Available online at www.sciencedirect.com
SCIENCE DIRECT®
European Journal of Medicinal Chemistry 38 (2003) 421–425
EUROPEAN JOURNAL OF MEDICINAL CHEMISTRY
www.elsevier.com/locate/ejmech

A novel orally active inhibitor of HLE

Márton Varga *, Zoltán Kapui, Sándor Bátori, Lajos T. Nagy, Lelle Vasvári-Debreczy, Endre Mikus, Katalin Urbán-Szabó, Péter Arányi

Chinoin Co. Ltd – Member of Sanofi-Synthelabo Group, Discovery Research, H-1045 Budapest, Tó utca 1-5, Hungary

Received 14 October 2002; received in revised form 19 December 2002; accepted 26 January 2003

Abstract

Human leukocyte elastase (HLE) is a serine proteinase, capable of degrading a variety of structural matrix proteins. SSR69071 2-[(4-isopropyl-6-methoxy-1,1-dioxido-3-oxo-1,2-benzisothiazol-2(3*H*)-yl)methoxy]-9-(2-piperidin-1-ylethoxy)-4*H*-pyrido[1,2-*a*]pyrimidin-4-one was selected as a novel orally active HLE inhibitor for treatment of chronic obstructive pulmonary diseases, asthma, emphysema, cystic fibrosis and several inflammatory diseases (WO 01/44245 A1) (J. Pharm. Exp. Ther., submitted for publication).

Keywords: α1-Protease inhibitor; Human leucocyte elastase; Polymorphonuclear leukocyte; Bronchoalveolar lavage; Chronic obstructive pulmonary disease; Adult respiratory distress syndrome

1. Introduction

Human leukocyte elastase (HLE) belongs to the chymotrypsin family of serine proteinases. The enzyme consists of a single polypeptide chain of 218 amino acid residues and four disulfide bridges. HLE is located in the azurophilic granules of polymorphonuclear leukocytes. HLE aids in the migration of neutrophils from blood to various tissues such as the airways in response to chemotactic factors [3].

In addition to elastin, HLE is capable of degrading a variety of proteins [4], including different types of collagens and structural matrix proteins [4]. Under normal circumstances the proteolytic activity of HLE is tightly controlled by its natural inhibitors such as a_1-protease inhibitor (α_1-PI) and the secretory leukocyte protease inhibitor [5]. In a number of pulmonary pathophysiological states relative insufficiency of endogenous elastase inhibitors may result in severe conditions, such as pulmonary emphysema [6], adult respiratory distress syndrome (ARDS) [7], chronic bronchitis [8], chronic obstructive pulmonary disease (COPD) [9], pulmonary hypertension [10] and other inflammatory diseases [11]. Therefore it is expected that an orally active HLE inhibitor could be useful for the treatment of these diseases.

We have synthesised a large number of compounds and investigated their HLE inhibitor activity both in vitro and in vivo [1]. SSR69071 was selected as the most potent orally active HLE inhibitor for the treatment of COPD, ARDS, cystic fibrosis, asthma and other inflammatory diseases [2]. For comparison ZD8321, a selective and orally active elastase inhibitor was synthesised and used as a reference compound in the biochemical and pharmacological studies [12].

2. Chemistry

Recently many HLE inhibitors of small molecular weight have been described and reviewed [4]. The mechanism-based inhibitors like β-lactame or saccharin

* Corresponding author.
E-mail address: marton.varga@sanofi-synthelabo.com (M. Varga).

0223-5234/03/$ - see front matter
doi:10.1016/S0223-5234(03)00046-1

derivatives represent a particular class of this type of compound because of their remarkably high in vitro potency. The structure of SSR69071 was designed to be a saccharin type mechanism-based inhibitor. Similar compounds were investigated earlier by Sterling–Winthrop [13].

The chemical stability of the inhibitor and its activity in the biological binding process are influenced by reactivity of the carbonyl group of the saccharin ring, which is determined by both the electron withdrawing pyridopyrimidine leaving group (activation) and the methoxy group (deactivation). On the other hand, the methoxy group improves the inhibitor activity by increasing the stability of the acyl–enzyme complex. It is believed that the isopropyl side chain provides selectivity of the compound. The basic piperidinoethoxy group increases the water solubility and the penetration of the inhibitor into the bronchoalveolar fluid.

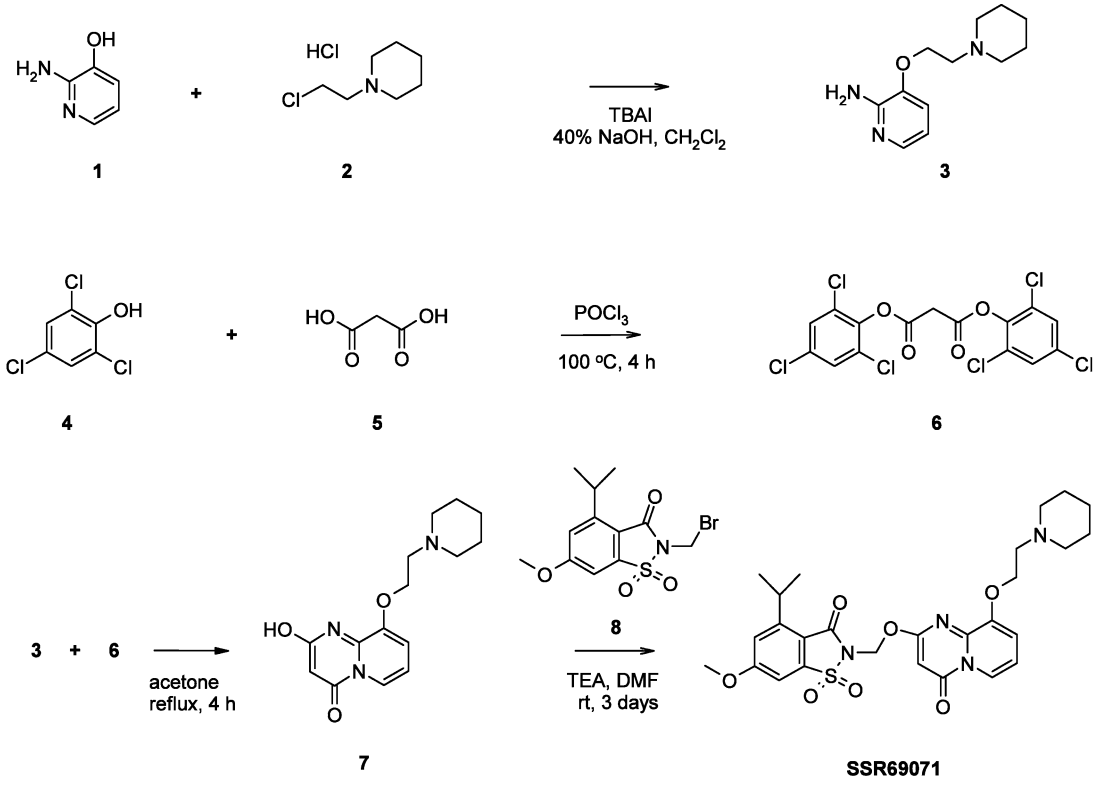
SSR69071 was synthesised according to Scheme 1. 2-Amino-3-hydroxypyridine (**1**) was reacted with 1-(2-chloroethyl)piperidine (**2**) in the presence of a phase transfer catalyst in the mixture of a suitable organic solvent and aqueous sodium hydroxide. The ring closure of 2-amino-3-(2-piperidin-1-ylethoxy)pyridine (**3**) and the active ester (**6**) of malonic acid (**5**) was performed by heating the reagents in acetone.

The hydroxy group of 2-hydroxy-9-(2-piperidin-1-ylethoxy)-4-oxo-4*H*-pyrido[1,2-*a*]-pyrimidine (**7**) was alkylated with 2-bromomethyl-4-isopropyl-6-methoxy-1,2-benzisothiazol-3(2*H*)-one 1,1-dioxide (**8**) in dimethylformamide in presence of triethylamine to provide SSR69071. The synthesis of the saccharine derivative (**8**) is described in ref. [13].

3. Biological results

On the basis of our results, SSR69071 is a potent inhibitor of HLE, with inhibition constant $K_i = 0.0168 \pm 0.0014$ nM and constant for inactivation process $k_{on} = 0.183 \pm 0.013\ 10^6\ Ms^{-1}$ [2]. The dissociation rate constant, k_{off} was $3.11 \pm 0.37\ 10^{-6}\ s^{-1}$. SSR69071 is a more potent elastase inhibitor than ZD8321. In the same experimental conditions the K_i value for ZD8321 was 5.57 ± 0.18 nM. SSR69071 displays a higher affinity for human elastase than for rat ($K_i = 3$ nM), mice ($K_i = 1.8$ nM) and rabbit ($K_i = 58$ nM) elastases.

The ex vivo activity of SSR69071 was determined in the bronchoalveolar lavage fluid (BAL) after oral administration in mice. The elastase inhibitory potency of BAL was examined in this experiment. SSR69071 has a dose-dependent efficacy with an $ED_{50} = 10.5\ mg\ kg^{-1}$ po after 1 h pre-treatment time. After 20 mg kg^{-1} oral treatment SSR69071 appeared in the BAL with an apparent fast absorption rate, a 73% inhibition of HLE being observed at 10 min post treatment. A maximum inhibitory activity (90%) was observed at 30



Scheme 1. Reaction scheme for the synthesis of SSR69071.

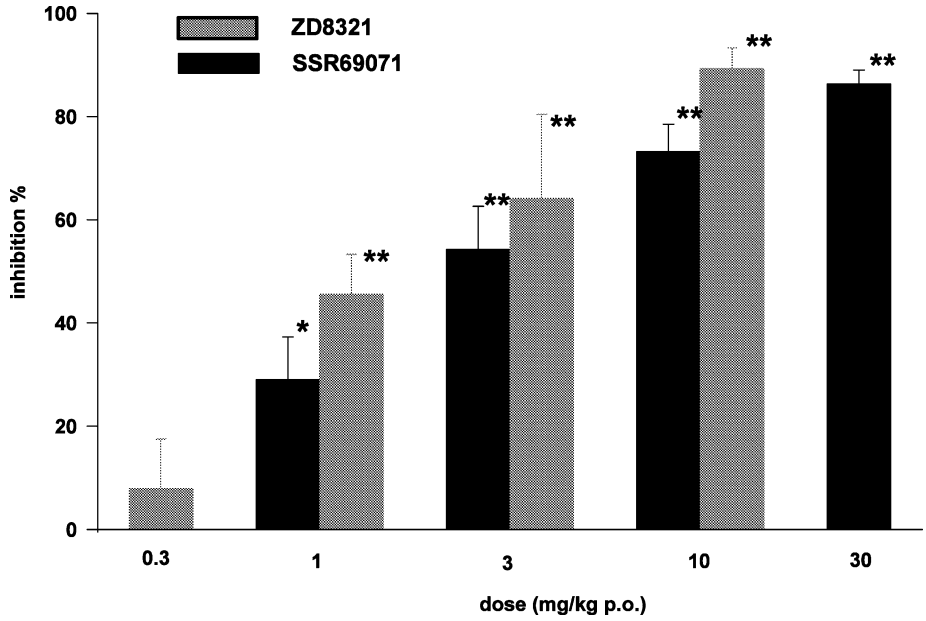


Fig. 1. Effect of ZD8321 and SSR69071 on HLE-induced lung haemorrhage in mice. SSR69071 was administered orally 30 min before HLE (10 U animal^{-1}) instillation. Values are means ± S.E.M. for 9–12 mice. Kruskal–Wallis test, * $P < 0.05$, ** $P < 0.01$.

min, the activity being decreased to 42% after 24-h pre-treatment time, indicating a long duration of action.

The ability of SSR69071 to protect animals from HLE-induced lung haemorrhage was evaluated in mice. Intratracheal instillation of HLE (10 IU) caused a severe lung haemorrhage. Oral administration of SSR69071 dose-dependently and potently inhibited the lung haemorrhage induced by HLE with an ID_{50} value of 2.8 mg kg^{-1} (Fig. 1). In the same experimental conditions, ZD8321 also produced good inhibition of haemorrhage with an ID_{50} value of 1.6 mg kg^{-1}.

4. Discussion

The recently synthesised SSR69071 compound is a potent, competitive and slow tight binding inhibitor of HLE in vitro with a K_i value of 16.8 pM. SSR69071 is more potent than FK706 [14], the thienooxazinone type inhibitors [15], TEI-8632 [16], ONO-5046 [17] and GW-311616A [18] according to the K_i values. It is believed that SSR69071 has a fast association rate due to the combined influence of the methoxy group and the leaving group and a slow dissociation rate caused by the stabilisation effect of the methoxy group. These effects explain the formation of a stable, slowly-reversible [HLE-Inhibitor] complex. These properties of SSR69071 suggest an extremely high activity and long duration of action in humans.

We examined the ability of SSR69071 to inhibit elastases from different species using a synthetic substrate. SSR69071 inhibited elastases from different species in a dose dependent manner, but at much higher concentrations compared to HLE. Because of the strong species selectivity (human vs. rodent elastase), the results obtained in rats and mice models may lead to underestimate the efficacy of SSR69071 in humans.

Ex vivo experiments in mice and the in vivo acute HLE induced mouse lung haemorrhage model demonstrated the remarkable oral activity of SSR69071 ($ID_{50} = 2.8$ mg kg^{-1} p.o.). SSR69071 has a good penetration into the lungs from the systemic circulation after oral administration on the basis of ex vivo study.

SSR69071 shows good oral activity. Only a limited number of published elastase inhibitors show oral activity and their active doses are relatively high, between 10–50 mg kg^{-1} [4].

In conclusion, SSR69071 has been shown to be a potent inhibitor of HLE, exhibiting good oral activity with a potency to treat inflammatory bronchopulmonary diseases such as COPD and chronic bronchitis.

5. Experimental

Kieselgel 60 (REANAL) was used for flash column chromatography. Melting points were determined with a BÜCHI 535 apparatus and are uncorrected. A Bruker IFS-28 was used to record the IR (KBr pellet) spectra. BRUKERDRX-400 or BRUKER AC-200 spectrometer was used for recording the ^{1}H spectra ($CDCl_3$ or DMSO-d_6 solutions; TMS standard); J values are quoted in Hz. Microanalysis was performed on CARLO ERBA MOD 1110 for C, H, N, instrument.

2-Amino-3-(2-piperidin-1-ylethoxy)pyridine (**3**) 110.12 g (1 mol) of **1** was dissolved in 500 mL of 40%-(w/w) aqueous NaOH. A solution of tetrabutylammonium iodide (2 g) in CH_2Cl_2 (500 mL) followed by 184.11 g (1 mol) of **2** were added to the stirred mixture. Stirring of the dark brown mixture was continued in argon atmosphere for 5 days at room temperature. CH_2Cl_2 (500 mL) and water (200 mL) were added. The organic layer was separated and the water was extracted with CH_2Cl_2 (3×150 mL). The combined organic solution was washed with water (3×200 mL), dried over $MgSO_4$ and evaporated. The red–brown crystalline product was recrystallised from acetone.

Yield: 144.71 g (65%) of **3** (m.p. 105–106 °C). NMR, δ_H (200 MHz, DMSO-d_6): 1.37 (m, 2H, $CH_2(CH_2CH_2)_2N$), 1.48 (m, 4H, $CH_2(CH_2CH_2)_2N$), 2.42 (m, 4H, $CH_2(CH_2CH_2)_2N$), 2.64 (t, 2H, $J = 5.8$, NCH_2CH_2O), 4.01 (t, 2H, $J = 5.8$, NCH_2CH_2O), 5.63 (s, 2H, NH_2), 6.47 (dd, 1H, $J = 7.7$; 5.0, 5-H), 7.03 (dd, 1H, $J = 7.7$, 1.2, 6-H), 7.51 (dd, 1H, $J = 5.0$, 1.2, 4-H).

Bis-2,4,6-trichlorophenyl malonate (**6**) 158.0 g (0.8 mol) of **4** and 41.6 g (0.4 mol) of **5** were added to $POCl_3$ (100 mL, 1.05 mol, 162 g). The mixture was stirred with mechanical stirrer under reflux for 4 h until the hydrogen chloride evolution ceased. The warm dense suspension was poured into iced water (500 g). The crystals were filtered off and added to a solution of water (100 mL) and concentrated aqueous $NaHCO_3$ (50 mL). After 30 min stirring the crystals were filtered, washed with water (3×40 mL) and dried in vacuum.

Yield: 180.9 g (98%) of **6** (m.p. 154–156 °C). NMR δ_H (200 MHz, $CDCl_3$): 4.05 (s, 2H, CH_2), 7.40 (s, 4H, $C_6H_2Cl_3O$).

2-Hydroxy-9-(2-piperidin-1-ylethoxy)-4-oxo-4H-pyrido[1,2-a]pyrimidine (**7**) 88.50 g (0.4 mol) of **3**, was added to dry acetone (550 mL). The mixture was refluxed and **6** (185.20 g, 0.4 mol) was carefully added to it in small portions. Reflux was continued for 2 h and after cooling to room temperature the suspension was kept at 5 °C for a night. The crystals were filtered off. After evaporating the mother liquor to half of its volume a second generation of product was obtained. The combined raw product was washed 3 times with ~ 30 mL of acetone. The product was purified by flash chromatography on silica gel. The remaining 2,4,6-trichlorophenol was eluted with CH_2Cl_2 and the pure product was obtained by elution with a mixture of ethanol–CH_2Cl_2 (1:1 v/v).

Yield: 69.31 g (60%) of **7** (mp 171–172 °C). NMR δ_H (200 MHz, DMSO-d_6): 1.39 (m, 2H, $CH_2(CH_2CH_2)_2N$), 1.50 (m, 4H, $CH_2(CH_2CH_2)_2N$), 2.50 (m, 4H, $CH_2(CH_2CH_2)_2N$), 2.83 (t, 2H, $J = 5.9$, NCH_2CH_2O) 4.27 (t, 2H, $J = 5.9$, NCH_2CH_2O), 5.16 (s, 1H, 3-H) 7.13 (t, 1H, $J = 7.3$, 7-H), 7.50 (d, 1H, $J = 7.3$, 8-H) 8.50 (d, 1H, $J = 6.4$, 6-H).

2-[(4-Isopropyl-6-methoxy-1,1-dioxido-3-oxo-1,2-benzisothiazol-2(3H)-yl)methoxy]-9-(2-piperidin-1-ylethoxy)-4H-pyrido[1,2-a]pyrimidin-4-one (**SSR69071**) hemihydrate. **7** (13.7 g, 45 mmol) was dissolved at room temperature in 100 mL of dry DMF, then triethylamine (7 mL) and **8** (15.6 g, 45 mmol) were added. The suspension was stirred for 60 h at room temperature in argon atmosphere. The precipitate was filtered off and the mother liquor was poured into iced water (200 mL). The suspension was filtered and the product was recrystallised from methanol.

Yield: 4.8 g (19%) of **SSR69071** (m.p. 138–139 °C). $C_{27}H_{32}N_4O_7S \cdot 0.5H_2O$ Calc. C, 57.33; H, 5.88; N, 9.90; S, 5.67. Found C, 57.41; H, 6.02; N, 9.77; S, 5.39; NMR δ_H (200 MHz, $CDCl_3$): 1.30 (d, 6H, $J = 6.8$, $(CH_3)_2CHO$), 1.47 (m, 2H, $CH_2(CH_2CH_2)_2N$), 1.62 (m, 4H, $CH_2(CH_2CH_2)_2N$), 2.64 (m, 4H, $CH_2(CH_2CH_2)_2N$), 2.98 (t, 2H, $J = 5.8$ NCH_2CH_2O), 3.96 (s, 3H, CH_3O) 4.23 (m, 1H, $J = 6.8$, $(CH_3)_2CHO$), 4.32 (t, 2H, $J = 5.8$ NCH_2CH_2O), 5.90 (s 1H,), 6.23 (s 2H,), 7.05 (t, 1H, $J = 7.4$, 7′-H), 7.14 (dd, 1H, $J = 7.7$, 1.3, 8′-H), 7.19-7.21 (m, 2H, 5-H, 7-H), 8.72 (dd, 1H, $J = 7.0$, 1.3, 6′-H).

The elastase assay, the determination of the kinetic parameters of elastase inhibition, the ex vivo inhibition of HLE activity in mice BAL and the in vivo model of HLE-induced lung haemorrhage in mice are described in details in ref. [2].

References

[1] P. Arányi, S. Batori, S. Dessilla, I. Hermecz, Z. Kapui, F. Levai, E. Mikus, M. Pascal, L. T. Nagy, B. Simonot, K. Urban-Szabo, M. Varga, L. Vasvari-Debreczy, Saccharin derivatives as orally active elastase inhibators, WO 01/44245 A1 (Priority date. 17-12-1999).
[2] Z. Kapui, M. Varga, K. Urban-Szabo, E. Mikus, T. Szabó, J. Szeredi, O. Finance, P. Arányi, J. Pharm. Exp. Ther. 305 (2003) 1–9.
[3] C. Delclaux, C. Delacourt, M. d'Ortho, V. Boyer, C. Lafuma, A. Harf, Am. J. Respir. Cell Mol. Biol. 14 (1996) 288–295.
[4] J.W. Skiles, A. Jeng, Exp. Opin. Ther. Patents 9 (1999) 869–895.
[5] C. Vogelmeier, R.C. Hubbard, G.A. Fells, H.P. Schnebli, R.C. Thompson, H. Fritz, R.G. Crystal, J. Clin. Invest. 87 (1991) 482–488.
[6] A. Janoff, Am. Rev. Respir. Dis. 132 (1985) 417–433.
[7] C.T. Lee, A.M. Fein, M. Lippmann, H. Holzmann, P. Kimbel, G. Weinbaum, N. Eng. J. Med. 304 (1981) 192–196.
[8] C.G. Llewellyn-Jones, T.A. Harris, R.A. Stockley, Am. J. Respir. Crit. Care Med. 153 (1996) 616–621.
[9] P.D. Piccioni, J.A. Kramps, A. Rudolphus, A. Bulgheroni, M. Luisetti, Chest 102 (1992) 1470–1476.
[10] K.N. Cowan, A. Heilbut, T. Humpl, C. Lam, S. Ito, M. Rabinovitsch, Nat. Med. 6 (2000) 698–702.
[11] E.O. Adeyemi, S. Neumann, V.S. Chadwik, H.J.F. Hodgson, M.B. Prepys, Gut 26 (1985) 1306–1311.
[12] C.A. Veale, P.R. Bernstein, C.M. Bohnert, F.J. Brown, C. Bryant, J.R. Damewood, R. Earley, S.W. Feeney, P.D. Edwards, B

Gomes, J.M. Hulsizer, B.J. Kosmider, R.D. Krell, G. Moore, D.J. Wolanin, S. Woolson, J. Med. Chem. 40 (1997) 3173–3181.

[13] (a) D.J. Hlasta, M.R. Bell, N.W. Boaz, J.J. Court, R.C. Desai, C.A. Franke, A.J. Mura, C. Subramanyam, R.P. Dunlap, Bioorg. Med. Chem. Lett. 4 (1994) 1801–1806;
(b) D.J. Hlasta, J.H. Ackerman, J.J. Court, et al., J. Med. Chem. 38 (1995) 4687–4692;
(c) R.C. Desai, D.J. Hlasta, G. Monsour, et al., J. Org. Chem. 59 (1994) 7161–7163 (EP 550111 A1).

[14] Y. Shinguh, A. Yamazaki, N. Inamura, K. Fujie, M. Okamoto, K. Nakahara, Y. Notsu, M. Okuhara, T. Ono, Eur. J. Pharmacol. 345 (1998) 299–308.

[15] M. Gütschow, L. Kuerschner, U. Neumann, M. Pietsch, R. Löser, N. Koglin, K. Eger, J. Med. Chem. 42 (1999) 5437–5447.

[16] H. Mitsuhashi, S. Asano, T. Nonaka, K. Masuda, M. Kiyoki, J. Pharm. Exp. Ther. 282 (1997) 1005–1010.

[17] K. Kawabata, M. Suzuki, M. Sugitani, K. Imaki, M. Toda, T. Miyamoto, Biochem. Biophys. Res. Comm. 177 (1991) 814–820.

[18] P. Norman, Drug News Perspect. 11 (1998) 431–437.