Archives of Biochemistry and Biophysics 475 (2008) 115–120

Contents lists available at ScienceDirect

Archives of Biochemistry and Biophysics

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

Inhibition of serine proteases by a new class of cyclosulfamide-based carbamylating agents

Qingliang Yang [a], Yi Li [a], Dengfeng Dou [a], Xiangdong Gan [a], Swathi Mohan [a], Christopher S. Groutas [a], Laura E. Stevenson [a], Zhong Lai [a], Kevin R. Alliston [a], Jiaying Zhong [a], Todd D. Williams [b], William C. Groutas [a,*]

[a] Department of Chemistry, Wichita State University, 1845 N Fairmount Avenue, Wichita, KS 67260, USA
[b] Mass Spectrometry Lab, The University of Kansas, Lawrence, KS 66047, USA

ARTICLE INFO

Article history:
Received 6 March 2008
and in revised form 15 April 2008
Available online 22 April 2008

Keywords:
Carbamylating agents
Cyclosulfamide
Human neutrophil elastase
Activity-based probe

ABSTRACT

A new class of carbamylating agents based on the cyclosulfamide scaffold is reported. These compounds were found to be efficient time-dependent inhibitors of human neutrophil elastase (HNE). Exploitation of the three sites of diversity present in the cyclosulfamide scaffold yielded compounds which inhibited HNE but not proteinase 3 (PR 3) or bovine trypsin. The findings reported herein suggest that the introduction of appropriate recognition elements into the cyclosulfamide scaffold may lead to highly selective agents of potential value in the design of activity-based probes suitable for investigating proteases associated with the pathogenesis of chronic obstructive pulmonary disease.

Chronic obstructive pulmonary disease (COPD) is a major health problem that affects 16 million people in the US, and is currently the fourth most common cause of death [1,2]. COPD is a complex disorder associated with an influx of neutrophils, macrophages and CD8+ T cells into the lungs. This is followed by the release of a range of pro-inflammatory chemokines and cytokines, adhesion molecules, transcription factors, as well as an array of proteases [3]. The pathogenesis of COPD is currently unknown, consequently there is a need for (a) a rigorous definition of the cellular and molecular mechanisms of the inflammatory and immune processes which play a role in the pathogenesis and progression of COPD and, (b) illuminating the identity and function(s) of the various proteases involved in COPD [4]. The identification and validation of new molecular targets would likely pave the way toward the development of new and improved therapeutic interventions [5].

During the course of exploratory studies related to the utilization of the cyclosulfamide scaffold in the design of reversible competitive inhibitors of COPD-relevant serine proteases [6], it was observed that urea-type cyclosulfamide derivatives inhibited HNE in a time-dependent manner. We report herein a new class of carbamylating agents (I) (Fig. 1) of serine proteases having three points of diversity and potentially amenable to the construction of activity-based probes [7].

Materials and methods

General

The ^{1}H and ^{13}C NMR spectra were recorded on a Varian XL-300 or XL-400 NMR spectrometer. A Hewlett–Packard diode array UV/VIS spectrophotometer was used in the *in vitro* evaluation of the inhibitors. Human neutrophil elastase, proteinase 3, cathepsin G and Boc-Ala-Ala-Nva thiobenzyl ester were purchased from Elastin Products Company, Owensville, MO. Bovine trypsin, methoxysuccinyl Ala-Ala-Pro-Val *p*-nitroanilide, succinyl Ala-Ala-Pro-Phe *p*-nitroanilide, 5,5′-dithio-bis(2-nitrobenzoic acid), and Nα-benzoyl-L-Arg *p*-nitroanilide were purchased from Sigma Chemicals, St. Louis, MO. Melting points were determined on a Mel-Temp apparatus and are uncorrected. Reagents and solvents were purchased from various chemical suppliers (Aldrich, Acros Organics, TCI America, and Bachem). Silica gel (230–450 mesh) used for flash chromatography was purchased from Sorbent Technologies (Atlanta, GA). Thin layer chromatography was performed using Analtech silica gel plates. The TLC plates were visualized using iodine and/or UV light.

Chemistry

Compounds **7a–g** were synthesized using the reaction sequence shown in Scheme 1a. Compounds **7a–g** and **8–9** are listed in Scheme 1a and b, respectively. The synthetic methodology employed in Scheme 1 is highly versatile and permits the facile introduction of a large number of diverse fragments at the R_1, R_2, and R_3 positions using commercially available natural and unnatural amino acids, carboxylic acids and isocyanates. Intermediate **4** can also be prepared directly from **3** using the Mitsunobu reaction.

Representative syntheses

Compound 1. A solution of *N*-chlorosulfonyl isocyanate (13.0 mL, 0.15 mol) in methylene chloride (200 mL) was cooled in an ice bath and a solution of *t*-butyl alcohol (11.12 g, 0.15 mol) in methylene chloride (100 mL) was added dropwise with stir-

* Corresponding author. Fax: +1 316 978 3431.
E-mail address: bill.groutas@wichita.edu (W.C. Groutas).

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doi:10.1016/j.abb.2008.04.020

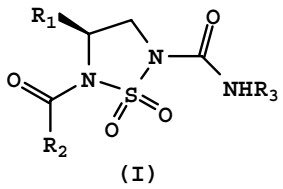


Fig. 1. General structure of inhibitor (I).

ring. After the solution was stirred for 10 min, the reaction mixture was transferred to a separatory funnel and added dropwise to a solution of (L) leucine methyl ester hydrochloride (27.25 g, 0.15 mol) and triethylamine (30.4 g, 0.30 mol) in methylene chloride (300 mL) kept in an ice bath. The resulting mixture was then stirred at room temperature overnight. The reaction mixture was washed with 5% HCl (2 × 75 mL) and brine (75 mL). The organic layer was dried over anhydrous Na_2SO_4 and then concentrated to yield a white solid (45.84 g, 94%): mp 89–91 °C; ^{1}H NMR ($CDCl_3$): δ 0.93 (t, 6H), 1.44 (s, 9H), 1.59 (m, 2H), 1.81 (m, 1H), 3.74 (s, 3H), 4.19 (m, 1H), 5.56 (d, 1H), 7.18 (bs, 1H).

Compound 2. To a solution of compound **1** (4.86 g, 15 mmol) in dry THF (20 mL) kept in a dry ice–acetone bath was added dropwise 2 M lithium borohydride in THF (7.5 mL, 15 mmol). After the reaction was stirred for 3 h, absolute ethanol (50 mL) was added dropwise. The reaction mixture was stirred overnight at room temperature, then cooled to 0 °C, and neutralized with 5% aqueous HCl. The resulting mixture was concentrated and then diluted with H_2O (10 mL). The resulting solution was extracted with ethyl acetate (3 × 25 mL), the organic phase was dried over anhydrous sodium sulfate, and then concentrated. The crude product was purified using flash chromatography (hexane/EtOAc, 75:25) to afford a white solid (1.93 g, 43%): mp 114–116 °C; ^{1}H NMR ($CDCl_3$): δ 0.92 (d, 6H), 1.36 (m, 1H), 1.42 (m, 1H), 1.44 (s, 9H), 1.71 (m, 1H), 3.47 (m, 2H), 3.71 (m, 1H), 5.50 (d, 1H).

Compound 3. To a solution of compound **2** (2.96 g, 10 mmol) in dry THF (25 mL) kept in an ice bath was added methanesulfonyl chloride (1.15 g, 10 mmol). Triethylamine (1.35 g, 13.3 mmol) was then added dropwise. After the reaction mixture was stirred for 4 h at room temperature, the solvent was evaporated and the residue was taken up in ethyl acetate (50 mL) and washed with 5% HCl (2 × 10 mL) and brine (10 mL). The organic phase was dried over anhydrous Na_2SO_4, then concentrated to yield an oil (3.32 g, 89%): ^{1}H NMR ($CDCl_3$): δ 0.92 (t, 6H), 1.42 (m, 2H), 1.44 (s, 9H), 1.71 (m, H), 3.03 (s, 3H), 3.71 (m, 1H), 4.19 (dd, 1H), 4.30 (dd, 1H), 5.40 (dd, 1H).

Compound 4. To a solution of compound **3** (3.74 g, 10 mmol) in dry acetonitrile (45 mL) was added dropwise a solution of DBU (1.83 g, 12 mmol) in dry acetonitrile (25 mL). The mixture was stirred for 5 h and the solvent was then evaporated. The residue was taken up in ethyl acetate (50 mL) and washed with 5% aqueous HCl (2 × 15 mL), 5% $NaHCO_3$ (2 × 15 mL) and brine (15 mL). The organic phase was dried over anhydrous sodium sulfate, filtered, and the solvent evaporated off. The crude product was purified using flash chromatography (hexane/EtOAc, 85:15) to afford a white solid (1.50 g, 54%): mp 97–99 °C; ^{1}H NMR ($CDCl_3$): δ 0.93 (d, 6H), 1.46 (m, 1H), 1.56 (s, 9H), 1.59 (m, 1H), 1.74 (m, 1H), 3.41 (t, 2H), 3.81 (m, 1H), 4.00 (dd, 1H), 4.27 (d, 1H).

Representative procedure for the synthesis of compounds 5a–g. To a solution of (3-methoxyphenyl) acetic acid (0.25 g, 1.5 mmol) in dry methylene chloride (8 mL) was added HATU (0.68 g, 1.8 mmol), followed by TEA (0.30 g, 3 mmol) and compound **4** (0.42 g, 1.5 mmol) in dry methylene chloride (5 mL). The reaction mixture was stirred at room temperature for 4 h and the solvent was evaporated off. The residue was taken up in ethyl acetate (50 mL) and washed with 5% aqueous HCl (2 × 8 mL), 5% $NaHCO_3$ (2 × 8 mL) and brine (2 × 8 mL). The organic phase was dried over anhydrous sodium sulfate, filtered and the solvent evaporated off to give a crude product which was purified using flash chromatography (hexane/EtOAc, 95:5) to give compound **5a** as an oil (0.32 g, 50%): ^{1}H NMR ($CDCl_3$): δ 0.92 (t, 6H), 1.57 (m, 2H), 1.58 (s, 9H), 1.63 (m,1H), 3.78 (m, 2H), 3.79 (s, 3H), 4.03 (q, 2H), 4.54 (m, 1H), 6.80–7.22 (m, 4H).

Compound 5b. White solid (44% yield), mp 98–100 °C; ^{1}H NMR ($CDCl_3$): δ 0.92 (t, 6H), 1.57 (m, 2H), 1.58 (s, 9H), 1.63 (m, 1H), 3.78 (m, 2H), 3.79 (s, 3H), 4.01 (q, 2H), 4.54 (m, 1H), 6.82–7.23 (m, 4H).

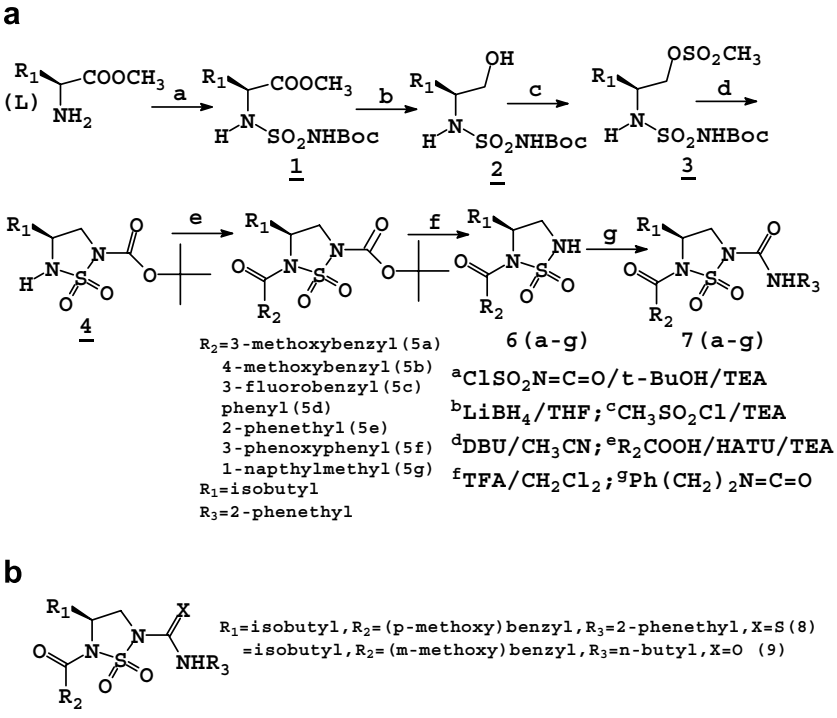
Compound 5c. Oil (22% yield). ^{1}H NMR ($CDCl_3$): δ 0.92 (t, 6H), 1.57 (m, 2H), 1.58 (s, 9H), 1.63 (m, 1H), 3.78 (m, 2H), 4.02 (q, 2H), 4.54 (m, 1H), 6.90–7.30 (m, 4H).

Compound 5d. Oil (10% yield). ^{1}H NMR ($CDCl_3$): δ 0.96 (dd, 6H), 1.58 (s, 9H), 1.62 (m, 2H), 1.96 (m, 1H), 3.63 (dd, 2H), 4.00 (dd, 2H), 4.99 (m, 1H), 7.45–7.79 (m, 5H).

Compound 5e. Oil (20% yield). ^{1}H NMR ($CDCl_3$): δ 0.94 (dd, 6H), 1.57 (m, 2H), 1.58 (s, 9H), 1.61 (m, 1H), 2.98 (m, 3H), 3.10 (m, 1H), 3.73 (m, 2H), 4.53 (m, 1H), 7.20 (m, 5H).

Compound 5f. Oil (13% yield). ^{1}H NMR ($CDCl_3$): δ 0.96 (dd, 6H), 1.58 (s, 9H), 1.60 (m, 2H), 1.96 (m, 1H), 3.63 (dd, 2H), 4.00 (dd, 2H), 4.99 (m, 1H), 6.99–7.58 (m, 9H).

Compound 5g. Oil (11% yield). ^{1}H NMR ($CDCl_3$): δ 0.92 (dd, 6H), 1.59 (m, 1H), 1.60 (s, 9H), 1.70 (m, 2H), 3.82 (m, 2H), 4.56 (q, 2H), 4.58 (m, 1H), 7.40–7.84 (m, 7H).



Scheme 1. Synthesis of compounds **7a–g.**