Nonpeptidic Inhibitors of Human Leukocyte Elastase. 6. Design of a Potent, Intratracheally Active, Pyridone-Based Trifluoromethyl Ketone[1]

Peter R. Bernstein,* Bruce C. Gomes,† Benedict J. Kosmider, Edward P. Vacek, and Joseph C. Williams†

Departments of Medicinal Chemistry and Pharmacology, ZENECA Pharmaceuticals, A Business Unit of ZENECA Inc., 1800 Concord Pike, Wilmington, Delaware 19897

Received August 17, 1994®

Further modification of the 3-amino substituent in a trifluoromethyl ketone-based series of 3-amino-6-phenylpyridin-2-ones that had been optimized for oral activity led to analogs that were potent intratracheal inhibitors in a model of HLE-induced lung damage in the hamster. The best 3-amino substituent for intratracheal activity is [4-[*N*-[(4-chlorophenyl)sulfonyl]-carbamoyl]phenyl]sulfonyl. At a 30 min prechallenge interval, compound **9**, which incorporates this substituent, had an ED_{50} of ~2 nmol/animal and, qualitatively, afforded a very similar dose–response relationship to that found with a peptidic trifluoromethyl ketone inhibitor, ICI 200,355.

Introduction

Human leukocyte elastase (HLE, EC 3.4.21.37)[2] is a serine proteinase that has been implicated as a causative or contributive agent in several pathological states.[3] This profile resulted in a wide-ranging search for low molecular weight inhibitors of HLE that could serve as therapeutic agents. Many types of inhibitors, having peptidic or nonpeptidic frameworks and either reversible or nonreversible mechanisms of action, resulted from this quest.[4]

We recently described the development of a series of heterocycle-containing, reversible, trifluoromethyl ketone-based inhibitors of HLE (Figure 1).[1] While high levels of in vitro potency were readily attained for these compounds (e.g., **1**), they originally lacked in vivo activity.[1a–c] Variation of the 3-amino substituent in these compounds led to pyridones that are active following oral (po) administration[1d] and further modification of the heterocyclic nucleus afforded pyrimidones[1e] that had an improved oral profile. During most of the effort that led to these compounds, there was only limited interest in examining their intratracheal profiles. This was because the intratracheal profiles of the earlier peptidic inhibitors from these laboratories (e.g., ICI 200,355 and ICI 200,880) were excellent, and our efforts were focused on finding orally active compounds. As part of the profiling of these pyridones we were curious to see if analogs that had good intratracheal profiles could also be developed.

Initially, we examined the intratracheal activity of some pyridones that were orally active (e.g., **11** and **12**, Table 2). These pyridones were inactive when dosed intratracheally 30 min prior to instillation of HLE at 100 nmol/animal. Under this protocol our peptidic standards (e.g., ICI 200,355) afforded complete inhibition (>95%). The result with the pyridones was not a complete surprise. Optimization of the N-substituent for improved oral activity should not necessarily lead to compounds that would also be intratracheally active, if given sufficiently prior to the elastase insult that absorption from the lung could occur. As a lead toward development of intratracheally active analogs, we turned to previous SAR studies from these labs. Those efforts had demonstrated, in the tripeptide-TFMK series of HLE inhibitors, that P_4-arylamides that contained an arylsulfonimide-based substituent (e.g., for ICI 200,880 P_4 = [4-(4-$ClC_6H_4SO_2NHCO$)C_6H_4CO]) were critical for optimal intratracheal activity.[5] However, in the pyridone-based inhibitors, variation of the corresponding 3-amino substituent revealed that arylamide-substituted *pyridones* were much less potent in vitro than their corresponding P_4-arylamide-substituted *peptides* (e.g., compare **3** to **4**, Table 1).[1d] This finding had argued against the preparation of pyridones that were substituted with (arylsulfonimide-containing) arylamides analogous to the P_4-arylamide in ICI 200,880 since they would probably have reduced potency in vitro. At about the same time it was also found that in the 3-aminopyridone series analogs with arylsulfonamide N-substituents were much more potent in vitro than the corresponding arylamides (e.g., compare **5** to **3**). This result contrasted with prior findings in the tripeptide series (e.g., compare **6** to **4**). Therefore, we chose to prepare pyridones substituted with (arylsulfonimide-containing) arylsulfonamides (e.g., 4-($ArSO_2NHCO$)$C_6H_4SO_2$), in the hope that they would retain good in vitro activity and like the peptidic (arylsulfonimide-containing) arylamides ICI 200,355 and ICI 200,880, would show excellent intratracheal activity.

Synthetic Chemistry

The target compounds were readily prepared (see Scheme 1) from amino ketone **7**[1d] in two steps. Acylation of **7** by treatment with 4-(chlorosulfonyl)benzoic acid and pyridine resulted in formation of the common intermediate, carboxylic acid **8**. Condensation of this acid by using 1-ethyl-3-(3-(dimethylamino)propyl)carbodiimide hydrochloride (WSCDI), 4-(*N*,*N*-dimethylamino)pyridine and either 4-chlorobenzenesulfonamide or 2-methylbenzenesulfonamide resulted in formation of sulfonimides **9** and **10**, respectively.

Results and Discussion

Table 2 contains the biological results for these compounds. In vitro evaluation of these compounds consisted of determination of their inhibition constants

† Department of Pharmacology.

® Abstract published in *Advance ACS Abstracts*, December 1, 1994.

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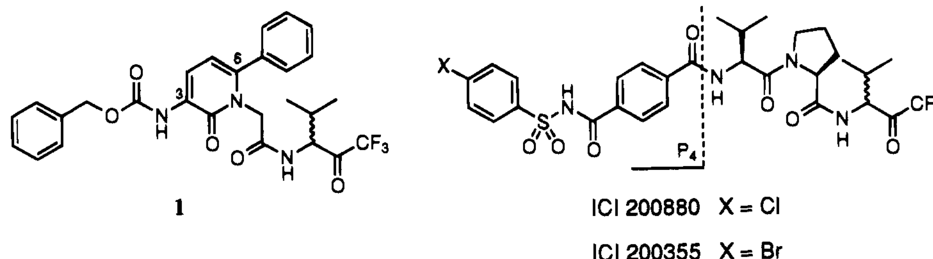


Figure 1. Structures of several human leukocyte elastase inhibitors.

Table 1. Comparison of the Effect of Varying the "P_4" *N*-Substituent on *in Vitro* Activity in Pyridone- and Peptide-Based Inhibitors

#	R =	K_i (nM)[a]	#	R =	K_i (nM)
3	O, CH_3O	480	4[b]	O, CH_3O	1.9
5	O S O, CH_3O	8.4	6[b]	O S O, CH_3O	8.0

[a] The inhibition constant (K_i) versus HLE was determined using the method described in ref 6. [b] This compound is reported in ref 5.

(K_i), against HLE-catalyzed hydrolysis of MeO-Suc-Ala-Ala-Pro-Ala-pNA, using the standard method.[6] The compounds exhibited time-dependent kinetics,[7] and the K_i values reported are the equilibrium numbers. In vivo efficacy was determined in the hamster using the acute hemorrhagic assay (AHA), a model based on lung damage induced by intratracheally administered HLE.[1d] The routine in vivo protocol used intratracheal administration of 100 nmol/animal of test compound 30 min prior to instillation of 50 μg of HLE. Several of these compounds were more fully investigated by examination of their intratracheal dose–response relationships at doses ranging from 1000 to 0.3 nmol/animal, as appropriate, in order to determine a 50% effective dose (ED_{50}) at the 30 min time point. Efficacy following po administration was determined in the same model with drug dosing intervals of either 30 or 90 min prior to instillation of the HLE.

Both **9** and **10** had good levels of in vitro activity and each showed essentially complete inhibition of HLE-induced hemorrhage at the standard screening dose of 100 nmole/animal. Evidence that the improved intratracheal effectiveness of these compounds was probably due to the sulfonimide and not to either their lower K_i (in comparison to **11**) or the presence of an *N*-arylsulfonamide substituent was supplied by benzenesulfonamide **12**. This analog was intratracheally inactive at 100 nmol/animal. However, arenesulfonamide **13**, which does not contain a sulfonimide substituent but is the most potent compound in vitro in the pyridone series,[1c] did show good intratracheal activity at 100 nmol/animal.

Oral evaluation of compounds **9** and **10** showed them to be inactive when dosed at 2.5 mg/kg 90 min prior to HLE challenge.[8] This result indicated a divergence between the physicochemical features necessary for good po or good intratracheal activity. Further profiling was achieved by generation of intratracheal dose-response curves for **9**, **13**, ICI 200,355, and the β-lactam-based inhibitor L680833[9] (see Figure 2). The curves generated for **9** and ICI 200,355 were very similar and led to estimated ED_{50} values of approximately 2 nmol/animal. In contrast **13** and L680833[10] were weaker following intratracheal administration and had ED_{50} values of 8 and 50 nmol/animal, respectively.

Experimental Section

General Methods. Proton NMR (^{1}H NMR) spectra were recorded on either a Bruker WM 250 (250 MHz) or Bruker WM 300 (300 MHz) instrument in the solvent indicated. Chemical shifts are reported in parts per million (δ) relative to internal tetramethylsilane. Peaks are reported as follows: s, singlet; d, doublet; t, triplet; b, broad; ex, exchanged by added deuterotrifluoroacetic acid or deuterium hydroxide. Mass spectra (MS) were recorded on a Kratos MS-80 instrument or Finnigan MAT-60 operating in the chemical ionization mode using methane as reagent gas. Melting points were determined on a Thomas-Hoover capillary melting point apparatus and are uncorrected. Combustion analyses for carbon, hydrogen, and nitrogen were performed on a Perkin-Elmer 241 instrument by the ZENECA Analytical Department, and are within $\pm$0.4% of theoretical values. Flash chromatography[11] was performed using the indicated solvent ratios (v/v) on Kieselgel 60 (230–400 mesh) supplied by E. Merck. Analytical thin-layer chromatography (TLC) was conducted either on prelayered silica gel GHLF plates, 250 μm thickness (Analtech, Newark, DE), or on Whatman $MKC_{18}F$ reversed-phase TLC plates (RP-TLC), 200 μm thickness. Visualization of the plates was accomplised by using UV light and/or phosphomolybdic acid–sulfuric acid charring. Tetrahydrofuran (THF) was distilled from sodium–benzophenone ketyl; dichloromethane and pyridine were distilled from calcium hydride. All other reagents were purified by standard methods (recrystallization or distillation) as needed.

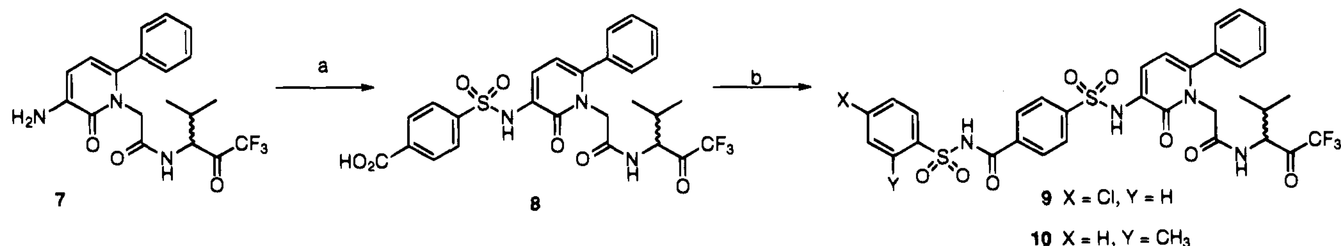
2-[3-[[(4-Carboxyphenyl)sulfonyl]amino]-2-oxo-6-phenyl-1,2-dihydro-1-pyridyl]-*N*-(3,3,3-trifluoro-1-isopropyl-2-oxopropyl)acetamide (8). In a 50 mL round-bottomed flask equipped with a magnetic stirrer was suspended amino ketone **7** (230 mg, 0.58 mmol) in THF (6 mL). The flask was cooled to 0 °C, and pyridine (0.24 mL, 2.9 mmol) was added followed by 4-(chlorosulfonyl)benzoic acid (150 mg, 0.7 mmol). After 2 h, additional sulfonyl chloride (50 mg, 0.23 mmol) was added, and the reaction mixture was allowed to stir overnight and warm to ambient temperature. The mixture was diluted with ethyl acetate (10 mL) and partitioned with half-saturated potassium diacid phosphate (12 mL). The aqueous phases were extracted two additional times with ethyl acetate, and the combined organic phases were washed with brine, dried over MgSO4, filtered, and concentrated to afford a gum which was purified by reverse-phase chromatography on 25 g of REGIS ODS packing material using a gradient of 90:10 water: methanol to 50:50 water:methanol. The appropriate fractions were combined, filtered, concentrated and dried in vacuo to afford a light yellow solid (0.286 g, 75%) of analytically pure **8**: mp > 250 °C; ^{1}H NMR δ 0.87 (dd, 6), 2.14 (m, 1), 4.40 (q, 2), 4.60 (dd, 1), 6.15 (d, 1), 7.35 (d, 1), 7.45 (m, 5), 8.00 (d, 2),

Table 2. Pharmacological and Chemical Characterization of Pyridone TFMKs

#	R Group	Molecular Formula	K_i (nM)[a]	Oral Activity[b] % inhibition / dose (mg/kg)	IT activity[b] % inhibition at 100 nmol / animal
9	p-ClC6H4SO2NHCO-C6H4-SO2	$C_{32}H_{28}ClF_3N_4O_8S_2 \cdot 1.0\,H_2O$	9.4±3.8	NS / 2.5[d]	96
10	o-MeC6H4SO2NHCO-C6H4-SO2	$C_{33}H_{31}F_3N_4O_8S_2 \cdot 0.5\,H_2O$	18.0±12	NS / 2.5[d]	97
11	HC(O)	c	26.0±3	33 / 2.5	NS
12	C6H5SO2	c	6.0±2	85 / 20, NS / 5	NS
13	CH3C(O)NH-C6H4-SO2	c	0.7±0.2	NS / 2.5	95

[a] The inhibition constant (K_i) versus HLE was determined using the method described in ref 6. [b] Percent inhibition of elastase-induced lung hemorrhage when the compound is dosed either orally or intratracheally at the indicated dose. The percent inhibition values reported reflect statistically significant differences ($P < 0.05$ using the standard student's t test) from the controls. Due to the variability of this assay, compounds active (at <95% inhibition levels) were tested at least two separate times. Only if two or more studies showed significant inhibition was the compound considered active and then the percent inhibition reported is the average of the individual values. The compounds were dosed 30 min prior to instillation of HLE unless otherwise indicated. NS means not statistically significant at the indicated dose. [c] The preparation of this compound is described in ref 1c. [d] This compound was only examined at a 90 min prechallenge dosing interval, see ref 8.

Scheme 1



[a] Reagents: (a) $4\text{-}HO_2CC_6H_4SO_2Cl$, pyridine/THF; (b) 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide, DMAP and either *o*-toluenesulfonamide (for **10**) or *p*-chlorobenzenesulfonamide (for **9**)/CH_2Cl_2.

8.10 (d, 2), 8.70 (d, 1), 9.98 (s, 1); MS *m/z* 580 (M + 1, 100); TLC $R_f = 0.36$, RP_{18}, 65:35 methanol:water. Anal. C, H, N.

2-[3-[[[4-[*N*-[(4-Chlorophenyl)sulfonyl]carbamoyl]phenyl]sulfonyl]amino]-2-oxo-6-phenyl-1,2-dihydro-1-pyridyl]-*N*-(3,3,3-trifluoro-1-isopropyl-2-oxopropyl)acetamide (9). Acid **8** (390 mg, 0.67 mmol), 1-ethyl-3-[3-(dimethylamino)propyl]carbodiimide (144 mg, 0.75 mmol), 4-chlorobenzenesulfonamide (143 mg, 0.74 mmol), and 4-(di-methylamino)pyridine (91 mg, 0.74 mmol) were dissolved in dichloromethane (3 mL). After 3.5 h, the reaction was diluted with ethyl acetate (25 mL), washed with 0.1 N hydrochloric acid (10 mL), water (10 mL), and saturated brine (10 mL), dried (sodium sulfate), and evaporated to a tan foam. This foam was purified by flash chromatography using a gradient eluent of dichloromethane to 1:25 ethyl acetate:dichloromethane. Appropriate fractions were combined and evaporated and the

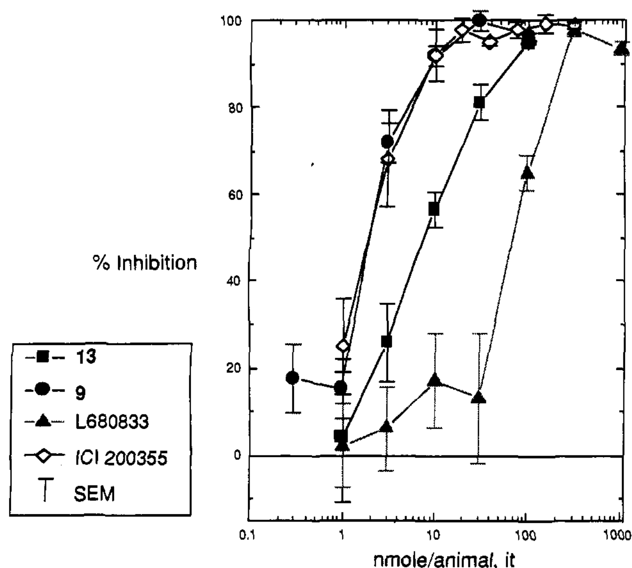


Figure 2. Intratracheal dose–response relationships of selected HLE inhibitors.

resulting solid taken up in dichloromethane, filtered, and evaporated. This solid was triturated with diethyl ether: hexane (1:1) and then dichloromethane to yield a white solid (125 mg, 24%) of analytically pure **9** monohydrate: mp 250–254 °C dec; ^{1}H NMR δ 0.85 (dd, 6), 2.12 (m, 1), 4.38 (ab q, 2), 4.60 (m, 1), 6.13 (d, 1), 7.38 (m, 6), 7.57 (d, 2), 7.91, (m, 4), 8.01 (d, 2), 8.70 (d, 1), 9.87 (s, 1); MS m/z 753 (M + 1). Anal. C, H, N.

2-[3-[[[4-[*N*-[(2-Methylphenyl)sulfonyl]cabarmoyl]phenyl]sulfonyl]amino]-2-oxo-6-phenyl-1,2-dihydro-1-pyridyl]-*N*-(3,3,3-trifluoro-1-isopropyl-2-oxopropyl)acetamide (10). A procedure analogous to that described for the preparation of sulfonimide (**9**) but using o-toluenesulfonamide instead of 4-chlorobenzenesulfonamide yielded a white solid (24% yield) of analytically pure **10** hemihydrate: mp 125–130 °C; ^{1}H NMR δ 0.79 (d, 3, J = 6.8 Hz), 0.85 (d, 3, J = 6.7 Hz), 2.11 (m, 1), 2.61 (s, 3), 4.33 (d, 1, J = 16.3 Hz), 4.44 (d, 1, J = 16.4 Hz), 4.59 (m, 1), 6.12 (d, 1, J = 7.0 Hz), 7.35–8.02 (m, 11), 8.68 (d, 1, J = 7.0 Hz), 10.01 (s, 1); MS m/z 733 (M + 1, 2); TLC R_f = 0.48, RP_{18}, 65:35 MeOH:H_2O, pH 6.7. Anal. C, H, N.

Acknowledgment. The authors sincerely thank Mr. R. A. Mumford for the generous sample of L680833 used in these studies.

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(2) Abbreviations: AHA, acute hemorrhagic assay; DMAP, dimethylaminopyridine; HLE, human leukocyte elastase; IT, intratracheal; TEA, triethylamine; TFMK, trifluoromethyl ketone; WSCDI, 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride.

(3) See detailed discussion in ref 1b and references, therein.

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JM9405405