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PHOSPHOROUS ACID ANALOGS OF L-680,833, A POTENT MONOCYCLIC β-LACTAM INHIBITOR OF HUMAN LEUKOCYTE ELASTASE

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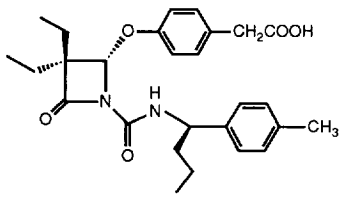
Abstract: Analogs of the monocyclic β-lactam human leukocyte elastase (HLE) inhibitor L-680,833 in which the carboxyl group is replaced by phosphorous acid moieties were synthesized and found to be potent inhibitors of the enzyme (k_{inact}/K_i in the range 217,000-1,326,000 $M^{-1}s^{-1}$). Cellular activity was demonstrated by inhibition of the generation of the *N*-terminal cleavage product Aα-(1-21) from the Aα chain of fibrinogen.

The inhibition of human leukocyte elastase (HLE, EC 3.4.21.37) by β-lactams has been the subject of numerous reports from these laboratories.[1] The target enzyme is a serine protease found in the azurophilic granules of polymorphonuclear leukocytes (PMN's) and has been implicated in pathology characterized by connective-tissue destruction, such as pulmonary emphysema, chronic bronchitis, cystic fibrosis, and rheumatoid arthritis. Potent and selective inhibitors of this enzyme may have considerable therapeutic potential for the treatment of these disorders. An extensive medicinal chemical effort in these laboratories led to the identification of a monocyclic β-lactam, namely 4-{[1-({[1-(R)-(4-methylphenyl)-butyl]amino}carbonyl)-3,3-diethyl-4-oxo-2-(S)-azetidinyl]oxy}-benzeneacetic acid L-680,833 (**1**), as a potent time-dependent inhibitor of HLE (k_{inact}/K_i 622,000 $M^{-1}s^{-1}$) having good oral bioavailability in several species.[2] We now report the synthesis and activity against HLE of several phosphorous acid analogs of **1** in which the carboxy group on the C-4 oxybenzeneacetic moiety is replaced by a phosphonic acid or alkyl- or aryl-phosphinic acid functionality. The incorporation of groups with acidities greater[3] than that of a carboxylate was examined to evaluate the effect of the lower pKa on the physical, biological, and pharmacokinetic properties of this class of compounds.



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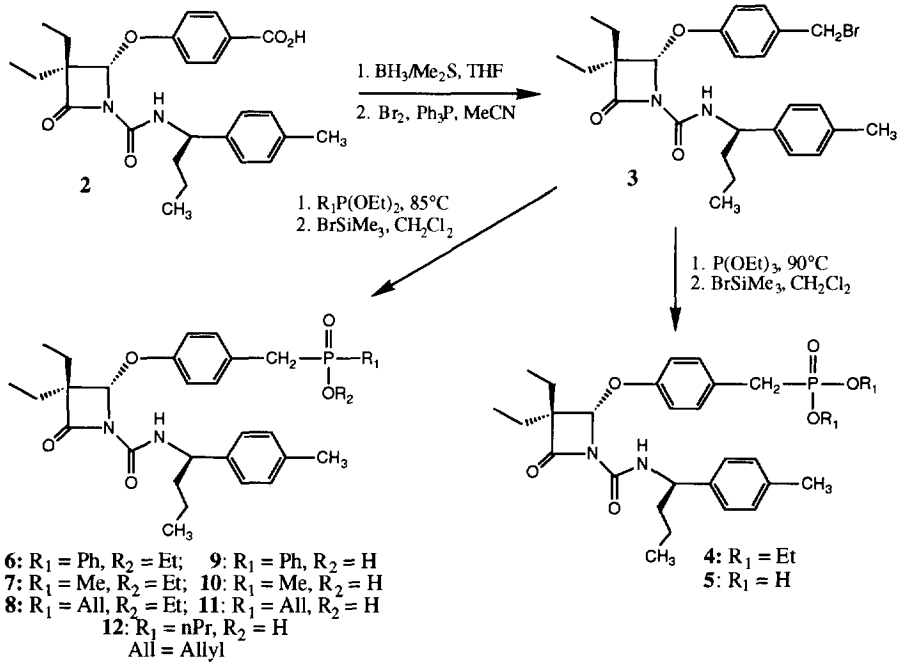
Chemistry

The synthesis of the phosphorous acid analogs is shown in the Scheme below. The starting material was the benzoic acid intermediate **2**.[4] Conversion of **2** into the benzylic bromide **3** was accomplished by reduction of the carboxy group with BH_3/Me_2S and subsequent treatment of the resulting alcohol with bromine/triphenylphosphine in acetonitrile. Arbuzov reaction of **3** with triethylphosphite afforded the intermediate diethyl phosphonate **4** in 81% yield, which upon treatment with bromotrimethylsilane (TMSBr) gave the free phosphonic acid analog **5** (21%). The various phosphinic acid analogs were obtained by Arbuzov reaction of **3** with the appropriate alkyl or aryl diethylphosphonite at 85°C to give the ethyl phosphinates **6-8** (41-93%). Cleavage of the ethyl ester group with TMSBr gave the free phosphinic acid analogs **9-11**. The n-propylphosphinic acid analog **12** was obtained in 64% overall yield by catalytic hydrogenation of the ethyl allylphosphinate **8** followed by treatment with TMSBr.[5]

Scheme



6: R_1 = Ph, R_2 = Et; **9**: R_1 = Ph, R_2 = H
7: R_1 = Me, R_2 = Et; **10**: R_1 = Me, R_2 = H
8: R_1 = All, R_2 = Et; **11**: R_1 = All, R_2 = H
12: R_1 = nPr, R_2 = H
All = Allyl

4: R_1 = Et
5: R_1 = H

Results and Discussion

The phosphorous acid analogs and their corresponding ethyl esters were initially evaluated for their ability to inhibit HLE-catalyzed hydrolysis of the substrate MeO-Suc-Ala-Ala-Pro-Ala-pNA. The compounds were found to exhibit time-dependent inhibition, and the data listed in the Table below are expressed in terms of the bimolecular rate constant k_{inact}/K_i in M^{-1} sec^{-1}. These novel monocyclic β-lactams were found to be potent inhibitors of HLE, with the highest activity expressed by the phenylphosphinic acid **9** (1,167,000 M^{-1} sec^{-1}), the diethylphosphonate **4** (1,164,000 M^{-1} sec^{-1}), and the ethyl allylphosphinate **8** (1,326,000 M^{-1} sec^{-1}) analogs. These structures are approximately two-fold more active than **1**. Alkylphosphinic acids **10-12** exhibited activities comparable to that of **1**, whereas the free phosphonic acid **5** was somewhat less active. In general, the phosphorous acid analogs described herein rank among the most potent HLE inhibitors reported to date. The inhibition of HLE by the phosphinic acids **9-12** was greater than that of the phosphonic acid **5**. In the phosphinic acid series, activity increased with the size of the alkyl/aryl group (Ph>nPr>All>Me). In the two cases where measured, the ethyl esters (**4** and **8**) were more potent than their corresponding free acids (**5** and **11**, respectively).

Table. Inhibition of Human Leukocyte Elastase by Phosphonic and Phosphinic Acid Analogs of L680,833

compd	k_{inact}/K_i, $M^{-1}s^{-1}$ (SD)[a]	Aα-(1-21)[a] % inhibition at dose shown	lung hem[b] % inhibition (SD)
1	622,000 (5000)[2]	IC_{50} = 9 μM	ED_{50} 1.5 mg/kg[c]
4	1,164,000 (55,000)	63 (3 μM)	15 (20)
5	217,000 (300)	61 (0.3 μM)	17 (13)
8	1,326,000 (187,000)	51 (3 μM)	64 (9)
9	1,167,000 (11,000)	34 (3 μM)	27 (19)
10	417,000 (21,000)	55 (3 μM)	-59 (30)
11	740,000 (19,000)	48 (3 μM)	19 (12)
12	906,000 (1,200)	50 (3 μM)	15 (8)

[a] See reference 2 for methodology. [b] % Inhibition in HLE-induced lung hemorrhage when compound was dosed orally at 10 mg/kg 2 hours before challenge by HLE; see references 1(b) and 6 for methodology. [c] Administered orally 5 hours before the enzyme.

Activity at the cellular level *in vitro* was assessed by the compounds' ability to inhibit generation of the specific *N*-terminal cleavage product Aα-(1-21) from the Aα chain of fibrinogen by HLE in whole blood

stimulated with A23187.[2] The data are presented in the Table above. All the analogs exhibited activity greater than or comparable to that of **1** (IC_{50} 9 μM[2]). The phosphonic acid analog **5** was the most potent analog giving 61% inhibition at 0.3 μM.

The compounds' *in vivo* activity following oral dosing was evaluated by measuring their ability to prevent tissue damage elicited in hamster lungs by intratracheal instillation of human HLE. With the exception of compound **8**, which showed 64% inhibition at 10 mg/kg, only marginal activity was observed. The (1-propionyloxy-2-methyl)-propyl ester prodrug of **12** (k_{inact}/K_i 1,048,000 $M^{-1}s^{-1}$) was prepared,[7] but without any enhancement of oral activity in the lung hemorrhage assay.

In conclusion, replacement of the carboxylic group in **1** led to the identification of a new class of potent HLE inhibitors, the 4-(4-phosphinomethyl)- and 4-(4-phosphonomethyl)-phenoxy-3,3-diethyl-2-azetidinones and their corresponding ethyl esters. The intrinsic potency of these compounds was reflected at the cellular level where they inhibited formation of Aα-(1-21) in whole blood stimulated with A23187. However, the compounds were only weakly active when dosed orally in the lung hemorrhage assay.

References and Notes

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3. The acidity of the phosphinic acid is estimated to be at least 100-times greater (pK<2.5) than that of the carboxylate group in **1** (pK~4.8). The first ionization of an organic phosphonic acid (pK <2.0) occurs at a lower pH than comparable phosphinic acids, while the second leading to the dianion occurs in the neutral pH range (pK 6.5-7.0).

4. Doherty, J.B.; Dorn, C.P.; Finke, P.E.; Hagmann, W.K.; MacCoss, M.; Shah, S.K. US Patent 5,229,381 (July 20, 1993).

5. Compounds gave NMR, MS, and analytical data consistent with their structures.

6. Fletcher, D.; Osinga, D.; Hand, K.; Dellea, P.; Ashe, B.; Mumford, R.; Davies, P.; Hagmann, W.; Finke, P.; Doherty, J.; Bonney, R. *Am. Rev. Respir. Dis.* **1990**, *141*, 672.

7. This prodrug ester was used successfully in the development of the phosphinic acid angiotensin-converting enzyme inhibitor fosinopril; see Powell, J.R.; DeForrest, J.M.; Cushman, D.W.; Rubin, B.; Petrillo, E.W. *Fed. Proc., Am. Soc. Exp. Biol.* **1984**, *43*, 733.

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