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ARTICLE

Synthesis and Characterization of Cystathionine-γ-lyase (CSE) Inhibitors 1-(1*H*-Tetrazol-5-yl)but-3-yn-1-amine

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Asian Journal of Organic & Medicinal Chemistry

Volume: 7 Year: 2022
Issue: 2 Month: April–June
pp: 159–162
DOI: https://doi.org/10.14233/ajomc.2022.AJOMC-P379

Received: 27 January 2022
Accepted: 31 May 2022
Published: 29 June 2022

ABSTRACT

Cystathionine-γ-lyase (CSE) 1-(1*H*-tetrazol-5-yl) but-3-yn-1-amine) is co-enzyme play an important role in *in situ* production of H_2S. In this study, herein reported the synthesis of a new molecule, which is inhibitors of CSE. Hydrogen sulfide is a signaling molecule in the form of gas, also it modulates a large number of mammalian physiological processes. Cystathionine-γ-lyase (CSE) catalyzes hydrogen sulfide synthesis and is a target for modulating under pathophysiological conditions. CSE is inhibited by propargylglycine (PPG), thus this study disclosed that it is useful for CSE inhibitors in the treatment of diseases where CSE inhibition provides therapeutic advantage to the patient having the disease.

KEYWORDS

Cystathionine-γ-lyase, Hydrogen sulfide, Propargylglycine.

INTRODUCTION

Cystathionine-β-synthase (CBS) and cystathionine-γ-lyase (CSE) are the two key enzymes, which were involved in the synthesis of hydrogen sulfide. Cystathionine-γ-lyase (CSE) the pyridoxal 5′-phosphate (PLP)-dependent synthesis of L-cysteine from cystathionine whilst CBS catalyzes the pyridoxal 5′-phosphate (PLP)-dependent conversion of homocysteine in cystathionine. Murine CSE gene regulation is well characterized but little is known about in the human counterpart. Recently, it has been studied out that CSE transcription is regulated by Farnesoid-X-Receptor (FXR). Cystathionine-γ-lyase (CSE) is inhibited by propargylglycine (PPG) so disclosed herein are methods for synthesizing such CSE inhibitors and methods for using such CSE inhibitors in the treatment of diseases where CSE inhibition provides therapeutic advantage to the patient having the disease. This study focuses on the recent aspects of the CSE and highlights the possibility that members of the nuclear receptors super-family might be involved in the regulation of hydrogen sulfide metabolism.

The only substrate for the generation of endogenous H_2S is L-cysteine, a sulfur-containing amino acid derived from alimentary sources, synthesized from L-methionine through the so-called “transsulfuration pathway” with homocysteine.

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This process is catalyzed by two enzymes: cystathionine-γ-synthase (CBS) and cystathionine-lyase (CSE). The endogenous production of H_2S in mammalian brain and heart tissues is attributed mainly to CBS whilst that in mammalian liver, kidney, intestine and vascular smooth muscle cells is ascribed largely to CSE [1-4]. Hydrogen sulfide providing a biological signal that modulates multiple physiological processes [5-7]. In mammal family animals, hydrogen sulfide is secrete by produced by two pyridoxal 5-phosphate, which are based on enzyme cystathionine β-synthase (CBS) and cystathionine-γ-lyase (CSE) [8-10]. The transculturation pathway enzymes are managed to cellular needs for cysteine *versus* hydrogen sulfide is an important aspect. CBS which has NO- and CO- reactive heme sensor [11-17]. It also has activity of a protein regulates CSE [18]. The physiological concentrations of substrates CSE is a quantitatively more promising source of hydrogen sulfide over CBS in some tissues.

In pathways of hydrogen sulfide generation in mammalian cells [19-26] (Fig. 1) there are three principal enzymes that contribute to the endogenous production of hydrogen sulfide and these are cystathionine-β-synthase, cystathionine-γ-lyase and 3-mercaptopyruvate. Both CSE and CBS are part of the reverse transsulfuration route. Biochemical pathway accountable for the transfer of methionine to cysteine and catalyze a multitude of reactions that gives H_2S, which convert L-homocysteine to L-homolanthionine (by CSE) and the conversion of L-cysta-thionine from L-homocysteine and L-cysteine, the conversion of L-cystathionine to L-cysteine (by CSE), the conversion of L-cysteine to pyruvate and ammonia (by CSE) and the conversion of L-cysteine to L-serine and L-lanthinonine (by CBS). The route involves the CSE-dependent conversion of cystine to L-thiocystenine which genrate hydrogen sulfide. The third hydrogen sulfide generating enzyme, 3-mercaptopyruvate sulfurtransferase (3-MST), is part of the cysteine catabolism pathway and uses 3-mercaptopyruvate (3-MP) as a substrate. 3-Mercaptopyruvate sulfurtransferase works in tandem with aspartate aminotransferase that also possesses cysteine amino-transferase activity (CAT) activity, generating 3-mercaptopyruvate from cysteine by a series of reductions that first involve the formation of bound sulfane sulfur. 3-Mercaptopyruvate, in addition to acting as a substrate of 3-mercaptopyruvate sulfurtransferase, can also produce hydrogen sulfide spontaneously. In some of the tissues and cells, d-cysteine can also be a significant gives hydrogen sulfide; it is converted to 3-mercaptopyruvate by D-amino acid oxidase (DAO). Pyridoxal 52-phosphate (PLP) is a cofactor for CSE, CBS and CAT [26].

EXPERIMENTAL

All the reagents and solvents were purchased from the commercial sources and used without further purification. Compound confirmed by TLC and analysis. NMR spectra were recorded with 300 MHz spectrometers for ^{1}H NMR and 100 MHz for ^{13}C NMR on Bruker Supercon Magnet Avance DRX-300 spectrometers in deuterated solvents with TMS as internal reference. Mass spectra and HRMS were taken in the ESI positive ion mode. The reaction progress was monitored by thin layer chromatography (TLC) on pre-coated silica gel plates. All compounds were identified and characterized by TLC, ^{1}H NMR, ^{13}C NMR, MS and HRMS.

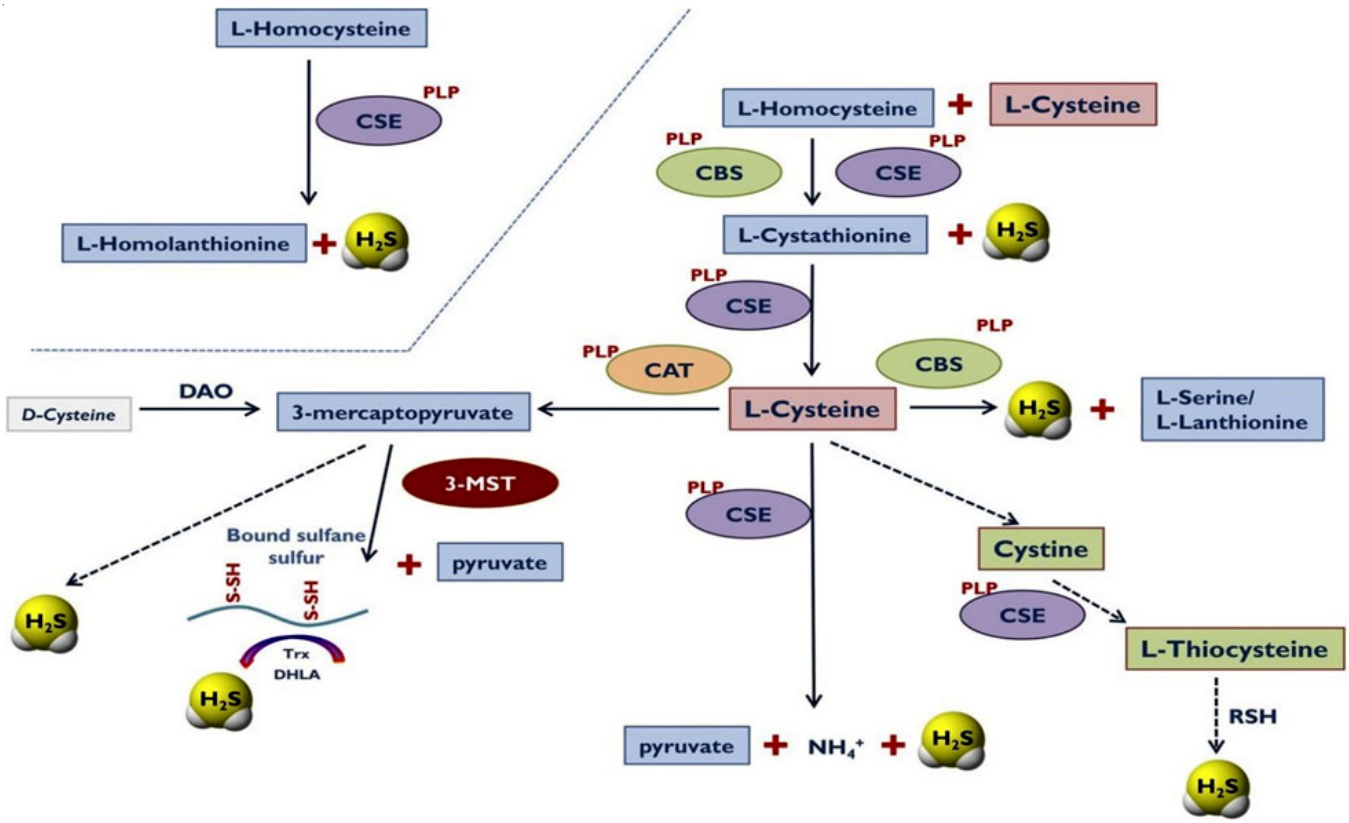
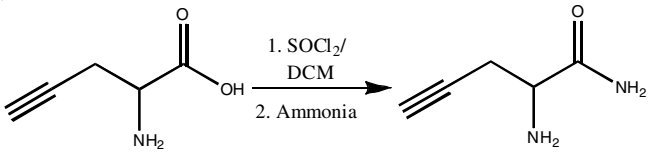


Fig. 1. Hydrogen sulfide generation in mammalian cells

Synthesis of 2-aminopent-4-ynamide (1b): 2-Aminopent-4-ynoic acid (2.8 g, 0.024 mol) were added in a round bottom flask with a MDC (50 mL) and thionyl chloride (4 mL, 0.036 mol). Reaction temperature raise to reflux for 5 h. Reaction mass cooled to 25-30 ºC followed by the addition of 7 M NH_3 in methanol (50 mL) at 0-5 ºC and finally stirred at 10 ºC for 12 h. Reaction mass concentrated to get 2-aminopent-4-ynamide (**Scheme-I**) as colourless solid. Yield: .52 g, 90%. ^{1}H NMR (300 MHz, DMSO) δ ppm: 8.38 (broad s, 2H), 7.81 (m, 2H), 3.85 (t, *J* = 5.85 Hz, 1H), 3.12 (t, *J* = 2.5Hz, 1H), 2.78 (m, 2H). HRMS: *m/z*: $[M + H]^+$ calculated for $C_5H_8N_2O$: 113.0577, found 113.0598.

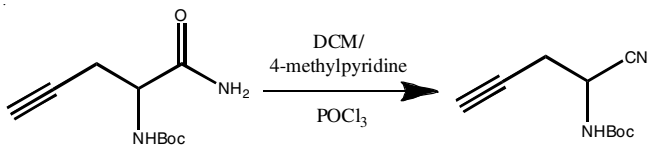


Scheme-I: Synthesis of 2-aminopent-4-ynamid

Synthesis of *tert*-butyl (1-amino-1-oxopent-4-yn-2-yl)-carbamate (1c): 2-Aminopent-4-ynamide (3 g, 2.36 mmol) added in a 100 mL round bottom flask with a THF (15 mL) fitted stir bar and then sodium carbonate (8.51 g, 80 mmol) solution and boc anhydride (7 g, 32.14 mmol) was added and then the reaction was stirred for 12 h at room temperature. Tetrahydrofuran was evaporated from reaction and compound was extracted in ethyl acetate (100 mL × 2). Ethyl actate layer was dried on sodium sulfate and concentrated *in vacuo* to yield *tert*-butyl (1-amino-1-oxo-pent-4-yn-2-yl)carbamate as white solid (**Scheme-II**). Yield: 6 g, 100%. ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm: 6.32 (brs, 1H), 5.68 (brs, 1H), 5.3 (brs, 1H), 2.78-2.86 (m, 1H), 2.58-2.65 (m, 1H), 2.10-2.11 (t, *J* = 2.5 Hz, 1H). 1.46 (s, 9H); HRMS: *m/z*: $[M + H]^+$ calculated for $C_{10}H_{16}N_2O_3$: 213.2405, found 213.5758.

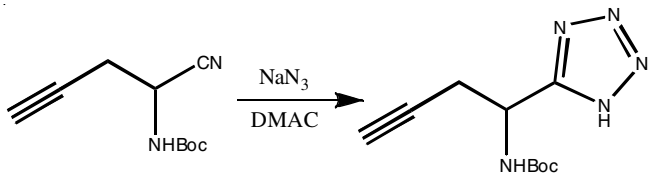
Scheme-II: *tert*-Butyl (1-amino-1-oxopent-4-yn-2-yl)carbamate

Synthesis of *tert*-butyl (1-cyanobut-3-yn-1-yl)carbamate (1d): *tert*-Butyl N-(1-carbamoylbut-3-yn-1-yl)carbamate (3 g, 14.14 mmol) were added in a 100 mL round bottom flask containing 4-methyl pyridine (8mL) followed by the addition of phosphoryl chloride (3.5 g, 21.0 mol) (in 25 mL DCM) and stirred it 1 h at 0 ºC. Allow the reaction to cool down at room temperature and stirred for 1 h. After that 50 mL water was added to reaction and extracted with ethyl acetate (50 mL × 3). The ethyl acetate was dried over sodium sulfate and concentrated *in vacuo* (**Scheme-III**). The compound was purified by column chromatography. The elute of column was ethyl acetate and hexane. The product (1.91 g, 70%) was isolated as colourless liquid. ^{1}H NMR (300 MHz, $CDCl_3$) ppm: 5.09 (brs, 1H), 4.82 (brs, 1H), 2.69-2.73 (m, 2H), 2.22 (t, *J* = 2.58Hz, 1H), 1.45 (s, 9H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 153.89, 117.45, 81.68, 73.34, 41.14, 28.16, 24.12. HRMS: *m/z*: $[M + H]^+$ calculated for $C_{10}H_{14}N_2O_2$: 195.1554, found 195.2884.



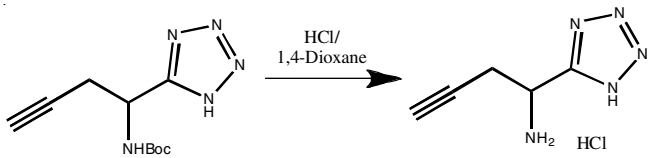
Scheme-III: *tert*-Butyl (1-cyanobut-3-yn-1-yl)carbamate

Synthesis of *tert*-butyl N-[1-(5*H*-1,2,3,4-tetrazol-5-yl)-but-3-yn-1-yl]carbamate (1e): *tert*-Butyl N-(1-cyanobut-3-yn-1-yl)carbamate (0.6g, 3.12 mmol) were added in a 100 mL round bottom flask with a DMF (5 mL) containing sodium azide (0.3 g, 4.6 mol) and ammonium chloride (0.24 g, 4.6 mmol) at room temperature and reaction was heated to 110 ºC for 8 h. DMF was evaporated from the reaction by flushing nitrogen gas. The compound was then purified by column chromatography by using deactivated silica gel. The elute of column was DCM and methanol and then *tert*-butyl N-[1-(5H-1,2,3,4-tetrazol-5-yl)but-3-yn-1-yl]carbamate (**Scheme-IV**) (0.400 g, 54%) was isolated as solid. ^{1}H NMR (300 MHz, MeOH-d_4) δ ppm: 5.12 (t, *J* = 6.6 Hz, 1H), 2.85 (m, 2H), 2.33 (br, 1H); 1.42 (s, 9H). HRMS: *m/z*: $[M + H]^+$ calculated for $C_{10}H_{15}N_5O_2$: 238.1254, found 238.1266.



Scheme-IV: *tert*-Butyl N-[1-(5*H*-1,2,3,4-tetrazol-5-yl)but-3-yn-1-yl]carbamate

Synthesis of 1-(1*H*-tetrazol-5-yl)-but-3-ynylamine hydrochloride (1f): *tert*-Butyl *N*-[1-(5*H*-1,2,3,4-tetrazol-5-yl)but-3-yn-1-yl]carbamate (0.24g, 0.99mmol) and dioxane·HCl (25mL) were taken up in a round bottom flask and stirred at 25-30 ºC for overnight. Dioxane as evaporated from reaction and 20 mL *n*-hexane wash given to reaction mass to get (5*H*-1,2,3,4-tetrazol-5-yl)but-3-yn-1-amine (**Scheme-V**). The product (185 mg, ~68%) was isolated as a white solid. ^{1}H NMR (300 MHz, MeOH-d_4) δ ppm: 5.14 (t, *J* = 6.3 Hz, 1H), 3.084-3.11 (dd, *J* = 3.7, 2.6 Hz, 2H), 2.62 (t, *J* = 2.6 Hz, 1H); HRMS: *m/z*: $[M + H]^+$ calculated for $C_5H_7N_5$: 138.0785, found 137.8911.



Scheme-V: 1-(1*H*-Tetrazol-5-yl)-but-3-ynylamine hydrochloride

RESULTS AND DISCUSSION

The inhibitors of cystathionine-γ-lyase (CSE-(1*H*-tetrazol-5-yl)but-3-yn-1-amine) were synthesized and characterized successfully. Total five steps were involved in the synthesis of 1-(1*H*-tetrazol-5-yl)-but-3-ynylamine hydrochloride. The Biological activity of single isomer of inhibitors of cystathionine-γ-lyase (CSE-(1*H*-tetrazol-5-yl) but-3-yn-1-amine) studied in patent WO2014/018569, WO2014/018570 and

WO2014/018571 [27-29]. By this method single isomer can be synthesized by using stereo specific 2-aminopent-4-ynoic acid.

Conclusion

In summary, an improved, scalable and time-effective synthetic process of inhibitors of cystathionine-γ-lyase (CSE-(1*H*-tetrazol-5-yl)but-3-yn-1-amine) is reported in good yields. This process can be easily applied to other modern small-molecule pharmaceuticals involving multistep cascade reactions.

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