Bioorganic & Medicinal Chemistry Letters 21 (2011) 5849–5853

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

Discovery of potent and novel *S*-nitrosoglutathione reductase inhibitors devoid of cytochrome P450 activities

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ARTICLE INFO

Article history:
Received 8 July 2011
Revised 26 July 2011
Accepted 26 July 2011
Available online 3 August 2011

Keywords:
GSNO
GSNOR
GSNOR inhibitor
Nitric oxide
Cytochrome P450
N6022

ABSTRACT

The pyrrole based **N6022** was recently identified as a potent, selective, reversible, and efficacious *S*-nitrosoglutathione reductase (GSNOR) inhibitor and is currently undergoing clinical development for the treatment of acute asthma. GSNOR is a member of the alcohol dehydrogenase family (ADH) and regulates the levels of *S*-nitrosothiols (SNOs) through catabolism of *S*-nitrosoglutathione (GSNO). Reduced levels of GSNO, as well as other nitrosothiols (SNOs), have been implicated in the pathogenesis of many diseases including those of the respiratory, cardiovascular, and gastrointestinal systems. Preservation of endogenous SNOs through GSNOR inhibition presents a novel therapeutic approach with broad applicability. We describe here the synthesis and structure–activity relationships (SAR) of novel pyrrole based analogues of **N6022** focusing on removal of cytochrome P450 inhibition activities. We identified potent and novel GSNOR inhibitors having reduced CYP inhibition activities and demonstrated efficacy in a mouse ovalbumin (OVA) model of asthma.

Endogenous *S*-nitrosothiols (SNOs) are important conductors of the biological influence of nitric oxide, from bronchodilation and vasodilation to controlling inflammation.[1–3] *S*-Nitrosoglutathione reductase (GSNOR), also known as the human ADH class III enzyme and formaldehyde dehydrogenase,[4,5] catalyzes the metabolism of the most abundant low molecular weight SNO, *S*-nitrosoglutathione (GSNO),[6,7] and has been identified as a potential drug target for the treatment of a broad range of diseases.[8–13] The therapeutic potential of GSNOR inhibitors has been demonstrated in animal models of asthma,[14–16] chronic obstructive pulmonary disease (COPD),[17] inflammatory bowel disease (IBD)[18] and high salt induced hypertension.[19]

We have recently reported the identification of **N6022** as a potent *S*-nitrosoglutathione reductase inhibitor in clinical development[20,21] for the treatment of acute asthma, and extended these observations with a report on structure–activity relationship of GSNOR inhibitors based on a pyrrole scaffold.[22] As a continuation of the series communication of the pyrrole based GSNOR inhibitors, we report here the studies to further improve the overall pharmacological properties of GSNOR inhibitors, focusing on the substitution of the imidazole moiety and replacement of this moiety with a variety of heterocycles in an attempt to eliminate the cytochrome P450 inhibitory activities previously reported GSNOR inhibitors.[20]

Most of the GSNOR inhibitors presented here were synthesized according to Scheme 1. The synthesis started from either commercially available ketones or the ketones prepared as described in the Supplementary data. In Scheme 1, condensation of ketone **1** and 2-furanaldehyde provided intermediate **2** in good yield.[23] Furan ring opening of intermediate **2** by HBr in ethanol under reflux conditions provided diketone **3**.[24] Pyrrole formation was achieved by condensation of the diketone **3** and 4-amino-3-methylbenzamide under acidic conditions to afford intermediate **4**.[25] For the synthesis of compounds **6a–6s**, where the Ar is phenyl, compounds **5a–5s** were synthesized either using the Suzuki coupling conditions for the C–C connected aromatic heterocyclic analogs, or coupling of heterocycles such as morpholine (**6o**) with intermediate **4** in the presence of copper iodide (I) and potassium carbonate in DMSO to form N–C connected compounds. The N–C connected analogs can also be synthesized using similar conditions, but using amides instead. Compounds **8a–8i** were prepared using substituted imidazoles as starting materials to couple with intermediate **4** either using L-proline[26] or *N*,*N*-dimethyl-cyclohexane-1,2-diamine[27] as a catalyst in the presence of copper iodide(I) and potassium carbonate in DMSO followed by hydrolysis of ester in aqueous lithium

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

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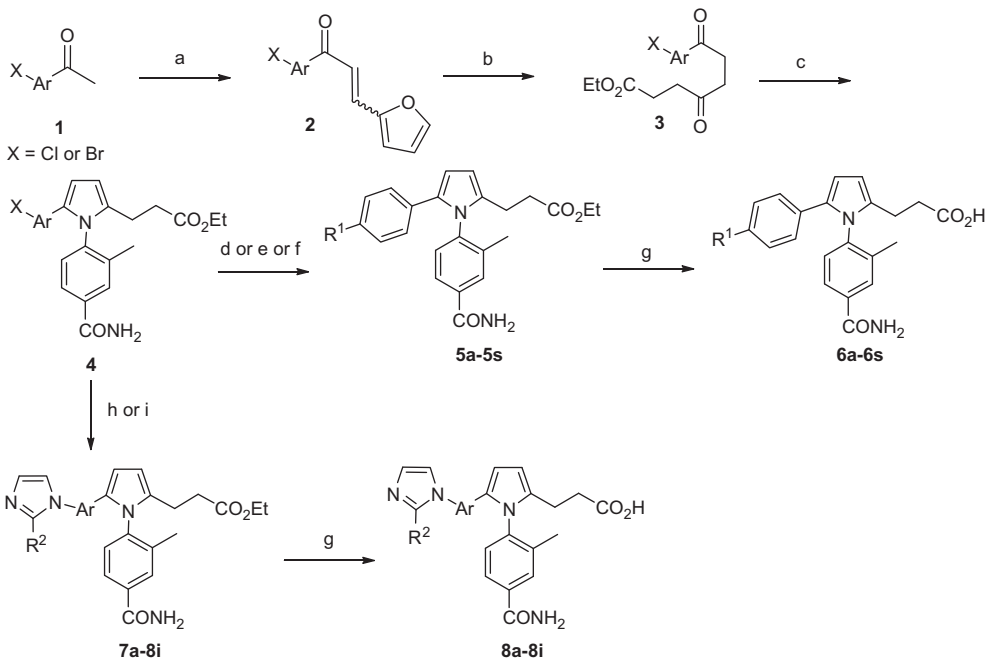
doi:10.1016/j.bmcl.2011.07.103



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/*p*TsOH/EtOH, reflux, overnight; (d) boronic acid or ester, Suzuki coupling conditions; (e) heterocycles/CuI/K_2CO_3/DMSO; (f) amide/CuI/K_3PO_4/DMEDA/dioxane; (g) LiOH; (h) imidazole/L-proline/CuI/K_2CO_3/DMSO; (i) imidazole/*N*,*N*-dimethyl-cyclohexane-1,2-diamine/CuI/K_2CO_3/DMSO, microwave.

hydroxide. The synthesis of compounds **9–24** are described in detail in the Supplementary data.

One issue we encountered during the lead optimization of GSNOR inhibitors was cytochrome P450 enzyme inhibition with compounds, such as **N6022**, containing a nitrogen linked imidazole moiety.[20] CYP inhibitions may be a concern in the drug development due to potential drug–drug interaction. Our initial approach was to replace the imidazole ring with other heterocycles in an attempt to avoid the CYP inhibition. A large set of five-membered heterocyclic analogs was synthesized and the GSNOR inhibitory activity was evaluated (Table 1). Overall, none of the new analogs exhibited better GSNOR inhibition than **N6022** although a number of compounds (**6a**, **6j**, and **10**) achieved sub-micromolar activity. To our surprise, the nitrogen containing analogs **12**–**16** exhibited substantially decreased GSNOR inhibitory activity. The thiadiazole analog **9** was completely devoid of enzyme inhibition activity while the thiazole comparator **10** achieved sub-micromolar potency. Substitution of imidazole at 5-position (**6k**) resulted in greater than a 2-log of loss in GSNOR inhibition activity. The best compound in the series was compound **6a** having GSNOR IC_{50} = 0.51 μM.

Next, six-membered ring heterocycles (**6l**–**6s**) were also used to replace imidazole. However, all of these compounds exhibited markedly decreased GSNOR inhibitory activity as shown in Table 2.

As a second approach to circumvent the CYP inhibition, we evaluated the crystal structure of the antifungal agent, ketoconazole, bound to CYP 3A4[28] in comparison with the crystal structure of **N6022** bound to the GSNOR enzyme.[20] We hypothesized that substitution at the 2-position of imidazole may hinder the binding of imidazole to CYP 3A4 enzyme, with the assumption that the imidazole moiety of **N6022** binds similarly to CYP 3A4. In addition, there is space in the **N6022**-GSNOR crystal structure to fit small groups. We started the SAR exploration with substitution of the imidazole ring at 2-position by a series of lower alkyl groups (Table 3). The overall CYP activity, including activity for CYP 3A4 and 2C19, was significantly reduced with 2-methyl (**8a**) and 2-ethyl (**8b**) substituted analogs as compared to non-substituted **N6022**.

Table 1
Imidazole replacement with five-membered rings

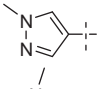
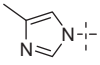
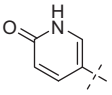
Compd	R^1	GSNOR IC_{50} (μM)
6a		0.51
6b		29.32
6c		6.52
6d		11.81
9		>100
10		0.76
11		53.49
6e		50.64
6f		10.15
6g		4.74
6h		2.66
6i		5.14
6j		0.68
6k		6.25

Table 1 (*continued*)

Compd	R^1	GSNOR IC_{50} (μM)
12		4.16
13		13.27
14		1.48
15		1.75
16		5.19

Table 2
Imidazole replacement with six-membered rings

Compd	R^1	GSNOR IC_{50} (μM)
6l		9.8
6m		23.92
6n		19.03
6o		8.17
6p		43.94
6q		8.78
6s		6.16

However, the GSNOR inhibition activity was also reduced by substitution, particularly with a larger group such as cyclopropyl (**17**) and isopropyl (**8c**). Hydroxymethyl (**18**) and trifluoromethyl (**19**) substitution at 2-postion of imidazole diminished the GSNOR inhibitory activity.

In order to maintain or improve the GSNOR inhibition activity and remove CYP inhibition, the phenyl ring connecting to the imidazole was replaced with a variety of heterocycles (Table 3). Replacement of phenyl ring on **N6022** with thiophene (**8d**, **8e**) maintained the GSNOR inhibition activity, but the CYP inhibition issue remained (**8d**). Substitution at 2-position of the imidazole with a methyl group (**8f**) not only maintained the GSNOR inhibition activity, also reduced the CYP inhibition to <30% as compared to >50% inhibition for **N6022** (Table 3). The thiophene regioisomer of **8f** (**8h**) also achieved a similar result. Heterocyclic analogs other than thiophene, such as furan (**20**), thiazole (**21**), pyridines (**22**, **23**) and pyrimidine (**24**) lost significant GSNOR inhibitory activity.

Selected GSNOR inhibitors were assessed for potential off-target activity with a panel of 54 transmembrane and soluble receptors, ion channels, and monoamine transporters. Off-target effects were estimated from the percent inhibition of receptor radio-ligand binding in the presence of 10 μM of test compound. 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. Limited off-target activity was observed towards the δ2 opiate receptor for compound **8d**, **8f**, and **8h** similar to **N6022** as reported earlier.[20] Compound **8h** demonstrated potent GSNOR inhibition and a clean profile toward CYP inhibition, but exhibited 69% inhibition against the opioid peptide receptor (Mu). Compounds **8d**, **8f**, and **8h** were also screened for cytotoxicity towards the A549 epithelial lung cell line. The IC_{50} values for **8f** and **8h** were >250 μM, and minimal cytotoxicity for **8d** (IC_{50} = 160 μM) was observed.

Compounds **8d**, **8f**, and **8h** were tested in mouse pharmacokinetic studies. Oral bioavailability of these compounds was 0.7%, 1.6%, and 0.49%, respectively, compared to 4.4% for **N6022** reported earlier.[20] The plasma clearance (CL) after intravenous (IV) administration was 54.1, 31.6, and 16.8 ml/min/kg for **8d**, **8f**, and **8h**, respectively, compared to 37.7 ml/min/kg for **N6022**.[20]

Compound **8d** was tested in a 5-day mouse toxicity study with intravenous QD dosing at 1, 10, or 50 mg/kg. The results of this study suggest that treatment of male CD-1 mice with **8d** for 5 days had no adverse effects. This study resulted in a no observable adverse effect level (NOAEL) of 50 mg/kg for IV treatment. Compound **8f** was also tested in a 5-day mouse toxicity study with IV BID dosing at 5, 25, or 50 mg/kg. To our surprise, the treatment of male CD-1 mice with **8f** for 5 days resulted in significant adverse effects on numerous study endpoints. In particular, histological findings demonstrated toxicity to the liver, spleen, and thymus of treated animals. The NOAEL for **8f** could not be established from the study and was considered to be <10 mg/kg/day.

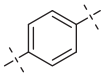
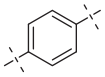
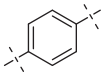
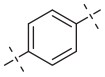
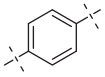
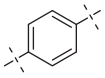
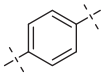
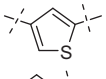
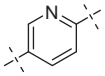
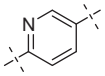
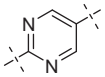
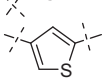
The efficacy of GSNOR inhibitors was assessed in an animal model of asthma, a disease influenced by dysregulated GSNOR and altered function of NO, GSNO, and SNOs. In a mouse model of ovalbumin-induced asthma,[29] compound **8d** attenuated methacholine-induced bronchoconstriction (airway hyper-responsiveness) and eosinophil infiltration into the lungs following a single IV dose administered 24 h prior to the methacholine challenge. Efficacy was observed at doses $\geqslant$0.01 mg/kg compound **8d**. In a similar study, compound **8f** also attenuated methacholine-induced airway hyper-responsiveness and eosinophil infiltration into the lungs following a single IV dose of 1 mg/kg administered 24 h prior to the methacholine challenge.

In conclusion, based on the crystal structure of GSNOR inhibitors and crystal structure of ketoconazole with CYP 3A4, substitution of the imidazole at 2-position with small alkyl group and replacement of the phenyl ring with thiophene led to potent GSNOR inhibitors that demonstrated significantly reduced CYP inhibition. These findings validate the initial hypothesis of the imidazole as a key pharmacophore for the binding of GSNOR inhibitors to cytochrome enzymes causing CYP inhibition. However, despite the improved in vitro profile of GSNOR inhibitor **8f**, this compound exhibited a less attractive pharmacological profile than **N6022** due to the surprise in vivo toxicity observed in the 5-day exploratory toxicity evaluation in mice. The studies described in this paper provided more insight into the understanding of GSNOR inhibition, the inhibitor–enzyme interaction, and structure–in vitro toxicity relationship of GSNOR inhibitors.

Acknowledgments

The authors are grateful to Dr. J. 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.

Table 3
SAR and cytochrome P450 activities of imidazole analogs

Compd	R^2	Ar	GSNOR IC_{50} (μM)	% Of CYP inhibition at 10 μM				
				1A2	2C9	2C19	2D6	3A4
N6022	H		0.020	85	81	95[a]	89	60
8a	Me		0.075	11	20	55	11	31
8b	Et		0.13	6	15	25	20	27
17	^{c}Pr		6.96	–	–	–	–	–
8c	^{i}Pr		Inactive	–	–	–	–	–
18	$HOCH_2$		2.31	–	–	–	–	–
19	CF_3		Inactive	–	–	–	–	–
8d	H		0.022	77	92	83	96	91
8e	H		0.026	–	–	–	–	–
20	H		0.90	–	–	–	–	–
21	H		1.57	–	–	–	–	–
22	H		0.36	–	–	–	–	–
23	H		2.30	–	–	–	–	–
24	H		0.15	–	–	–	–	–
8f	Me		0.017	−8	15	25	10	27
8g	Et		0.048	–	–	–	–	–
8h	Me		0.021	−11	13	11	12	18
8i	Me		0.048	–	–	–	–	–

[a] IC_{50} = 0.77 μM.[20]

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.2011.07.103.

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