Pergamon

Bioorganic & Medicinal Chemistry Letters 9 (1999) 1291–1294

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

SYNTHESIS AND IN VITRO AND IN VIVO EVALUATION OF THE 2-(6'METHOXY-3',4'-DIHYDRO-1'-NAPHTYL) -4H-3,1-BENZOXAZIN-4-ONE AS A NEW POTENT SUBSTRATE INHIBITOR OF HUMAN LEUKOCYTE ELASTASE

Antonio Arcadi[a], Cinzia Asti[b], Laura Brandolini[b], Gianfranco Caselli[b], Fabio Marinelli[a], Viviana Ruggieri[b],

[a] Dipartimento di Chimica, Ingegneria Chimica e Materiali, Università degli Studi di L'Aquila, Via Vetoio, Coppito Due, I-67100 L'Aquila, Italy

[b] Dompé Research Center, via Campo di Pile, I-67100 L'Aquila, Italy

Received 14 January 1999; accepted 29 March 1999

Abstract: the title 2-vinyl-4H-3,1-benzoxazin-4-one has been synthesised and tested for inhibitory activity against human leukocyte elastase. The compound has shown activity both *in vitro* towards human sputum elastase and *in vivo* in an hemorrhagic assay.

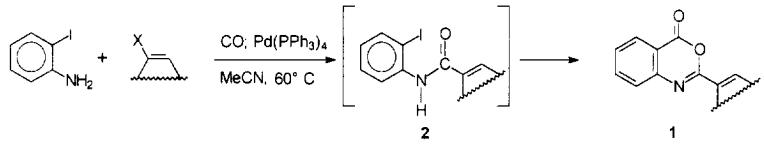
Human leukocyte elastase (HLE) is a potent serine proteinase which has been implicated in the chronic tissue destruction mechanisms of several diseases including emphysema, acute respiratory distress syndrome, atherosclerosis, and rheumatoid arthritis. HLE contained in the azurophil granules of human neutrophils is released into the extracellular environment by inflammatory stimuli. When ineffectively regulated by endogenous inhibitors, the resulting unrestrained proteolytic activity of HLE is capable of proteolytic degradation of structural components of connective tissues. Serine proteinases are attractive targets for medicinal chemists engaged in the design of enzyme inhibitors since the catalytic mechanisms of this class of enzymes have been extensively investigated over the past few decades. Many types of synthetic inhibitors have been reported in the literature and the inhibition of HLE has been reviewed.[1]

3,1-Benzoxazin-4-ones represent a class of heterocyclic acyl-enzyme inhibitors which has been reported to inhibit various serine proteinases, such as chymotrypsin, cathepsin G, C1r serine proteinase of the complement system, HSV-1 proteinase, porcine pancreatic elastase, and HLE.[2] In an extended study, Krantz et al.[3] have demonstrated the design of highly potent HLE inhibitors by introducing favourable substituents in both rings of the benzoxazinone skeleton. Small alkyl groups linked via heteroatoms to C-2 enhanced acylation and limited deacylation rates. Electron withdrawal by the 2-substituents led to an accelerated acylation, but also to a reduced stability as measured by alkaline hydrolysis rates. Moreover, both stability and inhibitory potency could be improved by substitution at the fused benzene ring. Recently the strategy to replace the benzene ring in benzoxazinones by thiophene, based on the consideration that the enhanced electron density at the thiophene carbon atoms might result in an improved intrinsic stability of an isosteric thieno[1,3]oxazin-4-one system, led to the synthesis of thieno[2,3-*d*][1,3]oxazin-4-ones[2] as HLE inhibitors with improved hydrolytic stability.

Corresponding author: e-mail: arcadi@aquila.infn.it ;fax ++39 0862433753

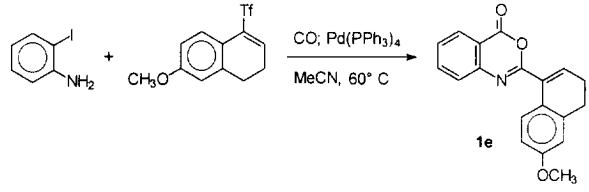
0960-894X/99/$ - see front matter
PII: S0960-894X(99)00168-7

The observation that *o*-acylamidophenyl iodides **2** can undergo an annellation reaction to 2-substituted-3,1-benzoxazin-4-ones when reacted in the presence of K_2CO_3 and a palladium catalyst under atmosphere of carbon monoxide resulted in a new approach[4] (Scheme 1) to 4H-3,1-benzoxazin-4-one derivatives containing an unsaturated unit linked to C_2. The project was aimed at investigating the possible formation of 2-aryl- and 2-vinyl- 4H-3,1-benzoxazin-4-ones through an *in situ* sequence combining the formation of *o*-acylamidophenyl iodides **2** with their carbonylative cyclization. Indeed, employing *o*-iodoaniline as building-block, unsaturated halides or triflates as precursors of the substituent at the 2-position and carbon monoxide, in the presence of K_2CO_3 and catalytic amounts of $Pd(Ph_3)_4$, such a process can be easily fulfilled to afford 2-substituted-4H-3,1-benzoxazin-4-ones **1** in good yield.



Scheme 1

On the basis of the results showing that 7-alkylidenecephalosporins[5] are potent inhibitors of HLE, we planned to investigate the in vitro antielastase activity[6] of 2-vinyl-4H-3,1-benzoxazin-4-ones and focused our efforts to the synthesis of **1e** (Scheme 2).[7] The mechanism of HLE inhibition by 7-alkylidenecephalosporines involves the possibility of double bond isomerization after serine attack/ring cleavage followed by an irreversible modification reaction within the active site. The interaction of benzoxazinones with serine proteases was established to occur *via* C4-attack to form (2-acylamido)benzoyl enzymes.



Scheme 2

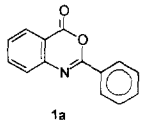
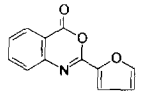
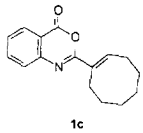
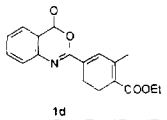
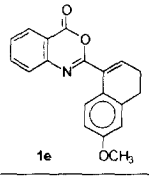
The 2-vinyl-4H-3,1-benzoxazin-4-ones **1c-d** are effective as inhibitor as the 2-aryl-4H-3,1-benzoxazin-4-ones **1a-b**[3] (Table 1). Compound **1e** revealed a high potency as demonstrated by its significant inhibitory effect at concentrations as low as 150 nM (percent inhibition: 75 nM, 5.7%; 150 nM, 15.1%; 300 nM 36.4%; 750 nM 55.5%). Thus, **1e** is more active compared to the previously described compounds.

The evaluation of the activity in vivo[8] of **1e**, given by the oral route to the mouse, demonstrated a significant inhibition of HSE induced haemorrhage as reported in Table 2.

In conclusion, this synthetic approach to 2-vinyl-4H-3,1-benzoxazin-4-ones enables an easy access to elastase inhibitors. Extension of this methodology to the preparation of the 2-(6'methoxy-3',4'-dihydro-1'-naphtyl)-4H-3,1-benzoxazin-4-one as a new potent substrate inhibitor of human leukocyte elastase has also

been demonstrated. Moreover, this is the first benzoxazinone derivative that demonstrated a relevant activity when administered *per os* in a predictive animal model.

Table 1. In Vitro Antielastase Activity of 2-aryl[c] and 2-vinyl-4H-3,1-Benzoxazin-4-ones

Tested compounds	% inhibition ± ES[a] 1.5 μM	% inhibition ± ES[a] 15 μM	% inhibition ± ES[a] 75 μM	IC_{50} (M) ± ES[b]
 1a	16.2 ± 6.7	45.7 ± 3.9	76 ± 3.2	$1.7x10^{-5}$ $\pm 0.20x10^{-5}$
 1b	22.6 ± 2.9	65.0 ± 4.6	88.7 ± 10.3	$5.5x10^{-6}$ $\pm 0.44x10^{-7}$
 1c	23.0 ± 3.2	64.5 ± 0.9	87.0 ± 2.8	$7.0x10^{-6}$ $\pm 0.10x10^{-6}$
 1d	6.0 ± 2.1	12.0 ± 7.1	20.8 ± 4.3	n.c.
 1e	69.9 ± 3.5	92.8 ± 2.0	99.6 ± 2.4	$6.1x10^{-7}$ $\pm 0.04x10^{-7}$

[a] Antielastase activity is calculated by comparing the different slopes of the progress curves. The regression coefficient of the curves (r) is calculated after 3 min from the beginning of the enzymatic reaction. The percentage of inhibition ± ES (n=3 in triplicate) is determined by the formula: $[1-(r_{inhibitor\ present}/\ r_{inhibitor\ absent})] \times 100$. [b] IC_{50} values ± ES (n=3 in triplicate) for each compound were calculated by the Allfit 2.0 programme. [c] The 2-aryl derivatives **1a** and **1b** have been previously reported by Krantz[3] and their pKi were respectively 5.02 and 5.75.

Table 2. Inhibition of HSE-induced lung haemorrhage by 1e administered in vivo by the oral route in the mouse[a]

	μl blood/2ml BALF (mean ± ES)		% inhibition (mean ± ES)
	EXP 1	EXP 2	
Saline	1.6 ± 0.5	1.25 ± 0.3	-
HSE	50.7 ± 10.9	76.9 ± 11.1	-
HSE + **1e** (10 mg/kg p.o.)	35.1 ± 11.2	-	31.5 ± 22.6
HSE + **1e** (30 mg/kg p.o.)	-	35.3 ± 4.7	55.0 ± 6.3

[a] number 5 animals in both experiments.

Acknowledgment: Part of this work is supported by the contract "Programma Nazionale di Ricerca sui Farmaci (seconda Fase), Tema 4" granted by the Italian Ministry of University and Scientific and Technological Research.

References and Notes

1. Bode, W.; Meyer, E.; Powers, J.C. *Biochemistry* **1989**, *28*, 1951; Bernstein, P.R.; Edwards, P.D.; Williams, J.C. *In Progress in Medicinal Chemistry*; Ellis, G.P., Luscombe, D.K. eds; Elsevier Science: New York, 1994; Vol. 31, pp 59; Hlasta, D.J.; Pagani, E.D. In *Annual Reports in Medicinal Chemistry*; Bristol, J.A., Ed.; Academic Press: New York, 1994; Vol. 29, pp 195; Edwards, P.D.; Bernstein, P.R. *Med. Res. Rev.* **1994**, *14*, 127.
2. Gilmore, J.L.; Hays, S. J.; Caprathe, B. W.; Lee, C. *Biorganic & Medicinal Chemistry Letters* **1996**, *6*, 679; Gütschow, M.; Neumann, U. *J. Med. Chem.* **1998**, *41*, 1729 and references therein.
3. Krantz , A.; Spencer, R. W.; Tam, T.F.; Liak, T.J.; Copp, L.J.; Thomas, E. M. *J. Med. Chem.* **1990**, *33*, 464.
4. Cacchi, S.; Fabrizi, G.; Marinelli, F. *Synlett* **1996**, 997.
5. Buynak, J.D.; Rao, S. A.; Ford, G.P.; Carver, C.; Adam, G.; Geng, B.; Bachmann, B.; Shobassy, S.; Lackey, S. *J. Med. Chem.* **1997**, *40*, 3423.
6. Enzyme inhibition assay method: as a source of HLE we used Elastase from Human Sputum (HSE, Elastin Products Inc): 110 µl of HSE, dissolved in 0.05M NaCl, 0.1M Hepes and 0.01% Brij 35, pH 7.5 were incubated with 10 µl of the tested compounds dissolved in DMSO for 30min at 24 °C (n = 3, in triplicate). The reaction was started by the addition of 100 µl of the chromogenic substrate MeOSuc-Ala-Ala-Pro-Val-pNa (Herbert J. M., Frehel D, Rosso M.P., Seban E., Castet C, Pepin O., Maffrand J.P., Le Fur G. *J. Pharm. Exp. Ther.* **1992**, *260*, 809). The final concentrations of the reagents were: HSE 75 nM, inhibitors 1,5 µM, 15 µM and 75 µM, (except for **1e** which was tested also at the concentrations of 75, 150, 300 and 750 nM), substrate 600 µM. The activity of the HSE against the specific substrate was monitored spectrophotometrically at A_{405nm} (Doherty, J.B.; Ashe, B.M.; Argenbright, L.W.; Baker, P.L.; Bonney, R.J.; Chandler, G.O.; Dahlgren, M.E.; Dorn, C.P. Jr.; Finke, P.E.; Firestone, R.A.; Fletcher, D.; Hagman, W.K.; Mumford, R.; O'Grady, L.; Maycock, A.L.; Pisano, J.M.; Shah, S.K.; Thompson, K.R.; Zimmerman, M. *Nature* **1986**, *322*, 192).
7. Preparation of **1e**: To a solution of 6-methoxy-3,4-dihidro-1-naphtyl triflate (0.820 g, 2.66 mmol) in anhydrous MeCN (8 mL) were added 2-iodoaniline (0.700 g, 3.19 mmol), K_2CO_3 (1.83g, 13.3 mmol) and $Pd(PPh_3)_4$ (0.061g, 0.046 mmol). The resulting suspension was purged with CO for few seconds, and stirred at 60 °C under a balloon of CO overnight. Then the mixture was cooled, diluted with EtOAc and washed with water. The organic layer was dried over Na_2SO_4 and evaporated under vacuum. The residue was chromatographed on silica gel, eluting with *n*-hexane-ethyl acetate (90/10 v/v) to afford 0.406 g (50 % yield) of **1e**: m.p. 116-118 °C; IR (KBr): 1780, 1610 cm^{-1}. ^1H NMR ($CDCl_3$): δ 8.24 (dd, J = 7.8 Hz, J = 1.5 Hz, 1H), 7.95 (d, J = 8.5Hz, 1H), 7.82 (dt, J = 8.2 Hz, J = 1.2 Hz, 1H), 7.70-7.65 (m,1H),7.56-7.48 (m, 1H), 7.28-7.23 (m, 1H), 6.85-6.76 (m, 2H), 3.84 (s, 3H), 2.80 (t, J = 8 Hz, 2H), 2.53-2.42 (s, 3H). ^{13}C NMR ($CDCl_3$): δ 159.1, 156.6, 146.7, 138.7, 137.2, 136.5, 130.0, 128.4, 128.3, 127.8, 127.3, 126.9, 123.5,117.2,113.7, 111.1, 55.3, 28.1, 23.7.
8. HSE-induced lung haemorrhage in mice (Balsamo, A.; Asti, C.; Belfiore, M.S.; Brandolini, L.; Cercignani, G.; Gentili, D.; Macchia, M.; Mantovanini, M.; Orlandini, E.; Rossello, A. *Eur J Med Chem* **1997**, *32*, 889): mice, under ketamine-xylazine (50mg/kg and 10 mg/kg i.p.) anaesthesia, were instilled intratracheally with 50 µl of saline (vehicle) or saline containing human sputum elastase (HSE, Elastin Products Company Inc) (3200 U/ml, corresponding to a solution of 16 µg/50 µl), after blunt dissection of the neck soft tissues to expose the trachea. Test compound (**1e**) was orally administered, 3h before enzyme challenge, at doses of 10 and 30 mg/kg as a suspension of DMSO in Methocel 0.5%; 1.5 h after HSE instillation, the animals were sacrificed by ether hyperanaesthesia and the bronchoalveolar lavage fluid (BALF) was obtained by gently washing of the lung cavities with repeated 600 µl saline-lavages, till a total volume of 2ml for each animal. The BALF was diluted with Na_2CO_3 and sonicated to ensure cell disruption. The blood content in BALF was determined spectrophotometrically using the haemoglobin absorbance at 414 nm. The amount of blood in each BALF sample was calculated by a standard curve obtained by progressive dilution of control mouse blood supplemented with Na_2CO_3 2% w/v. The haemorrhage was expressed as µl equivalents of blood in 2 ml of BALF. The protective effect of the compound was calculated as a percentage of inhibition of lung haemorrhage in treated versus control animals. The basal value (vehicle) was subtracted from each treatment group.