Bioorganic & Medicinal Chemistry Letters 25 (2015) 1070–1072

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

Marine natural products as inhibitors of cystathionine beta-synthase activity

Megan K. Thorson [a], Ryan M. Van Wagoner [a], Mary Kay Harper [a], Chris M. Ireland [a], Tomas Majtan [b], Jan P. Kraus [b], Amy M. Barrios [a,*]

[a] Department of Medicinal Chemistry, University of Utah, 30 South 2000 East, Salt Lake City, UT 84112, USA
[b] Department of Pediatrics, University of Colorado, Aurora, CO 80045, USA

ARTICLE INFO

Article history:
Received 13 August 2014
Revised 5 January 2015
Accepted 7 January 2015
Available online 14 January 2015

Keywords:
Hydrogen sulfide
Sulfur metabolism
Fluorogenic probe
Inhibitor screen

ABSTRACT

A library consisting of characterized marine natural products as well as synthetic derivatives was screened for compounds capable of inhibiting the production of hydrogen sulfide (H_2S) by cystathionine beta-synthase (CBS). Eight hits were validated and shown to inhibit CBS activity with IC_{50} values ranging from 83 to 187 μM. The majority of hits came from a series of synthetic polyandrocarpamine derivatives. In addition, a modified fluorogenic probe for H_2S detection with improved solubility in aqueous solutions is reported.

Cystathionine beta-synthase (CBS) is a type II fold pyridoxal-5′-phosphate (PLP) dependent enzyme that plays critical roles in sulfur metabolism. For example, CBS is responsible for regulating homocysteine levels. Mutations in the gene that encodes for CBS lead to homocystinuria, a genetic condition characterized by very high plasma homocysteine levels (>50 μM compared to <10 μM in the general population),[1] lens dislocation, skeletal abnormalities and vascular disease.[1,2] In addition to its clear role in homocystinuria, CBS plays a key role in the production of hydrogen sulfide (H_2S), a recently recognized 'gasotransmitter' with biological effects similar to, and often complementary to, those of nitric oxide (NO).[3–5] H_2S acts as a vasodilator, has anti-inflammatory properties and has been shown to be cardioprotective.[6–8] While a possible link between CBS-mediated homocysteine metabolism, H_2S production and cardiovascular health is intriguing, it has not been possible to obtain a detailed understanding of these potential connections because of a lack of chemical tools for monitoring and manipulating the activity of CBS.[9]

We have previously shown that an H_2S-selective, fluorogenic probe can be used to monitor CBS activity and provides a facile assay for CBS inhibitor screening.[10] Despite some recent progress in the field, there are only a few CBS inhibitors available,[10–13] and only two that have routinely been used for inhibition of CBS activity in vivo (aminooxyacetic acid, AOAA and hydroxylamine, HA).[11–13] Neither AOAA nor HA is selective for CBS over the other major H_2S-producing, PLP-dependent enzyme cystathionine gamma-lyase (CGL)[11] and there has been some controversy over their inhibitory potency against CBS.[12] Consequently there is a significant need for the development of potent, selective CBS inhibitors.

Natural products isolated from marine invertebrates have been an exceptional source of chemical diversity and pharmaceutical lead compounds.[14] With the aim of streamlining the initial screening process while maximizing the chemical diversity that can be sampled, our collaborators in the Ireland lab created a protocol for fractionating marine invertebrate extracts.[15,16] They also selected a series of chemically diverse, well-characterized natural products and synthetic derivatives for use in screening. In the work reported here, we screened this subset of the 'Marine Invertebrate Compound Library' consisting of 160 characterized marine natural products and 80 purified synthetic derivatives, termed 'MICL-240', for compounds capable of inhibiting CBS activity. From this library, we identified eight compounds with IC_{50} values below 200 μM (range: 83–187 μM).

Aromatic azides are now well-established H_2S-reactive functional groups, and the reduction of an aryl azide to an aryl amine

Abbreviations: CBS, cystathionine beta-synthase; CGL, cystathionine gamma-lyase; H_2S, hydrogen sulfide; PLP, pyridoxal-5′-phosphate; AOAA, aminooxyacetic acid; HA, hydroxylamine; MICL, Marine Invertebrate Compound Library; AzMC, 7-azido-4-methylcoumarin; AzCC, 7-azido-4-carbamoyl methyl coumarin.

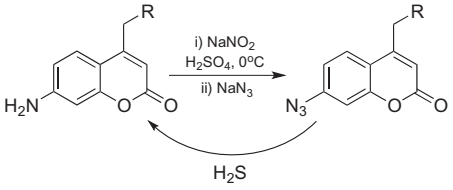
* Corresponding author. Tel.: +1 801 581 3198.
E-mail address: amy.barrios@utah.edu (A.M. Barrios).

http://dx.doi.org/10.1016/j.bmcl.2015.01.013
0960-894X/

with concomitant increase in fluorescence has been exploited in numerous H_2S-selective fluorogenic probes.[17,18] In previous work, we and others have shown that 7-azido-4-methylcoumarin (AzMC) undergoes a dramatic increase in fluorescence upon exposure to H_2S, with detection limits in the high nanomolar range (Scheme 1).[10,19] This probe has significant utility as an assay for CBS and CGL activity, readily detecting the H_2S produced by these enzymes in a high-throughput format, facilitating enzyme inhibition screens.[10] In the course of this work we noticed that, at higher concentrations of probe or low concentrations of DMSO, the AzMC probe would sometimes precipitate out of aqueous solution. This has not been a problem in our screening efforts, but could be an issue in cases where minimizing or eliminating DMSO is necessary. Therefore, we created a modified version of the probe with a carboxylate group rather than a methyl group at the 4-position (Scheme 1) for increased solubility in aqueous solutions. The new probe, 7-azido-4-carbamoylmethylcoumarin (AzCC) was synthesized as follows: 3-aminophenol was protected with ethyl chloroformate prior to a Pechmann condensation reaction to form the coumarin. After deprotection, the azide was formed from the amine as shown in Scheme 1.[10] The AzCC probe has an identical response to H_2S as the parent AzMC (Fig. 1), with the advantage of increased solubility in aqueous solution. In addition, with the carboxylate functionality, this probe could easily be conjugated to another moiety if desired.

With the aim of identifying novel CBS inhibitors, we screened the MICL-240 library looking for compounds that significantly decreased enzyme activity. As shown in Figure 2, several hits were identified in this initial screen using 2 μg of each compound. Compounds capable of reducing the activity of CBS by more than 50% were re-screened at 4 μg and background corrected. The hits that showed a concentration-dependent inhibition of enzyme activity were subjected to a series of secondary screens. The validated hits, with IC_{50} values, are shown in Figure 3.

For each compound, a dose–response curve was measured to obtain the apparent IC_{50} values for inhibition of the activity of full-length, wild-type CBS in the presence of 300 μM of the endogenous activator, *S*-adenosylmethionine. In addition, a series of control experiments was run to ensure that the hits did not interfere directly or indirectly with the assay. Specifically, the lead compounds shown in Figure 3 did not react with either the azidocoumarin probe or hydrogen sulfide to an appreciable extent (see Supporting information). Interestingly, the majority of the hits (hits **A6**, **A9**, **B7** and **C11** from the **MNP2** sub-library) are synthetic



Scheme 1. The 7-azidocoumarin analogs can be readily synthesized from the 7-aminocoumarin starting materials. H_2S-mediated reduction regenerates the fluorescent 7-aminocoumarin. When R=H, the probe is 7-azido-4-methylcoumarin (AzMC). When R=COOH, the probe is 7-azido-4-carbamoylmethylcoumarin (AzCC).

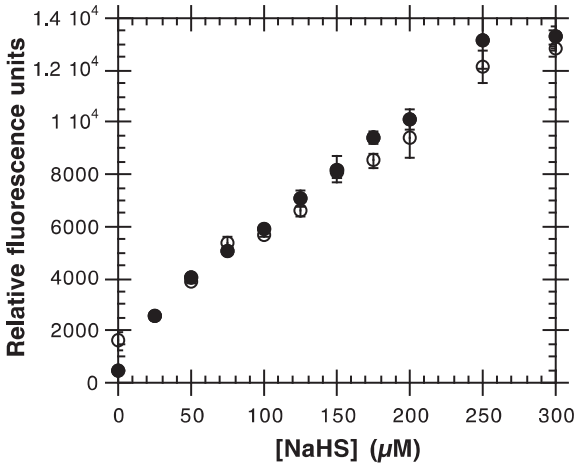


Figure 1. Hydrogen sulfide reduces both AzMC (10 μM, solid circles) and AzCC (10 μM, open circles) to the corresponding 7-aminocoumarins with a concomitant linear increase in fluorescence.

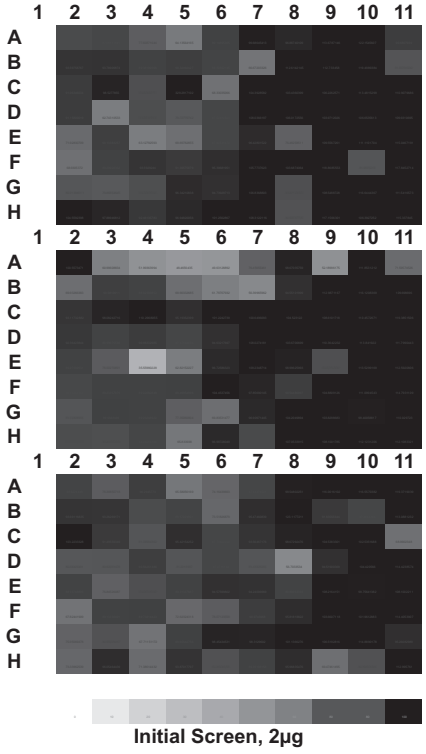


Figure 2. Heat map showing the results from the initial library screen. Lighter boxes represent strong inhibition of CBS activity and darker boxes represent weak or no inhibition.

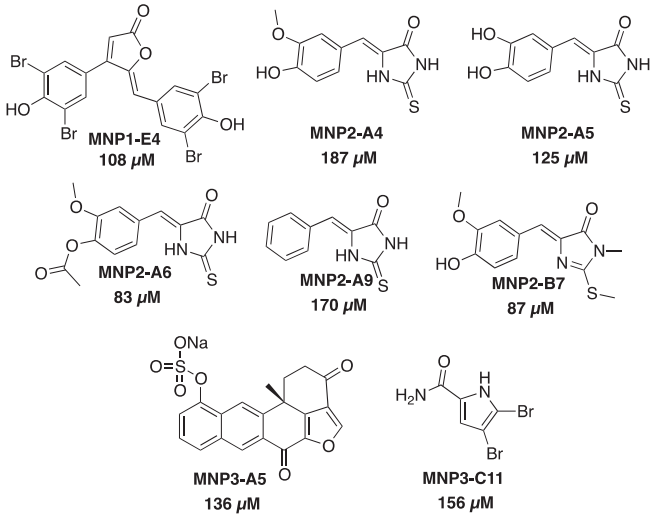


Figure 3. Top validated hits from the MICL-240 marine natural product library with IC_{50} values for inhibition of CBS activity.

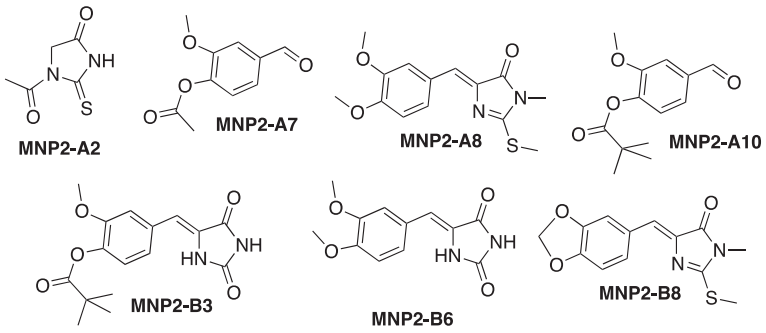


Figure 4. An analysis of structurally related compounds from MICL-240 lacking inhibitory activity against CBS provides insight into the SAR of the hit compounds.

compounds derived from polyandrocarpamines A and B, 2-aminoimidazolone compounds isolated from the ascidian *Polyandrocarpa* sp.[20] The MICL-240 library contained 17 compounds from this structural class in **MNP** plate 2, wells **A2**–**B8**. While several of these compounds inhibited CBS activity in our initial screen (Fig. 2), only 5 showed activity in both of the secondary screens (Fig. S1). An investigation of the structures of the validated hits compared to compounds that did not inhibit CBS activity in the initial screen provides some information about the structure–activity relationship of these compounds. As shown in Figure 4, compounds containing only the thiohydantoin or aromatic moiety are poor inhibitors of CBS activity (compounds **MNP2 A2**, **A7** and **A10**). In addition, compounds **MNP2**-**A8**, **MNP2**-**B6** and **MNP2**-**B8**, where both hydroxyl groups are etherified and the thiohydantoin either is methylated both at the 3 position and on the sulfur atom or replaced by a hydantoin moiety do not show up as hits. Thiohydantoin methylation alone (compound **MNP2**-**B7**) does not prevent enzyme inhibition while replacement of the thiohydantoin moiety with a hydantoin moiety does (**MNP2**-**B6**, **MNP2**-**B3**).

CBS plays a key role in biological sulfur metabolism and its importance in numerous physiological and pathological pathways is not yet fully appreciated. One of the key challenges in understanding the biological roles of this enzyme is a lack of potent, selective inhibitors that can be used to probe its activity in vivo. In this work, we have identified a handful of novel scaffolds for inhibiting CBS. The majority of the hits from our screen of the MICL-240 library were synthetic compounds derived from the polyandrocarpamines A and B, marine natural products isolated from the ascidian *Polyandrocarpa* sp. These scaffolds may serve as useful starting points for the development of potent and selective CBS-targeted tools necessary for advancing the field.

Acknowledgements

This work was supported by the American Heart Association grant 11GRNT7530023 to A.M.B. and award CA36622 from the National Institutes of Health to C.M.I.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2015.01.013.

References and notes

1. Kraus, J.; Janosik, M.; Kozich, V.; Mandell, R.; Shih, V.; Sperandeo, M.; Sebastio, G.; de Franchis, R.; Andria, G.; Kluijtmans, L.; Blom, H.; Boers, G.; Gordon, R.; Kamoun, P.; Tsai, M.; Kruger, W.; Koch, H.; Ohura, T.; Gaustadnes, M. *Hum. Mutat.* **1999**, *13*, 362.
2. Kraus, J. *Methods Enzymol.* **1987**, *143*, 388.
3. Kabil, O.; Banerjee, R. *J. Biol. Chem.* **2010**, *285*, 21903.
4. Szabo, C. *Nat. Rev. Drug Discov.* **2007**, *6*, 917.
5. Wang, R. *FASEB J.* **2002**, *16*, 1792.
6. Beard, R., Jr.; Bearden, S. *Am. J. Physiol. Heart Circ. Physiol.* **2011**, *300*, H13.
7. Li, L.; Hsu, A.; Moore, P. *Pharmacol. Ther.* **2009**, *123*, 386.
8. Li, L.; Rose, P.; Moore, P. *Annu. Rev. Pharmacol. Toxicol.* **2011**, *51*, 169.
9. Wallace, J. L.; Ferraz, J. G.; Muscara, M. N. *Antioxid. Redox Signal.* **2012**, *17*, 58.
10. Thorson, M.; Maitjan, T.; Kraus, J.; Barrios, A. M. *Angew. Chem., Int. Ed.* **2013**, *52*, 4641.
11. Zhou, Y.; Yu, J.; Lei, X.; Wu, J.; Niu, Q.; Zhang, Y.; Liu, H.; Christen, P.; Gehring, H.; Wu, F. *Chem. Commun.* **2013**, *49*, 11782.
12. Whiteman, M.; Le Trionnaire, S.; Chopra, M.; Fox, B.; Whatmore, J. *Clin. Sci.* **2011**, *121*, 459.
13. Whiteman, M.; Winyard, P. *Expert Rev. Clin. Pharmacol.* **2011**, *4*, 13.
14. Newman, D.; Cragg, G. *J. Nat. Prod.* **2012**, *75*, 311.
15. Bugni, T.; Harper, M.; McCulloch, M.; Reppart, J.; Ireland, C. *Molecules* **2008**, *13*, 1372.
16. Bugni, T.; Richards, B.; Bhoite, L.; Cimbora, D.; Harper, M.; Ireland, C. *J. Nat. Prod.* **2008**, *71*, 1095.
17. Lin, V.; Chang, C. *Curr. Opin. Chem. Biol.* **2012**, *16*, 595.
18. Lippert, A.; New, E.; Chang, C. *J. Am. Chem. Soc.* **2011**, *133*, 10078.
19. Chen, B.; Li, W.; Lv, C.; Zhao, M.; Jin, H.; Jin, H.; Du, J.; Zhang, L.; Tang, X. *Analyst* **2013**, *138*, 946.
20. Davis, R.; Aalbersberg, W.; Meo, S.; da Rocha, R.; Ireland, C. *Tetrahedron* **2002**, *58*, 3263.