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JANUS COMPOUNDS: DUAL INHIBITORS OF PROTEINASES

Michael R. Angelastro, Philippe Bey, Shujaath Mehdi,
Michael J. Janusz and Norton P. Peet*

Marion Merrell Dow Research Institute
2110 East Galbraith Road
Cincinnati, Ohio 45215

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Abstract: The concept of dual proteinase inhibition has been demonstrated with the preparation of unsymmetrical bis-terephthalamides.

With certain disease states the inhibition of more than one enzyme appears to be advantageous or necessary for effective therapy. For example, both human neutrophil elastase (HNE) and cathepsin G have been implicated in tissue destruction associated with several inflammatory diseases.[1] Neutrophil recruitment to inflammatory sites can release proteinases which mediate tissue damage by proteolysis, and it was recently shown that the degradation of cartilage matrix proteoglycan, a model of connective tissue degradation, could be blocked only by inhibiting both elastase and cathepsin G.[2]

In addition to inhibiting two enzymes using two specific inhibitors,[2,3] we considered two approaches for dual enzyme inhibition with one discreet inhibitor. One approach involved the design of an inhibitor containing a single recognition sequence which would bind to both enzymes. Problems with this approach included the availability of recognition sequences common to both enzymes, such that i) the affinity for either enzyme was not compromised, and that ii) the ratio of the two affinities was correctly adjusted to afford an acceptable concentration of the single compound which would inhibit the physiological proportions of the two enzymes.

A second approach, which we chose to pursue and report herein, was to design a single molecule bearing two separate recognition sequences, each of which would be recognized specifically by one enzyme. Thus, we selected a terephthalic acid unit as the center for the bifunctional molecule, and attached peptide-based inhibitors to the carboxy termini by coupling them to the amino termini of the inhibitors. We refer to these inhibitors as Janus compounds, named for the Roman god Janus who is "identified with doors, gates, and all beginnings and that is represented artistically with two opposite faces."[4] The advantage of this approach is that the affinity of each inhibitory moiety for its enzyme can be attenuated separately.

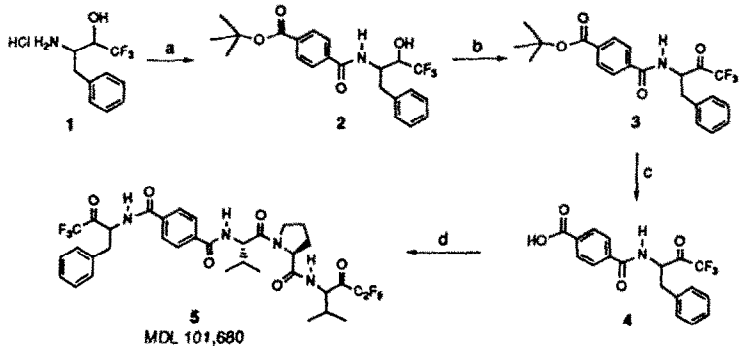
Chemistry

In Scheme I is shown the synthesis of MDL 101,680, a Janus compound designed to inhibit HNE and α-chymotrypsin. Trifluoromethyl amino alcohol hydrochloride salt 1[5] was coupled to terephthalic acid mono-t-butyl ester using the mixed anhydride coupling method (68%), and alcohol 2 was oxidized to the corresponding ketone 3 using the Dess-Martin

periodinane (72%).[6] Removal of the t-butyl group gave acid 4 (quantitative), which was coupled to HCl·Val·Pro·Val·CF_2CF_3 (mixed anhydride procedure) to give Janus compound 5 (35%).

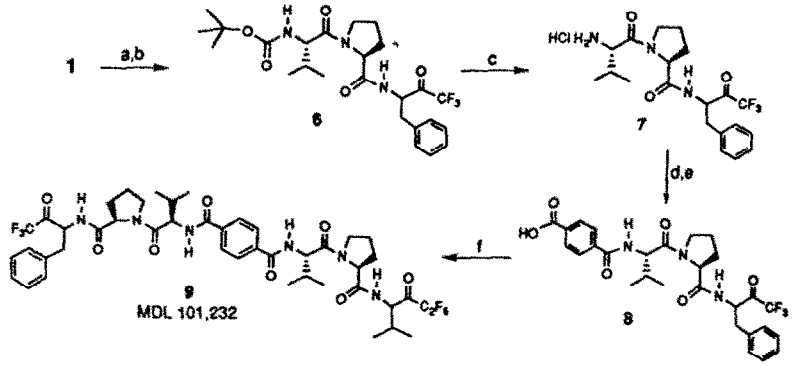
Synthesis of the Janus compound designed to inhibit HNE and cathepsin G, MDL 101,232, is shown in Scheme II. Coupling of 1 to Boc·Val·Pro·OH (mixed anhydride procedure) followed by oxidation (Dess-Martin periodinane[6]) of the resulting alcohol (97%) gave trifluoromethyl ketone 6 (94%). Removal of the N-protecting group gave amine hydrochloride 7 (quantitative), which was converted to terephthalic acid monoamide 8 by coupling to terephthalic acid mono-t-butyl ester (37%) followed by removal of the t-butyl group (97%). Coupling of 8 to HCl·Val·Pro·Val·CF_2CF_3[7] (mixed anhydride procedure) gave Janus compound 9 (18%).

Scheme I: Synthesis of MDL 101,680



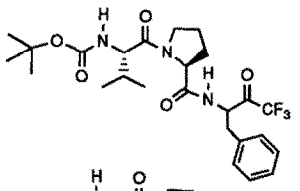
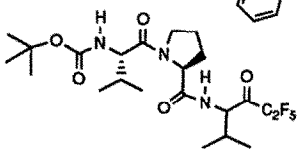
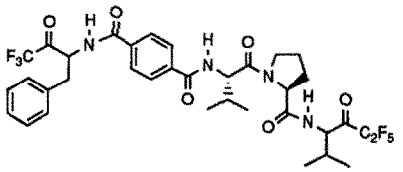
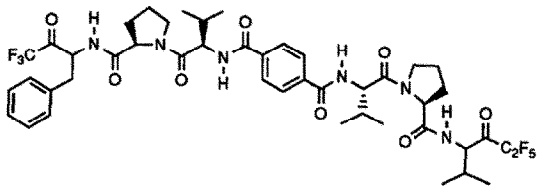
Reagents. a) Isobutyl chloroformate, N-methylmorpholine, *mono*-t-butyl terephthalate, CH_2Cl_2; b) Dess-Martin periodinane, CH_2Cl_2; c) HCl, EtOAc; d) Isobutyl chloroformate, N-methylmorpholine, HCl·ValProValCF_2CF_3.

Scheme II: Synthesis of MDL 101,232



Reagents: a) Isobutyl chloroformate, N-methylmorpholine, BocValProOH, CH_2Cl_2; b) Dess-Martin periodinane, CH_2Cl_2; c) HCl, EtOAc; d) Isobutyl chloroformate, N-methylmorpholine, *mono*-t-butyl terephthalate, CH_2Cl_2; e) HCl, EtOAc; f) Isobutyl chloroformate, N-methylmorpholine, HCl·ValProValCF_2CF_3.

Table I: Inhibition Constants for Janus Inhibitors

Cpd. No.	Pentafluoroethylketones	K_i values: Human Neutrophil Elastase	K_i values: Human Cathepsin G	K_i values: α-Chymotrypsin
6		—	1.7 μm	—
10		55 nm	—	—
5		4 nm	>1 mM	1.2 μm
9		15.9 nm	2.2 μm	—

Enzymology

Shown in Table I are the inhibition constants which were measured for the Janus inhibitors 5 and 9 using previously described assays.[3,8] An inhibitor was tested at three concentrations at a single substrate concentration and the K_i value was calculated from the initial steady-state rates v_o (control) and v_i (in the presence of inhibitor at the concentration [I]) using $v_o/v_i=1+([I]/K_{i,app})$ and $K_i=K_{i,app}/[1+([S]/K_M)]$. The potency of 5 for HNE (K_i value of 4 nM) is surprisingly good for a Val·Pro·Val sequence,[5] which suggests that the large N-protecting group attached to this sequence is enhancing the affinity. The α-chymotrypsin affinity for 5 (K_i value of 1.2 μM) is also quite good for the short phenylalanine recognition sequence, and perhaps this affinity could be improved with a longer sequence.

Potent inhibition of both HNE (K_i value of 15.9 nM) and human cathepsin G (K_i value of 2.2 μM) was effected with the Janus compound 9 containing both Val·Pro·Val and Val·Pro·Phe tripeptide sequences. The low micromolar affinity displayed by 9 for human cathepsin G is consistent with the K_i value of 1.1 μM reported for MeO·Suc·Val·Pro·Phe·-CO_2CH_3[5,9] which is our best inhibitor of that enzyme.

Comparison of the affinities of Janus compound **9** for human cathepsin G and HNE with the respective tripeptide inhibitory portions of **9**, which are terminated with N-t-Boc groups, e.g., compounds **6** and **10**, is also shown in Table I. The values of human cathepsin G displayed by **6** and **9** are essentially the same, whereas *9 is significantly more potent than 10* against HNE.

Pharmacology

Cartilage radiolabeled with ^{35}S has been used as a model system to investigate the role of neutrophils and neutrophil proteinases in the degradation of connective tissue matrix.[2,10,11] Degradation of radiolabeled cartilage by neutrophil lysate or stimulated neutrophils was only slightly inhibited when a specific synthetic inhibitor of elastase or cathepsin G was used individually. However, a combination of an elastase and a cathepsin G inhibitor virtually completely inhibited cartilage degradation by stimulated neutrophils or neutrophil lysate.[2]

The ability of the dual elastase and cathepsin G inhibitor, MDL 101,232, to block degradation of radiolabeled cartilage mediated by human neutrophil lysate or by zymosan stimulated neutrophils was measured as described previously.[2] Human neutrophil lysate at

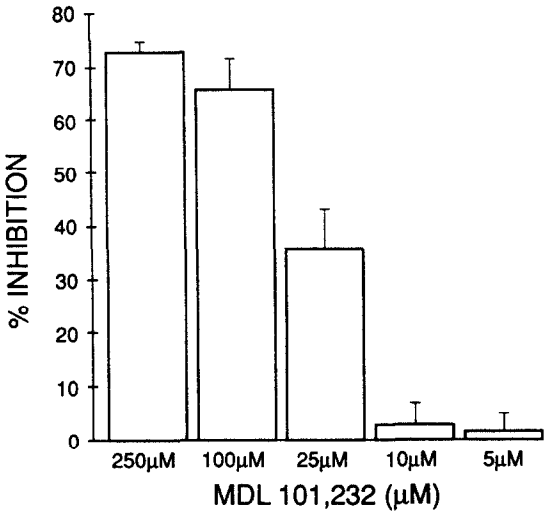


Figure 1. Cartilage matrix degradation mediated by human neutrophil lysate is inhibited by MDL 101,232. The supernatants from five replicate cultures containing radiolabeled cartilage disks were assayed for the release of radiolabeled proteoglycan. The data are expressed as percent inhibition of radiolabel released and are plotted as the mean ± SEM from three experiments.

a 1/100 dilution in serumless media degraded radiolabeled cartilage proteoglycan by 37% ± 2 (n=3) after incubation for 4 h at 37°C (Fig. 1). MDL 101,232 inhibited neutrophil lysate mediated cartilage degradation in a dose-related manner (Fig. 1). Human neutrophils were allowed to settle onto radiolabeled cartilage disks in the wells of microtiter plates. The neutrophils were stimulated with 1 mg/ml of opsonized zymosan and incubated at 37°C for 4 h in media containing 10% serum. Degradation of cartilage by stimulated neutrophils in the presence of serum antiproteases occurs in the serum-protected pericellular region between the neutrophils and the macromolecular substrate.[12-16] Stimulated neutrophils (1.0 X 10^6/well) degraded radiolabeled cartilage by 22% ± 2 (n=3) (Fig. 2). MDL 101,232 inhibited stimulated neutrophil mediated cartilage degradation in a dose related manner (Fig. 2). MDL 101,232 did not effect the viability of the neutrophils.

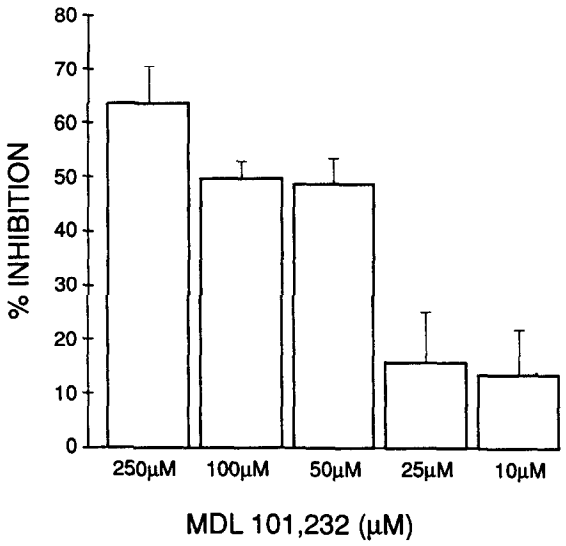


Figure 2. Cartilage matrix degradation mediated by stimulated human neutrophils is inhibited by MDL 101,232. The supernatants from five replicate cultures containing radiolabeled cartilage disks were assayed for the release radiolabeled proteoglycan. The data are expressed as percent inhibition of radiolabel released and are plotted as the mean ± SEM from three experiments.

Summary

Diacids can function as a centerpiece for building single compounds as dual proteinase inhibitors, termed Janus compounds. The concept has been demonstrated by dual inhibition of HNE/α-chymotrypsin and HNE/cathepsin G.

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7a. Synthesis of Boc·Val·Pro·Val·CF_2CF_3 was recently described. See Angelastro, M.R.; Burkhart, J.P.; Bey, P.; Peet, N.P. *Tetrahedron Lett.* **1992**, *33*, 3265. b. Both Janus compounds **5** and **9** exist as mixtures of 4 diastereomers due to epimerization at the centers α to the fluorinated ketones. The electron deficient ketones also exist in hydrated forms which adds additional complexity to the product mixtures.

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