSNOR activity, **preserves bio-available NO**

# Mechanisms Influencing Smooth Muscle and Inflammation

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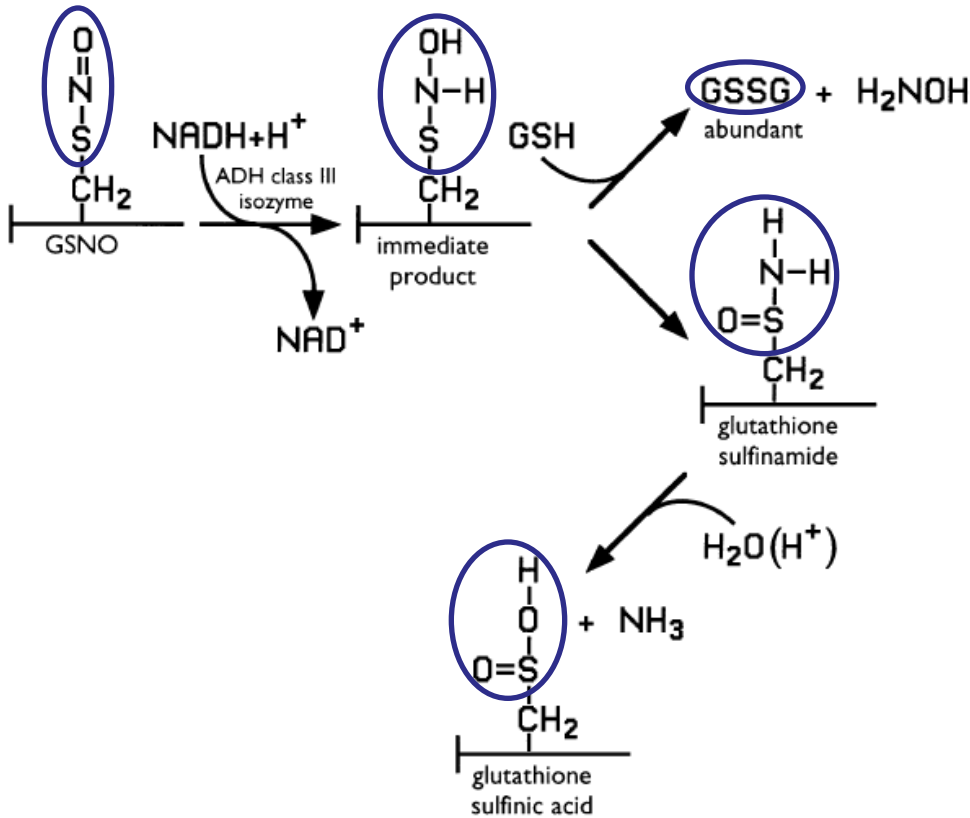
Broadly speaking, the mechanisms underlying disease modifying effects of GSNOR inhibition reside in two areas:

**Smooth Muscle:** Improving smooth muscle tone is relatively well established to reside within the NO-sGC-cGMP axis, which provides critical path signaling responsible for vaso- or bronchodilatory homeostasis.

**Inflammation:** While less appreciated, GSNO plays an important role in controlling inflammatory responses:

- by increasing the threshold for NF- $\kappa$ B activation
- as a potent anti-oxidant capable of neutralizing oxidant stress
- as an anti-bacterial agent
- by decreasing platelet aggregation
- by stabilizing caspase 3 and increasing the threshold for apoptosis

# ADH Class III Enzyme Also Known as GSNOR



**Scheme 3** Scheme for the ADH class III isozyme catabolism of GSNO included GSH

Jensen, D. E.; Belka, G. K.; Du Bois, G. C. S-nitrosoglutathione is a substrate for rat alcohol dehydrogenase class III isoenzyme. *Biochem. J.* **1998**, *331*, 659-668.

# Background of GSNOR Program

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- 100K compound library screened
- 10 series of hits have been identified
- Pyrrole series was selected to be followed up first
- **N6001** was identified as a hit
- Crystal structure of N6001 obtained

# SAR Development of Pyrrole Series

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## Structure of N6001

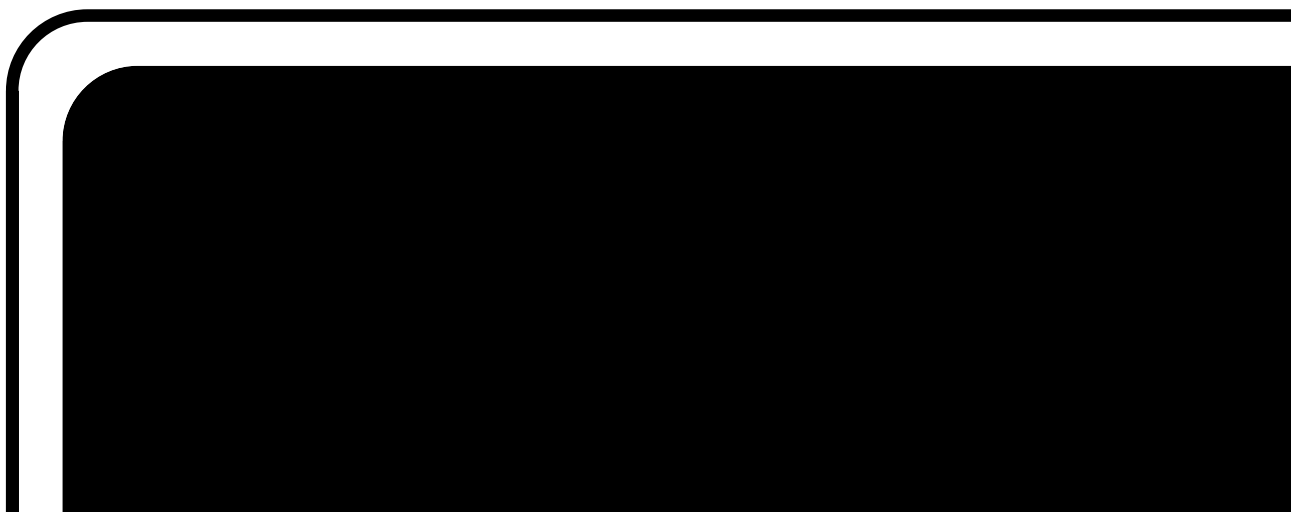
Strategy: Lead optimization of pyrrole series performed by evaluating all four major areas of the molecule

# Early SAR

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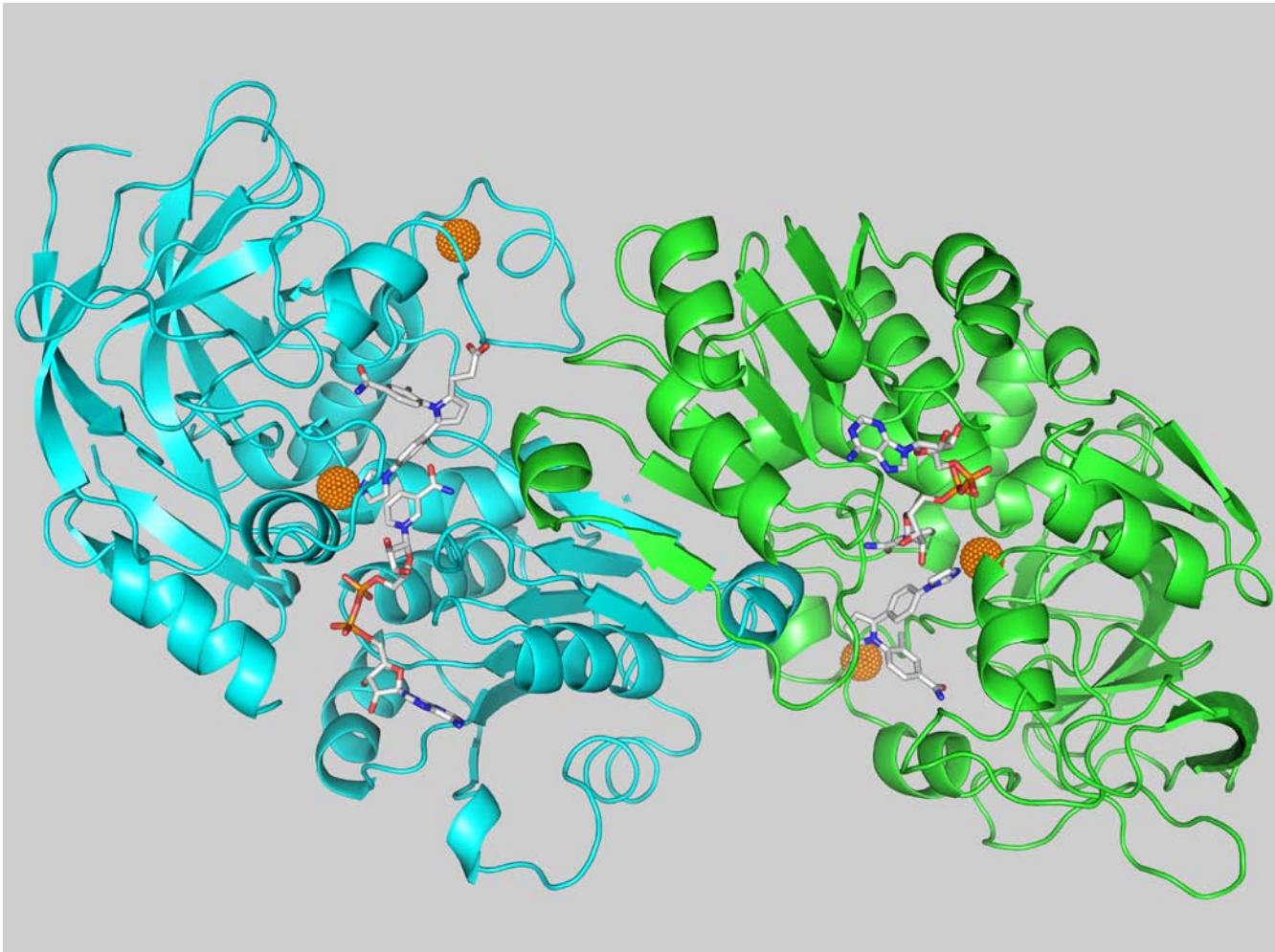
# Imidazole Containing Inhibitor

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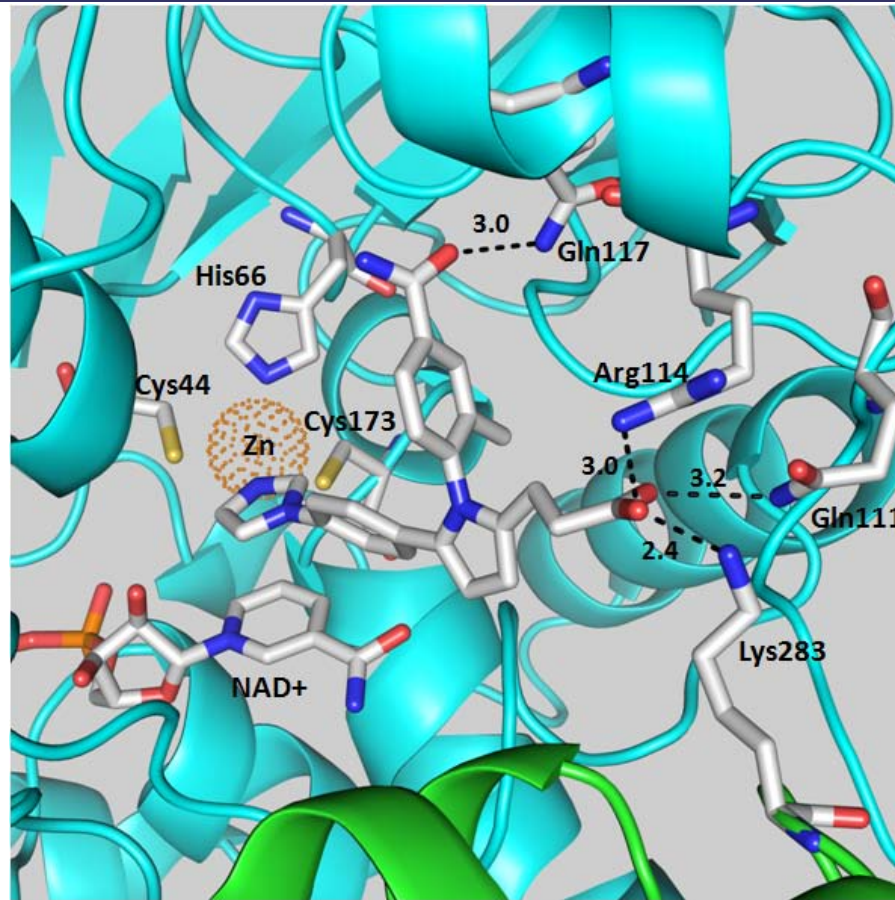
Replacement of the methoxy group in N6010 with an imidazole lead to a major breakthrough in potency

# Crystal Structure of N6022



The enzyme-ligand complex crystallized as a homodimer with N6022 binding to each GSNOR monomer

# Crystal Structure of N6022



Key interactions: 1) the imidazole in the inhibitor interacts with one of the structural zincs which is also coordinated to histidine 66, cysteine 44, and cysteine 173 to form the zinc coordination tetrahedral; 2) the carboxylic acid of N6022 hydrogen bonds to the glutamine 111 and forms a salt bridge with arginine 114 and lysine 283 from the second monomer; 3) the **N6022** carboxamide hydrogen bonds to glutamine 117; 4) the **N6022** imidazole ring forms a Pi-Pi interaction with the nicotinamide ring of NAD<sup>+</sup>.

# Lead Optimization Around N6022

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Further optimization:

- Maintain or improve the potency
- Reduce *in vitro* toxicity and P450 activity
- Improve oral bioavailability

# Pyrrole Regioisomers

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Pyrrole regioisomers maintained most of the inhibition activity

## Discovery of S-Nitrosogluthathione Reductase Inhibitors: Potential Agents for the Treatment of Asthma and Other Inflammatory Diseases

Xicheng Sun,<sup>\*,†</sup> Jan W. F. Wasley,<sup>†</sup> Jian Qiu,<sup>†</sup> Joan P. Blonder,<sup>†</sup> Adam M. Stout,<sup>†</sup> Louis S. Green,<sup>†</sup> Sarah A. Strong,<sup>†</sup> Dorothy B. Colagiovanni,<sup>†</sup> Jane P. Richards,<sup>†</sup> Sarah C. Mutka,<sup>‡</sup> Lawrence Chun,<sup>§</sup> and Gary J. Rosenthal<sup>†</sup>

<sup>†</sup>N30 Pharmaceuticals LLC, 3122 Sterling Circle, Suite 200, Boulder, Colorado 80301, United States

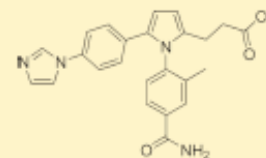
<sup>‡</sup>Simpharma LLC, 1 Stone Fence Lane, Guilford, Connecticut 06437, United States

<sup>§</sup>Emerald BioStructures, 7869 NE Day Road West, Bainbridge Island, Washington 98110, United States

### Supporting Information

**ABSTRACT:** S-Nitrosogluthathione reductase (GSNOR) regulates S-nitrosothiols (SNOs) and nitric oxide (NO) *in vivo* through catabolism of S-nitrosogluthathione (GSNO). GSNOR and the anti-inflammatory and smooth muscle relaxant activities of SNOs, GSNO, and NO play significant roles in pulmonary, cardiovascular, and gastrointestinal function. In GSNOR knockout mice, basal airway tone is reduced and the response to challenge with bronchoconstrictors or airway allergens is attenuated. Consequently, GSNOR has emerged as an attractive therapeutic target for several clinically important human diseases. As such, small molecule inhibitors of GSNOR were developed. These GSNOR inhibitors were potent, selective, and efficacious in animal models of inflammatory disease characterized by reduced levels of GSNO and bioavailable NO. N6022, a potent and reversible GSNOR inhibitor, reduced bronchoconstriction and pulmonary inflammation in a mouse model of asthma and demonstrated an acceptable safety profile. N6022 is currently in clinical development as a potential agent for the treatment of acute asthma.

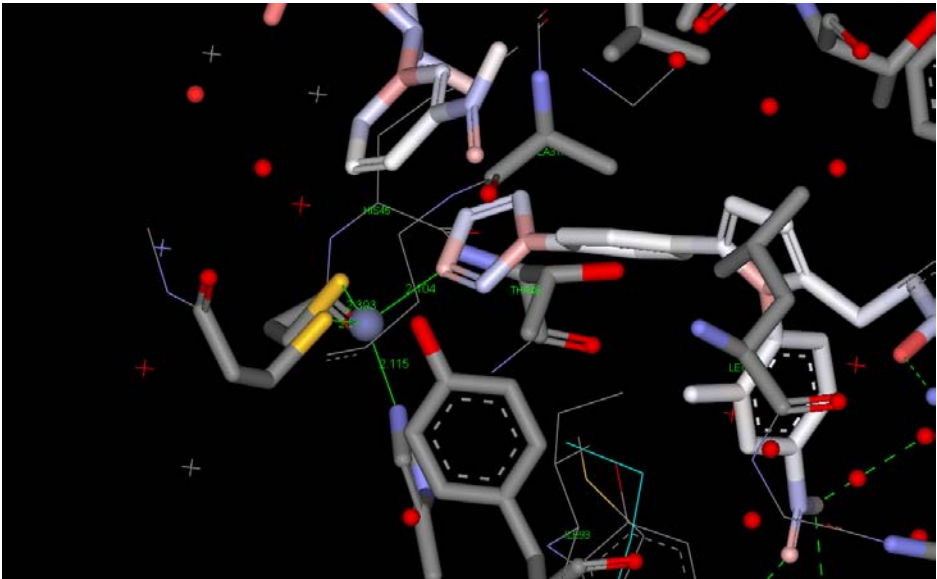
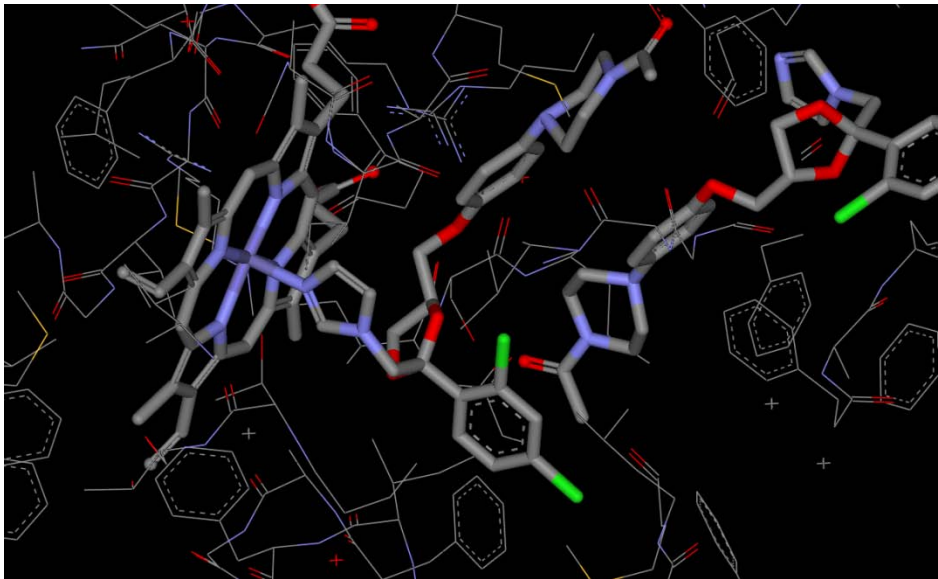
**KEYWORDS:** GSNOR, GSNO, N6022, asthma, pyrrole, nitric oxide



N6022  
GSNOR IC<sub>50</sub> = 20 nM

- Sun, X.; et al. ACS J. Med. Chem. Lett. Published online March 11, 2011; DOI: 10.1021/ml200045s
- Series of publications will follow this paper detailing the SAR of pyrrole based GSNOR inhibitors

# Removing Cyp P450 Activity of Imidazole Analogs



*Cyp450 3A4 + ketoconazole (dimer)*

*GSNOR + N6022 + NAD*



# Removing Cyp 450 Activity of Imidazole Analogs

ID	N6022	N6060	N6371
R	H	Me	Et
IC <sub>50</sub> (nM)	20	59	130
CYP450 (% of inhibition)			
1A2	85	11	6
2B6	ND	11	7
2C9	81	20	15
2C19	95	55	25
2D6	89	11	20
2E1	ND	37	0
3A4	60	31	27

assay concentration: 10 uM of compound

Introduction of alkyl group at the 2-position of imidazole reduced the P450 activities, but resulted in some loss of desired activity

# Thiophene Analogs

compd	IC <sub>50</sub> (nM)	1A2 (%)	2C9 (%)	2C19 (%)	2D6 (%)	3A4 (%)
N6022	20	85	81	95	89	60
N6501	21	-8	15	25	10	27
N6537	21	-11	13	11	12	18

Replacement of phenyl ring with thiophene maintains good GSNOR IC<sub>50</sub> and **reduces the P450 activity**

# Attempt to Develop Oral Agents

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Identify an orally bioavailable agent is one of our goals  
N6446 achieved 22% oral bioavailability

# Data Sheet of Advanced GSNORi

compd	Structure	IC <sub>50</sub> (nM)	Microsomal Stability (human)	Cytotoxicity (IC <sub>50</sub> , uM)	5-Day tox in mice NOAEL (mg/kg)	% F	Cerep Panel Score	P450 Score	Ames
N6338		160	93%	>250	50 (IV) 100 (PO)	18%	1/55	0/7	-
N6446		56	96%	>250	10 (IV) 100 (PO)	22%	1/55	0/7	-
N6547		55	93%	> 250	50 (IV)	2.7%	0/55	0/7	-

# Efficacy Achieved with GSNOR Inhibitors in Animal Models

compd	Asthma (OVA, in mice)	COPD (PPE induced in mice)	IBD (DSS induced in mice)
N6022	IV, single dose, 0.01 mg/kg	-	PO, QD, 10 days, 1 mg/kg
N6547	IV, single dose, 0.01 mg/kg	-	PO, QD, 10 days, 0.1 mg/kg PO, QD, 14 days, 10 mg/kg
N6338	PO, single dose, 0.1 mg/kg	PO, QD, 7-14 days, 0.1 mg/kg	-
N6446	IV, single dose, 0.1 mg/kg PO, single dose, 1 mg/kg	-	-

# N6022 Profile

Property	Value
Molecular weight	414.46
GSNOR IC <sub>50</sub>	20 nM
GSNOR Ki	4 nM
CeeTox Value	164 uM
Cerep Panel Score	1/54 (δ2, 66%)
Ames	negative
hERG	negative
Cytotoxicity IC <sub>50</sub>	>250 uM
<i>in vivo</i> toxicity in mouse	NOAEL: 30 mg/kg , 5-day
Microsomal Stability	>90% across three species
P450 inhibition	IC <sub>50</sub> = 0.77 μM, 2C19
P450 induction	negative
Oral bioavailability	0.6-4% across four species
Ova mouse efficacy in asthma, IV	EC <sub>50</sub> = 0.01 mg/kg

Based on the overall properties N6022 was advanced to pre-clinical development as IV agent to treat acute asthma

Currently in Phase I trials

# Summary SAR Pyrrole Series

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- Synthesized more than 400 analogs with variation on all four areas.
- Compounds achieved efficacy in multiple animal models
- A number of compounds achieved NOAEL > 30 mg/kg in 5-days IV toxicity
- N6022 was identified as preclinical candidate as IV agent to treat acute asthma and currently in Phase I clinical trial.
- Number of compounds could be back-ups pending further evaluations
- GSNOR proved to be a valid molecular target for the treatment of many diseases

# Acknowledgement

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*Emerald Biostructures*

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*Bolder Biopath*

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# Back-up slides

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