Pergamon

Bioorganic & Medicinal Chemistry Letters, Vol. 7, No. 8, pp. 1003-1006, 1997

0960-894X/97 $17.00 + 0.00

PII: S0960-894X(97)00145-5

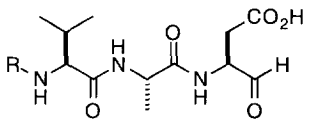
3-CHLORO-4-CARBOXAMIDO-6-ARYLPYRIDAZINES AS A NON-PEPTIDE CLASS OF INTERLEUKIN-1β CONVERTING ENZYME INHIBITOR

Roland E. Dolle,*,[†] Denton Hoyer,[‡] James M. Rinker,[η] Tina Morgan Ross,[ψ] Stanley J. Schmidt,[γ]
Carla T. Helaszek, and Mark A. Ator[φ]

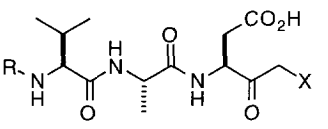
Sanofi Winthrop Inc., 9 Great Valley Parkway, P. O. Box 3206, Malvern, PA 19426

Abstract: The 3-chloro-4-carboxamido-6-arylpyridazines are a novel class of interleukin-1β converting enzyme (ICE) inhibitor . These agents are irreversible inhibitors with pyridazine **23** possessing a $k_{obs}/[I] = 355\ M^{-1}s^{-1}$. A structure-activity relationship for this non-peptide class of compounds and the putative mechanism for irreversible inactivation are described. © 1997 Elsevier Science Ltd.

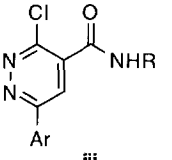
Interleukin-1β is a cytokine that elicits a potent inflammatory response in vivo.[1] The cytokine is produced in monocytic cells from a biologically inactive precursor by the specific action of interleukin-1β converting enzyme (ICE).[2] Inhibitors of ICE may be regarded as a potential new class of antiinflammatory agent.[3] Several laboratories have described the design, synthesis, and evaluation of inhibitors of this enzyme.[3] To date, all of these inhibitors are peptide-based, with their design driven in part by the enzyme's unique P_1 specificity preference for aspartic acid. The reversible aspartic acid aldehyde inhibitors **i** and the irreversible aspartic acid α-substituted methyl ketone inhibitors **ii** are examples of such peptide classes.[3] In this Letter, we describe the 3-chloro-4-carboxamido-6-arylpyridazines **iii** as a novel, non-peptide class of ICE inhibitor.



i



ii



iii

During the course of screening our proprietary compound collection, we discovered **1** to display inhibitory activity against ICE with an $IC_{50} = 3\ \mu M$. In an attempt to understand the functionality contributing to the activity of **1** with the aim of increasing the potency of this novel class of agent, a number of analogs were synthesized[4] (Table 1). The pyridyl analog **2** is devoid of activity, establishing the importance of the pyridazine ring in **1** for enzyme affinity. Likewise, the significance of the chlorine atom in **1** was confirmed when this group was replaced with either -H, -OMe or -SMe. Each of the analogs **3-5** (R^1 substituent, Table 1) are inactive against the enzyme.

These data, in conjunction with the known propensity of the 3-halopyridazines to suffer nucleophilic substitution,[4b,5] led to speculation that **1** may be an irreversible inhibitor of ICE. Indeed, pyridazine **1** displays

time-dependent inactivation with a $k_{obs}/[I]$ = 70 $M^{-1}s^{-1}$ (Table 1). The mechanism of ICE inactivation is hypothesized to involve direct alkylation of the enzyme's active site cysteine (thiol) by displacement of halogen (Figure 1).[6]

Modification of the 4-carboxamide (R^2 substituent, Table 1) to yield the ester **8** or the cyano analog **9** resulted in inhibitors with attenuated activity. Secondary amides, possessing either small hydrophobic (**11-13**) or hydrophilic groups (**14**) are as active as the lead compound **1**.[7a] In contrast, tertiary amides (e.g., **10**) are inactive.[7b] A severe non-bonded interaction would likely exist between the C(5)-proton and the N,N-dialkyl substituent on the C(4)-amide, disfavoring the formation of enolate **iv** (Figure 1). This may explain the loss of binding affinity observed for the tertiary amides as well as for the C(5)-methyl analog **6**.

Replacement of the C(6)-pyridyl ring (R^4 substituent, Table 1) with the non-aromatic chlorine atom leads to inactivity (analog **7**). However, the heterocycle may be replaced with other aryl rings, notably phenyl (**15**) and substituted phenyl (**16-25**). A series of C(6)-phenyl analogs possessing a range of electron-withdrawing and electron-donating groups (σ = -0.27 (4-OMe-Ph; **16**) to 1.00 (3-Cl-4-CN-Ph; **24**) were synthesized and evaluated against ICE. An excellent correlation exists between the ln IC_{50} values and the Hammett σ constant,[8] with the highest activity (lowest IC_{50}) residing with the pyridazines substituted with the more electron deficient phenyl rings (Figure 2). This trend in activity may be reconciled in light of the proposed mechanism for inactivation (Figure 1). Presumably, through an inductive effect of the electron-withdrawing C(6)-aryl substituent, the electrophilicity of the C(3)-chloro group is enhanced leading to greater reactivity toward the active site cysteine. On this premise, the highly electron deficient analog **25** was prepared which contains the 3,5-difluoro-4-methylsulfonyl substituted phenyl ring (calcd. σ = 1.42) at position C(6). This analog possesses a $k_{obs}/[I]$ = 355 $M^{-1}s^{-1}$, some 5-fold greater than pyridazine **1**. Finally, agents **1** and **11-25** are believed to be selective inhibitors of ICE as no inhibition was observed against cathepsin B and L, calpain I, or a panel of serine and metalloproteases at a screening concentration of 10 μM.

In summary, a novel non-peptidic class of ICE inhibitor has been described. These 3-chloro-4-carboxamido-6-arylpyridazines are time-dependent inhibitors, with the mechanism of inactivation believed to be a consequence of active site thiol alkylation. Potency enhancement in the series is achieved by introducing electron deficient aryls into the C(6) position of the pyridazine ring. This is exemplified by the 5-fold improvement in the second order rate constant measured for analog **25**. Finally, this class of inhibitor lacks an aspartic acid residue, which is an essential recognition element found in the peptide-based inhibitors.[3]

Figure 1. Putative Mechanism of ICE inactivation by the 3-Chloropyridazines.

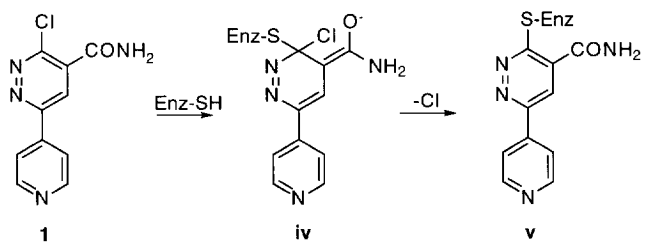
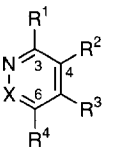


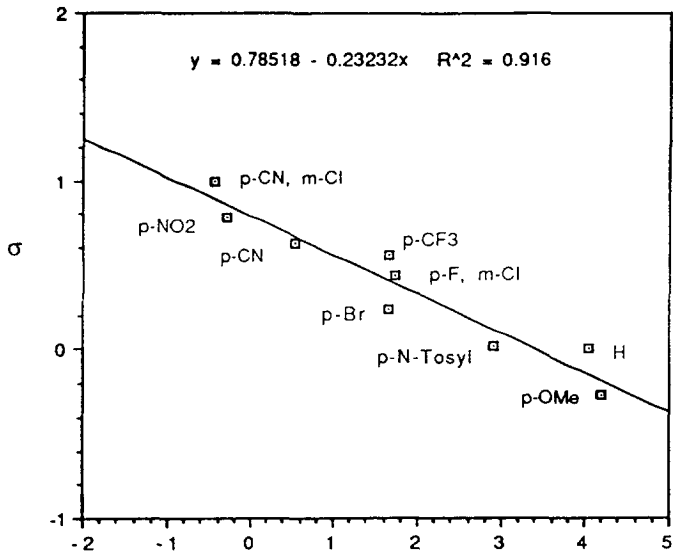
Table 1. Substituted Pyridazines and Kinetic Data Against ICE.

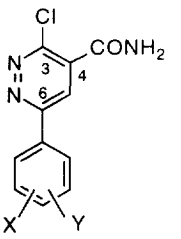


Comp No.	X	R^1	R^2	R^3	R^4	IC_{50} (μM)[a]	$k_{obs}/[I]$ ($M^{-1}s^{-1}$)[a,b]
1	N	Cl	$CONH_2$	H	4-pyridyl	3	70
2	CH	Cl	$CONH_2$	H	4-pyridyl	>250	nd[c]
3	N	H	$CONH_2$	H	4-pyridyl	>250	nd
4	N	OMe	$CONH_2$	H	4-pyridyl	>250	nd
5	N	SMe	$CONH_2$	H	4-pyridyl	>250	nd
6	N	Cl	$CONH_2$	Me	4-Cl-Ph	>250	nd
7	N	Cl	CONHMe	H	Cl	>250	nd
8	N	Cl	CO_2Et	H	4-pyridyl	13	10
9	N	Cl	CN	H	4-pyridyl	110	nd
10	N	Cl	$CONMe_2$	H	4-pyridyl	>250	nd
11	N	Cl	CONHMe	H	4-pyridyl	10	25
12	N	Cl	$CONHCH_2Ph$	H	4-pyridyl	4	50
13	N	Cl	$CONHCH_2$(2,4-Cl_2-Ph)	H	4-pyridyl	2	140
14	N	Cl	$CONHCH_2CO_2Et$	H	4-pyridyl	2	nd
15	N	Cl	$CONH_2$	H	Ph	57	<5
16	N	Cl	$CONH_2$	H	4-OMe-Ph	66	<5
17	N	Cl	$CONH_2$	H	4-($NHSO_2Ph$)-Ph	18	nd
18	N	Cl	$CONH_2$	H	4-CF_3-Ph	5	40
19	N	Cl	$CONH_2$	H	3-Cl-4-F-Ph	5.5	45
20	N	Cl	$CONH_2$	H	4-Cl-Ph	5	nd
21	N	Cl	$CONH_2$	H	4-Br-Ph	5	nd
22	N	Cl	$CONH_2$	H	4-CN-Ph	1.5	nd
23	N	Cl	$CONH_2$	H	4-NO_2-Ph	0.7	nd
24	N	Cl	$CONH_2$	H	3-Cl-4-CN-Ph	0.5	225
25	N	Cl	$CONH_2$	H	3,5-F_2-4-(SO_2Me)-Ph	0.3	355

[a]For the kinetic determinations see ref 9. [b]Standard error: <10%. [c]nd = not determined

Figure 2. C(6)-Substituent Hammett Correlation.





REFERENCES AND NOTES

Present addresses: †Pharmacopeia, Inc., 101 College Road East, Princeton, NJ 08540. ‡Ciba-Geigy Corp., RES 310, 556 Morris Ave., Summit, NJ 07901. ⁿ3-Dimensional Pharmaceuticals, Inc., Eagleview Corporate Center, 665 Stockton Drive, Suite 104, Exton, PA 19341. ᵠJanssen Research Foundation, Spring House, PA 19477. ᵞNycomed R&D Inc., 466 Devon Park Dr., P.O. Box 6630, Wayne, PA 19087. ᵩCephalon, Inc., 145 Bradywine Parkway, West Chester, PA 19380

1. (a) Dinarello, C. A. *Blood* **1991**, *77*, 1627. (b) Dinarello, C. A.; Wolff, S.M. *N. Engl. J. Med.* **1993**, *328*, 106. (c) Dinarello, C. A. *FASEB J.* **1994**, *8*, 1314.
2. (a) Black, R. A.; Kronheim, S. R.; Sleath, P. R. *FEBS Lett.* **1989**, *247*, 386. (b) Kostura, M. J.; Tocci, M. J.; Limjuco, G.; Chin, J.; Cameron, P.; Hillman, A. G.; Chartrain, N. A.; Schmidt, J. A. *Proc. Nat. Acad. Sci.* **1989**, *86*, 5227.
3. For a leading review on ICE see: Ator, M. A.; Dolle, R. E. *Curr. Pharmaceutical Design* **1995**, *1*, 191.
4. For the synthesis of these agents, see the detailed experimentals presented in: (a) Dolle, R. E. Hoyer, D.; Ross, T. M.; Rinker, J. R.; Schmidt, S. L.; Ator, M. A. Eur. Pat. Appl.: 628550 (1995). (b) Lesher, G. Y.; Dickinson, W. B. US Patent: 4,590,194 (1986). (c) Albright, J. D.; Envoy, F. J.; Moran, D. B. *J. Heterocyclic Chem.* **1978**, *15*, 881.
5. (a) Yanai, M.; Takeda, S.; Mitsuoka, T. *Chem. Pharm. Bull. Japan* **1977**, *25*, 1708. (b) Wermurth, C.-G.; et. al. *J. Med. Chem.* **1989**, *32*, 528.
6. Protection against inactivation by increasing [S] is observed, consistent with the active-site directed reaction.
7. The L- and D-phenylalanine amide analogs, R^2 = CONH-(L)-Phe-NH_2 and R^2 = CONH-(D)-Phe-NH_2 wherein R^4 = 4-Cl-Ph are inactive. (b) The piperidine amide R^2 = CO-N-piperidine; R^4 = 4-Cl-Ph is inactive.
8. Hansch, C.; Leo, A. *Exploring QSAR*; American Chemical Society, Washington, DC, 1995.
9. Assay for the IC_{50} determinations see ref 4a. Assay for determining the second order rate constant: Dolle, R. E.; Prouty, C. P.; Prasad, C. V. C.; Cook, E.; Saha, A.; Ross, T. M.; Salvino, J. M.; Helaszek, C. T.; Ator, M. A. *J. Med. Chem.* **1996**, *39*, 2438.

(*Received in USA* 6 *February* 1997; *accepted* 12 *March* 1997)