Pergamon

Bioorganic & Medicinal Chemistry Letters, Vol. 4, No. 20, pp. 2399-2404, 1994

0960-894X/94 $7.00+0.00

0960-894X(94)00347-5

SYNTHESIS OF NEW THIAZINOINDOLE DERIVATIVES AND THEIR EVALUATION AS INHIBITORS OF HUMAN LEUKOCYTE ELASTASE AND OTHER RELATED SERINE PROTEASES

GIUSEPPE ROMEO, FILIPPO RUSSO & SALVATORE GUCCIONE
Istituto di Chimica Farmaceutica e Tossicologica, Università degli Studi di Catania, Italy
Renee Chabin^, David Kuo^ & W. B. Knight*
^*Department of Enzymology, Merck Research Labs, Rahway, NJ, 07065, USA*
and **Dept. of Biochemistry, Glaxo Res. Inst., RTP, NC, 27713, USA.*

Abstract. A novel thiazinoindole tricyclic ring system was designed as potential inhibitors of serine proteases. The compounds were synthesized by ring closure at 80-90°C in poliphosphoric acid of the appropriate N'-alkyl or aryl substituted indolylthiourea derivatives. Members of this class of compounds inhibited human leukocyte elastase (K_i= 30-40 μM) and α-chymotrypsin.

Human leukocyte elastase (HLE) is a glycosylated, strongly basic serine protease found in the azurophilic granules of human polymorphonuclear leukocytes (PMN). HLE is released from PMN upon inflammatory stimuli and has been implicated in the pathogenesis of a number of disease states such as pulmonary emphysema, rheumatoid arthritis, adult respiratory distress syndrome, glomerulonephritis and cystic fibrosis[1-6]. Increased proteolysis, especially elastinolysis, may occur in the lung parenchyma as a result of an imbalance between HLE and its major endogenous inhibitor, α_1-proteinase inhibitor, because of either an acquired or an inherited deficiency of the inhibitor[1-6].

A rather compelling picture of protease catalyzed hydrolysis of amides and esters has emerged, featuring formation and breakdown of tetrahedral and acyl-enzyme intermediates [7,8]. This mechanistic framework, centered on carbonyl chemistry, makes the acyl-enzyme a natural focal point for rational drug design[7-9]. Therefore, based upon the criteria of bioisostere replacements and molecular hybridization we initiated a research program to develop acylating agents for serine proteases, in which different heterocycles provide "support" for reactive moieties, such as oxazinones, thiazinones or oxadiazinones[10-13]. The latter cyclic systems were chosen because of their analogy to the oxazinones and isatoic anhydride which were reported to be HLE inhibitors[9,14]. In addition, reports from other laboratories suggested the potential of indole "supported" oxazinone derivatives as serine proteases inhibitors[15] and the activity of 2-amino substituted benzoxazinones as inhibitors and alternate substrates of elastase.[9] Therefore, the compounds developed in this work could be used as inhibitors of other pathogenic serine proteases[1-7,14-19]. Given the nonpolar character of these compounds they might also inhibit serine proteases with specificity for hydrophobic residues proximal to the scissile bond, such as α-chymotrypsin. While this protease is not a therapeutic target, it is a prototype for this protease family, which includes human Cathepsin-G[18,19]. The physiological role of the latter is unknown,

but it is found in the same type of granule as HLE and therefore, may be involved in some of the same disease states as HLE[7]. The promising results obtained with pyrazolothiazine-4-one and thione derivatives[10-13] together with the reported binding of the indole nucleus in the hydrophobic specificity pocket of α-chymotrypsin[8] induced us to synthesize the title compounds (**A** in Scheme 1). Considering the different character of the indole nucleus when compared with the pyrazole nucleus[10-13], these compounds can be used to probe the role(s) of the "heterocycle support moiety". They also allow us to ascertain the structural features of the inhibitors that result in optimum inhibitory activity versus the target (HLE) and related enzymes[7] .

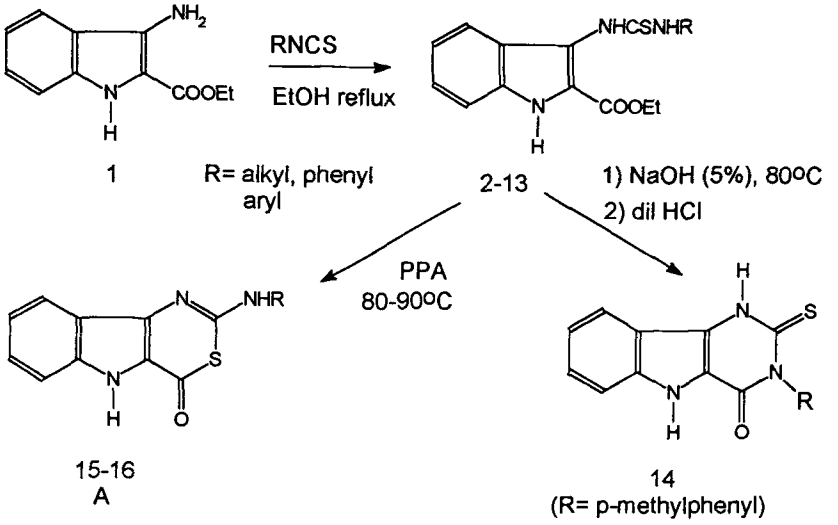
Methods. All chemicals were purchased from Aldrich, Sigma and Fluka Chemical Companies and were used without further purification. Peptide p-nitroanilide substrates were purchased from Chemical Dynamics Corp. and Bachem Chem. Co. HLE (Elastin Products Co.), human α-chymotrypsin (Worthington) and Cathepsin-G (Athens Research and Technology) were assayed according to Knight et al.[20, 21]. Synthetic reactions were monitored by TLC on silica gel plates (60 F254 Merck) with ethyl acetate:cyclohexane (1:1) as the eluant using UV irradiation (254-365 nm) for detection. The purity of the products was assessed similarly. Melting points were determined in open capillaries on a Gallenkamp apparatus and are uncorrected. Elemental combustion analyses were performed on a Carlo Erba Mod. EA 1108 Analyzer instrument by Dr. S. Di Marco of the Microanalysis Laboratory of Istituto di Chimica Farmaceutica e Tossicologica, University of Catania and were within ± 0.4 of the theoretical values for C,H and N. Infrared spectra were obtained with a Perkin-Elmer 281 infrared spectrometer as KBr discs. ^{1}H NMR spectra were recorded on a Bruker WP 80 spectrometer (DMSO-d6) operating at a frequency of 80 MHz. All ^{1}H chemical shifts are reported as ppm downfield from Me_4Si as the internal standard in DMSO-d_6. The structure of compounds **5-16** was confirmed further by examining the products resulting from treatment of the thiourea system present in **2-13** to alkaline conditions[22]. For example, treatment of N'-(p)tolyl-N-3-[(2-ethoxycarbonylindolyl)] thiourea (**6**) with base yielded 3-(p)-tolyl-substituted-3,5-dihydro-2-mercapto-4H-pyrimido[5,4-b]indol-4-one (**14**).

The synthesis of 2-amino substituted [1,3]thiazino[5,4-b]indol-4-ones was accomplished by ring closure of the appropriate N'-alkyl or aryl-N-3[(2-ethoxycarbonylindolyl)]thioureas[23] (**2-13**) in polyphosphoric acid (PPA) at 80-90 °C (see Scheme 1). The thioureas were prepared using a published procedure by refluxing 3-amino-1H-indol-2-carboxylic acid ethyl ester[24] **1** with alkyl, or aryl isothiocyanates in ethanol.

Synthesis of N'-substituted-N-3[(2-ethoxycarbonylindolyl)]thioureas (**2-13,** Table 1)**.** The appropriate alkyl, or aryl isothiocyanate (0.01 mol) was added to a solution of 3-amino-1H-indol-2-carboxylic acid ethyl ester **1**[24] (1.48g , 0.01 mol) in 20-30 ml of ethanol and the resulting mixture was heated at reflux until the starting material was consumed (typically 30-40 min). The crude product separated from the reaction mixture and was collected by filtration, washed with a small amount of ethanol and recrystallized from solvent.

Synthesis of 3-(p)tolyl-substituted-3,5-dihydro-2-mercapto-4H-pyrimido[5 ,4-b]indol-4-one **14.** Compound **14** was prepared by cyclization of the thiourea derivative **6.**[24] A solution of **6** (1.5 g, 0.0016 mol) in 15 ml of aqueous NaOH (5%) was heated at 80 °C in a steam bath for 1h. After cooling the solution was adjusted to pH 4 with dilute HCl and the resulting solid was collected by filtration and washed (3x) with cold water.

Recrystallization from aqueous 1% acetic acid gave 0.35 g (yield 81%) of compound **14** ($C_{17}H_{13}N_3OS$; Anal. Found: C,66.29;H,4.33; N, 13.62, calc. C,66.45;H, 4.26;N, 13.67 %; mp >300°C; IR 3200,3120 (NH),1660 (C=O)).



Scheme 1. Synthetic Route to [1,3]Thiazino[5,4-b]indol-4-ones

Table 1. *Physical properties of N'-substituted N-3[(2-ethoxycarbonylindolyl)]thioureas*

Cpd	R	m p,°C	recryst solvent	% yield	IR (cm^{-1}, KBr) NH	C=O	formula	Analysis (ratio obs/calc C:H:N)
2	CH_3	>300	DMF/H_2O	64	3400,3315	1665	$C_{13}H_{15}N_3O_2S$	0.9956:0.993:0.9881
3	C_2H_5	191-2	EtOH	71	3425,3315	1670	$C_{14}H_{17}N_3O_2S$	1.001:0.949:1.013
4	n-C_3H_7	>300	EtOH/H_2O	67	3395,3295	1665	$C_{15}H_{19}N_3O_2S$	0.9986:0.971:0.9898
5	C_6H_5[b]	184-5	AcOH	83	3320,3270	1655	$C_{18}H_{17}N_3O_2S$	0.9997:1.04:0.9895
6	$C_6H_4CH_3$(p)	188-9	EtOH	85	3325	1660	$C_{19}H_{19}N_3O_2S$	1.002:0.983:1.013
7	C_6H_4Cl(p)	179-80	EtOH	85	3320,3290	1655	$C_{18}H_{16}ClN_3O_2S$	0.9984:0.986:1.003
8	C_6H_4Br(p)	181-2	EtOH/H_2O	91	3330,3290	1655	$C_{18}H_{16}BrN_3O_2S$	1.001:1.00:0.989
9	C_6H_4F(p)	177-8	EtOH	88	3360,3320	1655	$C_{18}H_{16}FN_3O_2S$	1.006:1.03:1.002
10	C_6H_4F(o)	184-5	EtOH	88	3370,3320	1650	$C_{18}H_{16}FN_3O_2S$	1.007:0.989:1.007
11	C_6H_4F(m)	166-7	EtOH/H_2O	77	3330,3280	1650	$C_{18}H_{16}FN_3O_2S$	1.002:1.007:1.006
12	$C_6H_4CF_3$(m)	170-1	EtOH/H_2O	89	3390,3280	1690	$C_{19}H_{16}F_3N_3O_2S$	1.000:0.957:0.9855
13	$C_6H_4NO_2$(p)	184-5	Toluene	89	3320	1660	$C_{18}H_{16}N_4O_4S$	1.001:0.962:1.008

a. The elemental analysis is expressed as the ratio of the observed divided by the calculated. *b.* This compound was previously reported[23].

Table 2. *Physical properties of 2-amino substituted [1,3]Thiazino[5,4-b]indol-4-ones*

Cpd	R	m p,°C	recryst solvent	yield %	IR (cm^{-1}, KBr) NH	C=O	formula	Analysis (ratio obs/calc C:H:N)
15	CH_3	247-8	EtOH/H_2O	31	3240	1650	$C_{11}H_9N_3OS$	1.004:0.982:0.9895
16	C_2H_5	182-3	EtOH/H_2O	48	3385,3280	1650	$C_{12}H_{11}N_3OS$	0.9989:1.04:0.982
17	n-C_3H_7	157-8	EtOH/H_2O	47	3400,3290	1655	$C_{13}H_{13}N_3OS$	1.001:1.020.9870
18	C_6H_5	201-2	EtOH/H_2O	58	3440,3300	1665	$C_{16}H_{11}N_3O_3S$	1.003:0.968:1.015
19	$C_6H_4CH_3$(p)	227-8	Toluene	46	3280	1645	$C_{17}H_{13}N_3OS$	1.006:1.021:0.9890
20	C_6H_4Cl(p)	243-4	Toluene	80	3400,3330	1675	$C_{16}H_{10}ClN_3OS$	1.001:1.05:0.9906
21	C_6H_4Br(p)	244-5	EtOH/H_2O	88	3360,3290	1675	$C_{16}H_{10}BrN_3OS$	1.002:0.989:1.007
22	C_6H_4F(p)	226-7	Benzene	94	3420,3280	1645	$C_{16}H_{10}FN_3OS$ x(C_6H_6)$_1$	0.9966:0.963:1.007
23	C_6H_4F(o)	214-5	EtOH/H_20	50	3450,3275	1660	$C_{16}H_{10}FN_3OS$	0.9985:0.969:1.008
24	C_6H_4F(m)	230-1	EtOH/H_2O	86	3430,3270	1645	$C_{16}H_{10}FN_3OS$	1.002:0.997:1.016
25	$C_6H_4CF_3$(m)	179-80	EtOH/H_2O	52	3300	1640	$C_{17}H_{10}F_3N_3OS$ x(H_2O)$_1$	0.9998:1.13:1.018
26	$C_6H_4NO_2$(p)	>300	DMF/H_20	68	3350,3290	1660	$C_{16}H_{10}N_4O_3S$	1.003:1.02:0.9994

Table 3. *Inhibition of Human Leukocyte Elastase , Cathepsin G and α-Chymotripsin from 2-amino substituted Thiazinoindole-4- ones* **15-26.**

Compd	R	Inhibitor conc., μM	K_i, μM[a] HLE	% inhibition[b] Cat G	α-Chymotripsin
15	CH_3	-	NI[c]		
16	C_2H_5	-	NI[c]		
17	n-C_3H_7	-	NI[c]		
18	C_6H_5	64	43±3	11.1±6.3	13.3±0.7
19	$C_6H_4CH_3$(p)	16	≥78±5		
20	C_6H_4Cl(p)	23	29±4		
21	C_6H_4Br(p)	13	≥30±6		
22	C_6H_4F(p)	51	≥104±13		
23	C_6H_4F(o)	32	≥88±5		
24	C_6H_4F(m)	16	≥72±2		
25	$C_6H_4CF_3$(m)	13	≥30±3	5.3±3.4	34.2±1.2
26	$C_6H_4NO_2$(p)	15	≥45±6		

a. The K_i was estimated assuming competitive inhibition[21]. NI represents not inhibitory at the limit of solubility. When the concentration of inhibitor (limited by the solubility) was less than the calculated dissociation constant, then the inhibition observed was less than 50 %. Under these conditions the calculated value is greater than or equal to the K_i .

b. Cat-G and α-chymotrypsin were assayed with 20 μg/ml inhibitor (approximately 60 μM).

Synthesis of 2-amino substituted [1,3]Thiazine[5,4-b]indol-4-one **(15-26, Table 2)**

A slurry of the appropriate N'-substituted-N-3[(2-ethoxycarbonylindolyl)]thioureas[23] **2-13** (6 mmol) in 45-50 g of poliphosphoric acid (PPA) was heated at 80-90 °C for 3 h. After cooling the reaction mixture was poured into ice water, then yellow suspension was neutralized by treating with aqueous sodium bicarbonate (10% w/v) and extracted (3-4x) with 60 ml portions of diethyl ether. The pooled organics were dried over sodium sulphate and rotary evaporated *in vacuo* to an amorphous solid. Recrystallization from the appropriate solvent yielded the title compounds. All the spectral data are in agreement with the assigned structures or consistent with the literature data[23]. For example the ^{1}H-NMR spectra of **21** displayed resonances at 11.97(br s, 1H, NH), 10.30(br s, 1H, NH) and 7.98-7.26(m, 8H, aromatic protons) while that of **22** yielded resonances at 11.93(br s, 1H, NH), 10.23(br s, 1H, NH) and 7.96-7.36(m, 8H, aromatic protons).

CONCLUSIONS

The 2-amino substituted thiazinoindoles yielded apparent reversible inhibition of HLE. The inhibition did not appear to be time dependent. Therefore, if these compounds form acyl-enzymes it is either a reversible process or the intermediate is rapidly hydrolyzed. The compounds would be considered alternate substrates in the latter case. The observations that the alkyl substituted analogs were less active than the aryl substituted compounds suggests that the activity may favor a hydrophobic interaction with some site of the enzyme, different from the S_1[25] binding pocket. Of course, another interpretation of the lack activity with the alkyl derivatives, is that the inherent carbonyl reactivity is greater with the aryl analogs. Unfortunately, due to the limitations of solubility it is difficult to draw any firm conclusions from the SAR of substitutions on the phenyl ring. While none of the test compounds displayed significant inhibition (>20%) versus Cathepsin-G, **25** displayed significant activity versus α-chymotrypsin **(Table 3)**. These data demonstrate that this compound can distinguish between two enymes of very similar substrate specificity[26,27]. While the compounds reported in this work are of limited potency, they represent a new structural class of serine protease inhibitors from which more potent and specific inhibitors may be derived. Futhermore, members of this class could be developed as inhibitors of other serine proteases with different specifities [10]. Future studies will be aimed at defining the mechanism of action and designing similar compounds that produce stable acyl-enzymes.

Acknowledgements. This work was supported by a grant (40%) from Italian Ministero della Università e della Ricerca Scientifica e Tecnologica (MURST).

REFERENCES

1. Janoff, A. *Am. Rev. Respir. Dis.* **1985**, *132*, 417.
2. Ekerot, L.; Ohlsson, K. *Adv. Exp. Med. Biol.*,**1984**,*167*, 335.
3. Merritt, T.A.; Cochrane, C.G.; Holcomb, K.; Bohl, B.; Hallman, M.; Strayer, D.; Edwards, D.; Gluck, L., *J. Clin. Invest.* **1983**, *72*, 656.
4. Sanders, E.; Davies, M.; Coles, A. *Renal. Physiol.* **1980**, *3*, 355.
5. Jackson, A.H.; Hill, S.L.; Afford, S.C.; Stockley, R.A. *J. Respir. Dis.* **1984**, *65*, 114.
6. Suter, S.; Schaad, L.; Roux, L.; Nydegger, V.E.; Waldvogel, F. A. *J. Infect. Dis.* **1984**, *149*, 523.
7. Polgar L. *Mechanisms of Protease Action*; CRC Press: Boca Raton, 1989; pp. 87-113.

8. Henderson, R.H. *J. Mol. Biol.* **1970**, *54*, 341.
9. Krantz, A.; Spencer, R. W.; Tam, T.F.; Liak, T. J.; Copp, L. J; Thomas, E. M.; Rafferty, S. P. *J. Med. Chem.* **1990**, *33*, 464 and references therein.
10. Guccione, S. *Tesi di Dottorato di Ricerca (Italian Ph.D.)* **1992**.
11. Guccione, S.; Russo, F.; Romeo, G.; Andrisano, V.; Recanatini, M.; Chabin, R.; Kuo, D.; Knight, W. B. *XII International Symposium on Medicinal Chemistry* **1992**, 360; Guccione, S.; Knight, W.B. *Annu. Drug Data Report* **1993**, *15*, 320, and references therein.
12. Guccione, S.; Russo F.; Romeo, G.; Vicentini, C.B.; Guarneri, M.; Giori, P.; Chabin, R.; Kuo, D.; Knight, W. B. *XII International Symposium on Medicinal Chemistry* **1992**, 252.
13. Vicentini, C. B.; Veronese, A. C.; Manfrini, M.; Guccione, S.; Guarneri, M.; Giori, P. *J. Heterocyclic Chem.* **1993**, *36*, 10.
14. Moorman, A. R.; Abeles, R. H. *J. Am. Chem. Soc.* **1982**, *104*, 6785.
15. Gallaschun, R. J.; Schnur, R. C. *J. Hetereocyclic Chem.* **1992**, *29*, 369.
16. Gutschow, M.; Neumann, U.; Leistner, S. *Pharmazie* **1993**, *48(H.5)*, 394
17. *Pulmonary Emphysema and Proteolysis*; Mittman, C.; Taylor, J. C. Eds., Academic Press: NY, 1988;Vol.2.
18. *Biochemistry, Pathology and Genetics of Pulmonary Emphysema*; Bignon, J.; Scarpa, G. L., Eds.; Pergamon Press: NY, 1981.
19. Stockley, R. A.; Morrison, H., M.; Tetley, T. *Hoppe-Seyler' s Z. Physiol. Chem.* **1984**, *365*, 587.
20. Knight, W. B.; Green, B. G.; Chabin, R.; Gale, P.; Maycock, A. L.; Weston, H.; Kuo, D.; Westler, W. M.; Dorn, C. P.; Finke, P. E.; Hagmann, W. K.; Hale, J. J.; Liesch, J.; MacCoss, M.; Navia, M.; Shah, S. K.; Underwood, D.; Doherty, J. B. *Biochemistry* **1992**, *31*, 8160.
21. The apparent inhibition constants were calculated from concentrations of inhibitor yielding 20-70 % inhibition assuming competitive inhibition according to $v_i/v_o = 1/(1+[I]/K_i)$. The concentration of substrate (0.2 mM) used in the assay was >10 fold below the K_m. If the inhibition is not competitive then the values are actually IC_{50}'s. If $\approx$ 50 % inhibition was not observed then the values are likely a lower estimate of the potency.
22. Errede, L. A.; Oien, H. T.; Yarian, D. R. *J. Org. Chem.* **1977**, *42*, 12.
23. Russo, F.; Guccione, S.; Santagati N. A.; Santagati, A.; Caruso, A.; Leone, M. G.; Felice, A.; Attaguile, G.; Roxas, M. A. *Farmaco* **1988**, *43*, 409.
24. Unangst, P. C. *J. Heteterocyclic Chem.* **1983**, *20*, 495.
25. Stein, R. L.; Strimpler, A. M.; Hori, H.: Powers, J. C. *Biochemistry* **1987**, *26*, 1301.
26. Starkey, P. M.; Barrett, A. J. *Biochem J.* **1976**, *155*, 273.
27. Boudier, C.; Jung, M. L.; Stambolieva, N.; Bieth, J. G. *Arch. Biochem. Biophys.* **1981**, *210*, 790.

(*Received in USA* 5 *May* 1994; *accepted* 8 *September* 1994)