Bioorganic & Medicinal Chemistry Letters 19 (2009) 3525–3530

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Compelling P1 substituent affect on metalloprotease binding profile enables the design of a novel cyclohexyl core scaffold with excellent MMP selectivity and HER-2 sheddase inhibition

David M. Burns [a,*], Yun-Long Li [a,*], Eric Shi [a], Chunhong He [a], Meizhong Xu [a], Jincong Zhuo [a], Colin Zhang [a], Ding-Quan Qian [a], Yanlong Li [b], Richard Wynn [b], Maryanne B. Covington [b], Kamna Katiyar [b], Cindy A. Marando [b], Jordan S. Fridman [b], Peggy Scherle [b], Steve Friedman [b], Brian Metcalf [a,b], Wenqing Yao [a]

[a] Incyte Corporation, Department of Medicinal Chemistry, Wilmington, DE 19880, USA
[b] Incyte Corporation, Department of Discovery Biology, Wilmington, DE 19880, USA

ARTICLE INFO

Article history:
Received 3 April 2009
Revised 27 April 2009
Accepted 30 April 2009
Available online 5 May 2009

Keywords:
HER-2 sheddase
Metalloprotease
Hydroxamic acid
P1
P1′
S1
ADAM-10

ABSTRACT

A serendipitous discovery that the metalloprotease binding profile of a novel class of 2-carboxamide-3-hydroxamic acid piperidines could be significantly attenuated by the modification of the unexplored P1 substituent enabled the design and synthesis of a novel 2-carboxamide-1-hydroxamic acid cyclohexyl scaffold core that exhibited excellent HER-2 potency and unprecedented MMP-selectivity that we believe would not have been possible via conventional P1′ perturbations.

Drugs targeting the human epidermal growth factor receptor-2 (HER-2) have received a significant amount of attention as the first individualized targeted biological therapy for the treatment of cancer, particularly monoclonal antibodies, such as trastuzumab and pertuzumab, and tyrosine kinase inhibitors, such as lapatinib.[1] While these advancements are significant milestones in the effective treatment of cancer they are not optimal due to drug resistance and non-responsiveness.[2] Emerging biological and clinical evidence suggests that successful cancer treatment regimes will incorporate a combination of drugs that target multiple mechanisms, akin to HAART for HIV treatment.[3] In lieu of this strategy we have recently demonstrated in a preclinical setting that inhibition of the HER-2 sheddase can decrease ErbB/Akt signaling events and tumor cell proliferation with an increase in survival when administered as a monotherapy and positive synergistic effects when administered with clinically relevant cancer therapeutics, such as trastuzumab.[4] Thus a HER-2 sheddase inhibitor may serve as a useful therapeutic for the treatment of HER-2 positive cancers, such as breast, ovarian, non-small cell lung, colon and pancreatic tumors.

Recently we have identified ADAM-10 as a major source of HER-2 ectodomain sheddase activity in HER-2 overexpressing breast cancer cells; however, at the onset of the project the protease that was responsible for HER-2 shedding had not been identified.[5] In an effort to isolate the unknown HER-2 sheddase, the MMP-selective compound **1** was chosen from our previously disclosed novel class of (6*S*,7*S*)—7-[(hydroxyamino) carbonyl]-6-carboxamide-5-azaspiro[2.5]octane-5-carboxylates to be tethered to a solid support to extract the unknown protease from the extracellular matrix (Fig. 1).[6] We envisioned that we could attach an analog of compound **1** to a resin by placement of an appropriate linker at C-5 without affecting the metalloprotease binding profile, since it was believed that the P1 substituent was primarily solvent exposed. Thus compounds **3** and **4** were prepared as mixtures of diastereoisomers at C-5 and were shown to have a two-fold improvement in HER-2 shedding potency compared to **1**. (Table 1).

Although Wyeth recently disclosed the results of an SAR investigation that focused on variation of the P1 substituent to obtain a potent and selective TACE inhibitor, at the inception of the study

* Corresponding authors. Tel.: +1 302 498 6887; fax: +1 302 425 2704 (D.M.B.).
E-mail address: dburns@incyte.com (D.M. Burns).

0960-894X/$ - see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2009.04.143

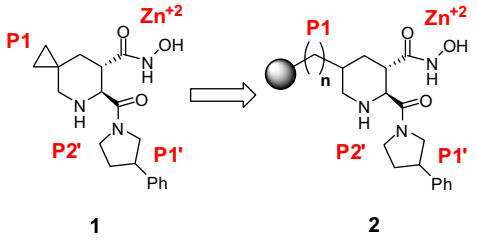
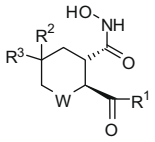


Figure 1. Design of HER-2 sheddase scavenger resin.

described herein there were limited publications on the SAR of the P1 group of metalloprotease inhibitors.[9,10] Conventional SAR studies of metalloproteases focused primarily and often entirely on the S1′ pocket to achieve both potency and selectivity.[11,12] This serendipitous discovery that the potency of a sheddase inhibitor could be attenuated by modifying the P1 substituent prompted further investigation and the results of this study will be presented. In the following discussion HER-2 shedding potency describes the cellular inhibition of HER-2 sheddase and ADAM-10 potency denotes the enzymatic inhibition of HER-2 sheddase. The ADAM-10 data is used to evaluate the MMP selectivity, since the ADAM-10 and MMP assays are all enzymatic.[5,8]

Table 1
Investigation of the SAR of the P1 substituent



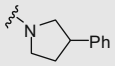
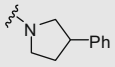
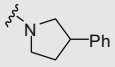
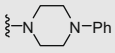
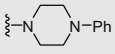
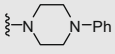
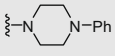
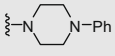
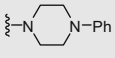
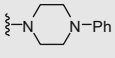
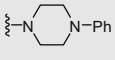
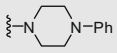
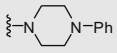
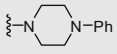
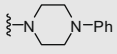
Compound	R^1	W	$R^{2,a}$	R^3	HER-2 shedding	Enzymatic binding IC_{50}^c (nM)				
					IC_{50}^b (nM)	ADAM-10	MMP-1	MMP-2	MMP-3	MMP-9
1		NH	———Spiro-cyclopropyl———		359	323	>5000	998	>5000	>5000
3		NH	$-CH_2C(O)NH(CH_2)_5NHCbz$	H	137	302	>5000	1277	4999	1788
4		NH	$-CH_2C(O)NH(CH_2)_5NH_2$	H	162	161	>5000	1662	>5000	>5000
5		NH	———Spiro-cyclopropyl———		52	120	>5000	200	>5000	650
6		NH	$-CH_2C(O)$pyrrolidin-1yl	H	28	19	—	8	—	—
7		NMe	———Spiro-cyclopropyl———		230	310	>5000	150	>5000	>5000
8		NMe	$-CH_2C(O)$pyrrolidin-1yl	H	194	167	—	92	—	—
9		NMe	$-CH_2C(O)$piperidin-1yl	H	102	77	—	81	—	—
10		CH_2	H	H	470	1545	>5000	180	—	
11		CH_2	———Spiro-cyclopropyl———		100	196	>5000	106	>5000	577
12		CH_2	$-CH_2C(O)$pyrrolidin-1yl	H	99	64	>5000	271	>5000	1443

Table 1 (*continued*)

Compound	R^1	W	$R^{2,a}$	R^3	HER-2 shedding	Enzymatic binding IC_{50}[c] (nM)				
					IC_{50}[b] (nM)	ADAM-10	MMP-1	MMP-2	MMP-3	MMP-9
13		CH_2	$-CH_2C(O)$piperidin-1yl	H	60	58	>5000	321	>5000	1876
14		CH_2	$-CH_2C(O)$4-Me-piperazin-1-yl	H	100	30	>5000	68	>5000	2500
15		CH_2	$-CH_2C(O)$morpholin-1-yl	H	208	83	—	236	—	—
16		CH_2	$-CH_2C(O)$pyrrolidin-3*R*-OH-1yl	H	226	111	—	156	—	—

[a] Compounds **3**–**9** and **12**–**16** were 50/50 and 40/60 mixtures of diastereoisomers, respectively.
[b] Values are obtained from a BT-474 cellular HER-2 ECD shedding assay, see Ref. 7.
[c] See Ref. 8 for a description of the enzymatic assays.

To ensure that the observed enhancement in HER-2 sheddase activity resulting from modification of the P1 group was experimentally significant, we prepared a second pair of compounds, namely **5** and **6**, and again observed that the acetamide derivative **6** is two-fold more potent than the parent cyclopropyl compound **5**. We speculated that installation of an appropriate P1 group may be particularly beneficial in improving the HER-2 potency of core scaffolds that were initially deemed less promising than the N–H piperidine core, even after optimal P1' substituents were attached.

Previously, we reported that N-methylation of the core piperidine nitrogen resulted in a substantial loss in potency, as exhibited by comparison of compounds **5** and **7**, which precluded the *N*-methyl piperidine core from consideration as a viable scaffold.[6] To test our hypothesis that a moderately potent core could be converted to a cogent scaffold by perturbation of the P1 substituent we prepared the 5-(2-oxo-2-pyrrolidin-1-ylethyl)piperidine analog **8**. We were pleased to observe a two-fold increase in HER-2 enzymatic potency (ADAM 10) in comparison to the parent spiro-cyclopropyl compound **7**, albeit the cellular potency (HER-2 shedding) was only modestly improved. Exchanging the P1 pyrrolidine ring of compound **8** with a piperidine ring to afford analog **9** resulted in a two-fold boost in both cellular and enzymatic potency in comparison to **8**. Comparison of analog **9** to the parent spiro-cyclopropyl compound **7** reveals a two-fold and four-fold improvement in cellular and enzymatic potencies, respectively. In addition to the improved HER-2 shedding potency, compound **9** is equipotent in ADAM-10 and MMP-2 binding, which is an improvement in selectivity in comparison to the parent compound **7** that has a two-fold higher affinity towards MMP-2 versus ADAM-10. Reexamination of compounds **1** and **4** reveals a three-fold improvement in MMP-2 selectivity. These results might be a manifestation of a difference between the HER-2 sheddase and MMP-2 within the S1 pocket or S1 cleft, which may be further explored and exploited to achieve the difficult task of obtaining MMP-2 selectivity. The attainment of an MMP selective compound is desirable to avoid potential adverse side effects such as musculoskeletal toxicity, which has been a clinical liability for numerous MMP and TACE inhibitors.[12]

The cyclohexyl scaffold had been investigated and was initially found to be less promising than the spiro-piperidine scaffold as demonstrated by comparison of compounds **5** and **10**. Previously we disclosed that the installation of a spiro-cyclopropyl group at C5 of the piperidine core series increased both the cellular and enzymatic potency by five-fold, which was attributed to the spiro-cyclopropyl group conferring conformational rigidity to the piperidine ring.[6] Application of this strategy to the cyclohexyl template had a similar effect on the potency as shown by compound **11**. To further investigate the influence of the P1 group on the binding profile the 4-(2-oxo-2-pyrrolidin-1-ylethyl)cyclohexyl analog **12** was prepared and was shown to have a three-fold increase in enzymatic HER-2 potency compared to the spiro-cyclopropyl compound **11**, albeit the HER-2 cellular potency remained constant. Replacement of the spiro-cyclopropyl group of **11** with the P1 methylene amide moiety reduced the affinity towards MMP-2 by approximately three-fold conferring analog **12** with four-fold selectivity against MMP-2. This was a compelling improvement to the spiro-cyclopropyl compound **11** which was two-fold more potent against MMP-2 then ADAM-10. Comparison of analog **12** to the unsubstituted parent compound **10** revealed an even more pronounced increase in enzymatic potency and MMP-2 selectivity, in addition to a five-fold increase in HER-2 cellular potency.

In an effort to study the SAR of the P1 group a variety of P1 substituted analogs of compound **10** were prepared and the results are discussed below. Enlargement of the P1 substituent of **12** to the 2-oxo-2-piperidin-1-ylethyl group **13** resulted in approximately a two-fold increase in HER-2 cellular potency and a small increase in MMP-2 selectivity. The observed two-fold enhancement in cellular potency when changing from the pyrrolidine to the piperidine P1 ring for compounds **12** and **13** was also observed in the *N*-methyl piperidine analogs **8** and **9** and may be a manifestation of an increase in lipophilicity. Increasing the polarity of the P1 group of **13** by introduction of a heteroatom to afford the 2-(4-methylpiperazin-1-yl)-2-oxoethyl analog **14** and the 2-morpholin-4-yl-2-oxoethyl analog **15** resulted in a two and three-fold decrease in cellular potency, in agreement with the previous observation that a lipophilic group is favored for HER-2 cellular potency. Compounds **14** and **15** also suffered from a two-fold loss in MMP-2 selectivity. These results may be indicative of a divergence between HER-2 sheddase and MMP-2 within the S1 region, with HER-2 sheddase preferring hydrophobic groups and MMP-2 preferring hydrophilic groups. Increasing the polarity of the 4-(2-oxo-2-pyrrolidin-1-ylethyl) cyclohexyl compound **12** by the introduction

of a hydroxyl group at the 3-position of the pyrrolidine to afford analog **16** resulted in a two-fold loss in HER-2 shedding potency and a three-fold loss in MMP-2 selectivity, in agreement with the previously proposed hypothesis.

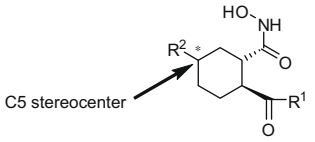
Compound **13** was identified as a lead compound within the cyclohexyl core scaffold series and in an effort to further increase the potency the diastereoisomers were separated and the 5-*S*-diastereoisomer **17** was found to be 10-fold more potent than the 5-*R*-epimer **18** (Table 2). In an effort to increase the selectivity towards MMP-2 and MMP-9 the P1′ group was modified to the 4-(3-isopropylphenyl)-piperazin-1-yl amide **19**, which was recently shown to have an excellent selectivity profile in the piperidine scaffold.[13] Compound **19** had a greater than three-fold loss in cellular potency that was inconsequential in lieu of the 36- and five-fold accession in selectivity towards MMP-2 and -9, respectively, in comparison to the corresponding parent *S*-epimer **17**.

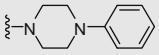
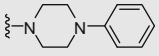
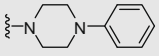
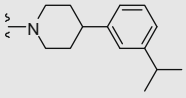
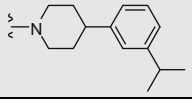
Based on the data presented thus far it appears that the P1 substituent is interacting with either the S1 pocket or the cleft of the pocket and is not simply solvent exposed, as initially believed. This hypothesis is supported by the observed divergence between HER-2 sheddase and MMP-2 with regards to the preferred polarity of the P1 group. However, it can not be discounted that the P1 group may also be inducing global conformational changes, which may contribute to permutations of the P1′ group and thus result in attenuation of the metalloprotease binding profile.

The general synthetic route used to prepare the P1 substituted piperidine scaffold compounds is outlined in Scheme 1. Ozonolysis of the previously disclosed 5-methylenepiperidine **21** afforded the desired ketone, which was subsequently subjected to the Wittig reaction with methyl(triphenylphosphoran-ylidene)acetate.[6] The α,β-unsaturated ester **22** was reduced and the Bn and Cbz protecting groups were removed under an atmosphere of H_2(g) in the presence of 10% Pd/C. The P1′ substituent was installed by BOP reagent-mediated amide coupling to afford **23**. The C5 β-ester was hydrolyzed under basic conditions and subjected to amide coupling to install the P1 group to afford **24**. The *tert*-butyl protecting group was removed by treatment with TFA followed by BOP-mediated amide coupling to afford the desired hydroxamic acid **3**. The Cbz protecting group of compound **3** was removed by treatment with H_2(g) in the presence of 10% Pd/$BaSO_4$ to afford the free amine **4**.

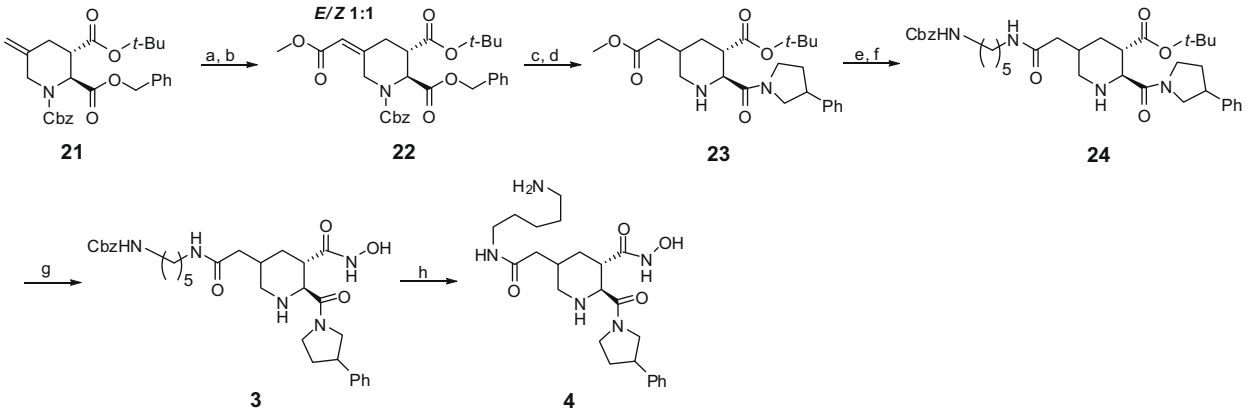
The general synthetic route for the P1 substituted cyclohexyl core analogs **12**–**20** is outlined in Scheme 2. The synthesis began with the enantioselective methanolytic desymmetrization of the cyclic meso anhydride **25** using pig liver esterase to afford the mono-carboxylic acid **26**.[14] The carboxylic acid **26** was converted to the *tert*-butyl ester and the remaining methyl ester was chemoselectively hydrolyzed under basic conditions to afford **27.** Epimerization at the C6 stereogenic center of **27** was achieved by treatment with potassium *tert*-butoxide to produce a 1:4 mixture of diastereoisomers **28** and **29**. Iodolactonization of the alkene followed by reduction of the remaining iodide by treatment with triethylsilylhydride and AIBN afforded **30** and the corresponding diastereoisomer, which were separated by conventional flash column chromatography. The lactone **30** was ring opened and the resulting carboxylic acid was protected as the benzyl ester. Swern oxidation of the secondary alcohol afforded the ketone **31**, which was subjected to the Wittig reaction and a series of sequential

Table 2
Investigation of the stereochemistry at C5

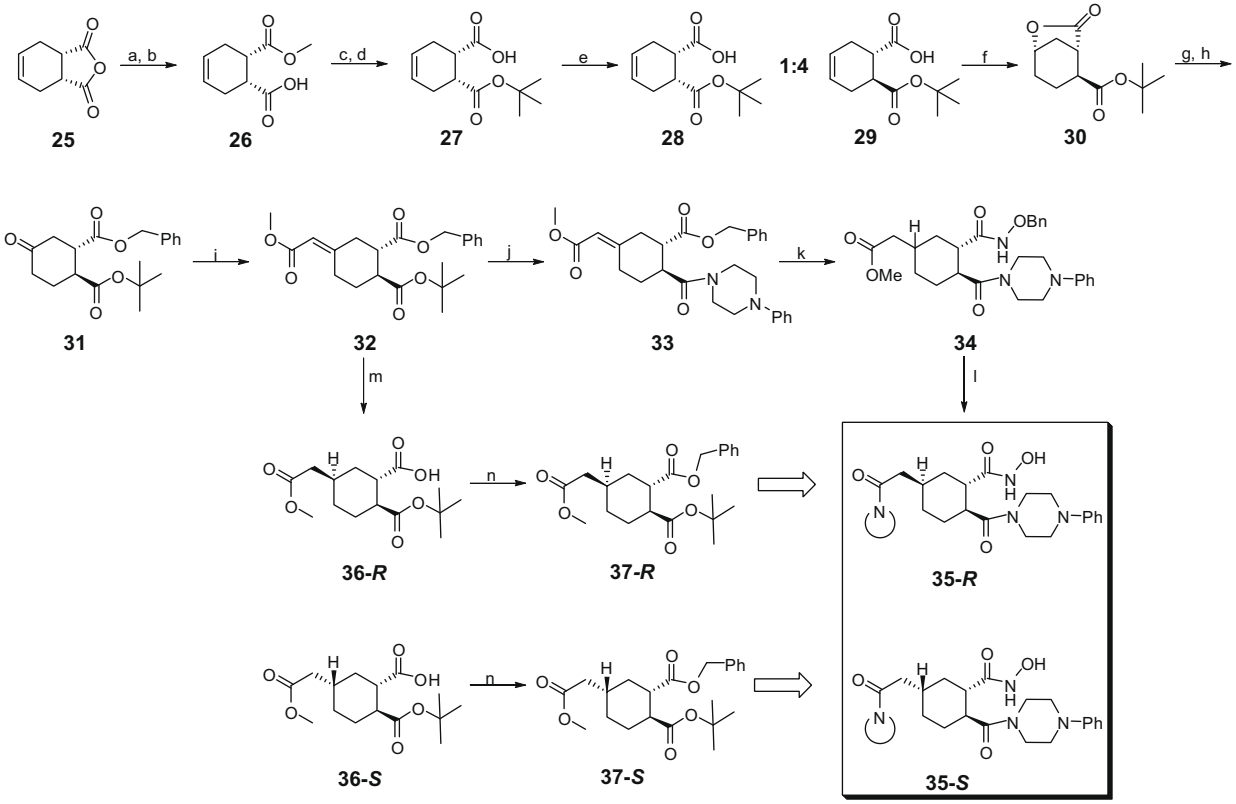


Compound	R^1	R^2	C5	HER-2 shedding IC_{50}[a] (nM)	Enzymatic binding IC_{50}[b] (nM)				
					ADAM-10	MMP-1	MMP-2	MMP-3	MMP-9
13		–CH_2C(O)piperidin-1yl	S/R	60	58	>5000	321	>5000	1876
17		–CH_2C(O)piperidin-1yl	S	18	15	>5000	100	>5000	683
18		–CH_2C(O)piperidin-1yl	R	170	165	—	1090	—	—
19		–CH_2C(O)piperidin-1yl	S	67	21	>5000	>5000	>5000	>5000
20		–CH_2C(O)piperidin-1yl	R	NA[c]	915	—	>5000	—	—

[a] Values are obtained from a BT-474 cellular HER-2 ECD shedding assay, see Ref. 7.
[b] See Ref. 8 for a description of the enzymatic assays.
[c] Not active.



Scheme 1. Reagents and conditions: (a) O_3 (90%); (b) $MeOC(O)CH{=}PPh_3$, PhMe, reflux (80%); (c) H_2 (g) 10% Pd/C (93%); (d) BOP, $(i\text{-Pr})_2EtN$, DMF, 3-Ph-pyrrolidine (88%); (e) LiOH, THF/H_2O (100%); (f) BOP, $(i\text{-Pr})_2EtN$, DMF, $BnOC(O)NH(CH_2)_5NH_2$ (66%); (g) (i) TFA; (ii) BOP, $(i\text{-Pr})_2EtN$, DMF, $NH_2OH{\cdot}HCl$ (80%); (h) H_2(g) 5% $Pd/BaSO_4$, MeOH (84%).



Scheme 2. Reagenst and conditions: (a) $MeOH/SOCl_2$, rt, 16 h (93%); (b) pig liver esterase, phosphate buffer/10% acetone pH 8, rt, 7d (100%); (c) 2-methylpropene, H_2SO_4, DCM, rt, 3d (91%); (d) 2 N NaOH/MeOH, rt, 2d (100%); (e) *t*-BuOK, THF, 0 °C to rt, 2 h (100%); (f) (i) I_2, KI, $NaHCO_3$, DCM/H_2O, rt, 2d; (ii) AIBN, Et_3SiH, PhMe (75%); (g) (i) LiOH, THF/H2O; (ii) BnBr (96%); (h) DMSO, ClC(O)C(O)Cl, Et_3N, DCM (98%); (i) $MeOC(O)CH{=}PPh_3$, PhMe, reflux (85%); (j) (i) TFA, DCM; (ii) BOP, NMM, DMF, 4-Ph-piperazine (94%); (k) (i) H_2(g), Pd/C, MeOH; (ii) BOP, NMM, DMF, $NH_2OBn{\cdot}HCl$ (88%); (l) (i) LiOH, THF/H_2O; (ii) PyBOP, NMM, DMF, RR'NH; (iii) H_2(g), $Pd/BaSO_4$ (~70%); (m) $[Rh(I)(cod)Cl]_2$, (*S*)-BINAP, MeOH, H_2(g) (90%, 96% de **36-*R***) n) DBU, BnBr, CAN (97%).

deprotection/BOP-mediated amide coupling reactions analogous to that described above for the synthesis of the core piperidine analogs.

The pure diastereoisomer analogs **18**–**21** were initially prepared from the chiral separation of either the triester intermediate **32** or the amide **34** and the stereochemical assignments were deduced from extensive NOE, COSY, HSQC, and HMBC NMR experiments. Reduction of the alkene using the conventional catalyst 10% Pd/C under an atmosphere of H_2(g) afforded a 40:60 ratio of the **36-*R*** and **36-*S*** diastereoisomers. The diastereomeric ratios were based on chiral HPLC analysis of the triester derivatives **37-*R*** and **37-*S***. In an effort to stereoselectively form both diastereoisomers, a variety of homogeneous catalysts and ligands, both chiral and achiral, were examined under a variety of reaction conditions. All of the reaction conditions examined thus far have resulted in a preference for the formation of diastereoisomer **36-*R***. For example, the ratio of diastereoisomers using $[Rh(I)(cod)Cl]_2$ in the presence of the chiral ligands (*S*)- and (*R*)-BINAP afforded a 95:5 and 80:20 ratio of the **36-*R*** and **36-*S*** diastereoisomers, respectively. Unfortunately, the preferred **36-*R*** diastereoisomer of the homogeneous

catalyzed reduction is the less active HER-2 sheddase inhibitor, which has led to an ongoing investigation to prepare the active isomer stereoselectively.

In summary we have demonstrated that perturbation of the P1 substituent may be a useful tool to modify the metalloprotease binding profile of a HER-2 sheddase inhibitor and may serve to augment the influence of conventional P1′ permutations, particularly when P1′ modifications have reached a plateau. The apparent divergence between HER-2 sheddase and MMP-2 within the S1 pocket and/or surrounding cleft is of particular utility, since there is a high level of homology within the S1′ subsite and achieving MMP selectivity via P1′ alterations can prove to be an arduous task. The encouraging results presented thus far prompted a comprehensive study of the SAR with regards to the P1 group and the results of this study are impending.

Acknowledgments

The authors would like to thank Mei Li, Laurine Galya, Jin Liu, and Karl Blom of the analytical group, Gengjie Yang and Xiangdong Liu from the drug discovery biology group, and Qiyan Lin of the medicinal chemistry group for their assistance in conducting the necessary experiments and providing helpful discussions.

References and notes

1. (a) Jarvis, L. M. *Chem. Eng. News* **2006**, *84*, 21; (b) Engel, R. H.; Kaklamani, V. G. *Drugs* **2007**, *67*, 1329; (c) Bartsch, R.; Wenzel, C.; Zielinski, C. C.; Steger, G. G. *Bio. Drugs* **2007**, *21*, 69; (d) Ross, J. S.; Schenkein, D. P.; Pietrusko, R.; Rolfe, M.; Linette, G. P.; Stec, J.; Stagliano, N. E.; Ginsburg, G. S.; Symmans, W. F.; Pusztai, L.; Hortobagyi, G. N. *Am. J. Clin. Pathol.* **2004**, *122*, 598.
2. (a) Nahta, R.; Esteva, F. J. *Cancer Lett.* **2006**, *232*, 123; (b) Plosker, G. L.; Keam, S. J. *Drugs* **2006**, *66*, 449; (c) Kamath, S.; Buolamwini, J. K. *Med. Res. Rev.* **2006**, *26*, 569; (d) Johnston, J. B.; Navaratnam, S.; Pitz, M. W.; Maniate, J. M.; Wiechec, E.; Baust, H.; Gingerich, J.; Skliris, G. P.; Murphy, L. C.; Los, M. *Curr. Med. Chem.* **2006**, *13*, 3483.
3. (a) Muller, A. J.; Scherle, P. A. *Nat. Rev.* **2006**, *6*, 613; (b) Spector, N.; Xia, W.; El-Hariry, I.; Yarden, Y.; Bacus, S. *Breast Cancer Res.* **2007**, *9*, 205; (c) Jackisch, C. *Oncologist* **2006**, *11*, 34; (d) Baselga, J.; Arteaga, C. L. *J. Clin. Onc.* **2005**, *23*, 2445.
4. (a) Fridman, J. S.; Caulder, E.; Hansbury, M.; Liu, X.; Yang, G.; Wang, Q.; Lo, Y.; Zhou, B.-B.; Pan, M.; Thomas, S. M.; Grandis, J. R.; Zhou, J.; Yao, W.; Newton, R. C.; Friedman, S. M.; Scherle, P. A.; Vaddi, K. *Clin. Cancer Res.* **2007**, *13*, 1892; (b) Zhou, B.-B. S.; Peyton, M.; He, B.; Liu, C.; Girard, L.; Caudler, E.; Lo, Y.; Baribaud, F.; Mikami, I.; Reguart, N.; Yang, G.; Li, Y.; Yao, W.; Vaddi, K.; Gazdar, A. F.; Friedman, S. M.; Jablons, D. M.; Newton, R. C.; Fridman, J. S.; Minna, J. D.; Scherle, P. A. *Cancer Cell* **2006**, *10*, 39; (c) Liu, X.; Fridman, J. S.; Wang, Q.; Caulder, E.; Yang, G.; Covington, M.; Liu, C.; Marando, C.; Zhou, J.; Li, Y.; Yao, W.; Vaddi, K.; Newton, R. C.; Scherle, P. A.; Friedman, S. M. *Cancer Biol. Ther.* **2006**, *5*, 648.
5. Liu, P. C. C.; Liu, X.; Li, Y.; Covington, M.; Wynn, R.; Huber, R.; Hillman, M.; Yang, G.; Ellis, D.; Marando, C.; Katiyar, K.; Bradley, J.; Abremski, K.; Stow, M.; Rupar, M.; Zhuo, J.; Li, Y.-L.; Lin, Q.; Burns, D.; Xu, M.; Zhang, C.; Qian, D.-Q.; He, C.; Sharief, V.; Weng, L.; Agrios, C.; Shi, E.; Metcalf, B.; Newton, R.; Friedman, S.; Yao, W.; Scherle, P.; Hollis, G.; Burn, T. C. *Cancer Biol. Ther.* **2006**, *5*, 657.
6. Yao, W.; Zhou, J.; Burns, D. M.; Xu, M.; Zhang, C.; Li, Y.-L.; Qian, D.-Q.; He, C.; Weng, L.; Shi, E.; Lin, Q.; Agrios, C.; Burn, T. C.; Caulder, E.; Covington, M. B.; Fridman, J. S.; Friedman, S.; Katiyar, K.; Hollis, G.; Li, Y.; Liu, C.; Liu, X.; Marando, C. A.; Newton, R.; Pan, M.; Scherle, P.; Taylor, N.; Vaddi, K.; Wasserman, Z. R.; Wynn, R.; Yeleswaram, S.; Jalluri, R.; Bower, M.; Zhou, B.-B.; Metcalf, B. *J. Med. Chem.* **2007**, *50*, 603.
7. Cellular assay for HER-2 shedding: BT474 cells, a human breast cell cancer line that constitutively sheds HER-2, were incubated with compound for 72 h at 37 °C, 5% CO_2. Supernatants were tested for inhibition of HER-2 sheddase by commercial ELISA. Compound inhibition was determined relative to cells cultured alone and compound activity was reported as the inhibitor concentration required for 50% inhibition (IC_{50} values) of HER-2 sheddase. Internal standards INCB46 and INCB4601 were used to determine the standard deviations. The average IC_{50} values are expressed in nM with the corresponding standard deviation and number of experiments performed (n) to determine these values. INCB46: 48 ± 20 (n = 105); INCB4601: 106 ± 43 (n = 13).
8. ADAM-10: Rosendahl, M. S.; Ko, S. C.; Long, D. L.; Brewer, M. T.; Rosenzweig, B.; Hedl, E.; Anderson, L.; Pyle, S. M.; Moreland, J.; Meyers, M. A.; Kohno, T.; Lyons, D.; Lichenstein, H. S. *J. Biol. Chem.* **1997**, *272*, 24588; MMP-1: Templeton, N. S.; Brown, P. D.; Levy, A. T.; Margulies, I. M. K.; Liotta, L. A.; Stetler-Stevenson, W. G. *Cancer Res.* **1990**, *50*, 5431; MMP-2 and MMP-9: Chandler, S.; Coates, R.; Gearing, A.; Lury, J.; Wells, G.; Bone, E. *Neurosci. Lett.* **1995**, *201*, 223; MMP-3: Saus, J.; Quinones, S.; Otani, Y.; Nagase, H.; Harris, E. D., Jr.; Kurkinen, M. *J. Biol. Chem.* **1988**, *263*, 6742. An internal standard INCB4601 was used in the enzymatic assays to determine the standard deviations. The average IC_{50} values are expressed in nM with the corresponding standard deviation and number of experiments performed (n) to determine these values. ADAM-10: 115 ± 32 (n = 69); MMP-2: 781 ± 293 (n = 60).
9. (a) Condon, J. S.; Joseph-McCarthy, D.; Levin, J. I.; Lombart, H.-G.; Lovering, F. E.; Sun, L.; Wang, W.; Xu, W.; Zhang, Y. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 34; (b) Park, K.; Aplasca, A.; Du, M. T.; Sun, L.; Zhu, Y.; Zhang, Y.; Levin, J. I. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 3927.
10. (a) Levin, J. I.; Chen, J.; Du, M.; Hogan, M.; Kincaid, S.; Nelson, F. C.; Venkatesan, A. M.; Wehr, T.; Zask, A.; DiJoseph, J.; Killar, L. M.; Skala, S.; Sung, A.; Sharr, M.; Roth, C.; Jin, G.; Cowling, R.; Mohler, K. M.; Black, R. A.; March, C. J.; Skotnicki, J. S. *Bioorg. Chem. Lett.* **2001**, *11*, 2189; (b) Hanessian, S.; Bouzbouz, S.; Boudon, A.; Tucker, G. C.; Peyroulan, D. *Bioorg. Chem. Lett.* **1999**, *9*, 1691; (c) Curtin, M. L.; Garland, R. B.; Davidsen, S. K.; Marcotte, P. A.; Albert, D. H.; Magoc, T. J.; Hutchins, C. *Biorg. Med. Chem. Lett.* **1998**, *8*, 1443; (d) Chapman, K. T.; Wales, J.; Sahoo, S. P.; Niedzwiecki, L. M.; Izquierdo-Martin, M.; Chang, B. C.; Harrison, R. K.; Stein, R. L.; Hagmann, W. K. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 329.
11. Excellent reviews: (a) Babine, R. E.; Bender, S. L. *Chem. Rev.* **1997**, *97*, 1359; (b) Brown, S.; Meroueh, S. O.; Fridman, R.; Mobashery, S. *Curr. Top. Med. Chem.* **2004**, *4*, 1227; (c) Levin, J. I. *Curr. Top. Med. Chem.* **2004**, *4*, 1289; (d) Skiles, J. W.; Gonnella, N. C.; Jeng, A. Y. *Curr. Med. Chem.* **2004**, *11*, 2911.
12. (a) Fisher, J. F.; Mobashery, S. *Cancer Met. Rev.* **2006**, *25*, 115; (b) Bachmeier, B. E.; Iancu, C. M.; Jochum, M.; Nerlich, A. G. *Exp. Rev. Anticancer Ther.* **2005**, *5*, 149; (c) Coussens, L. M.; Fingleton, B.; Matrisian, L. M. *Science* **2002**, *295*, 2387.
13. Yao, W.; Zhuo, J.; Burns, D. M.; Li, Y.-L.; Qian, D.-Q.; Zhang, C.; He, C.; Xu, M.; Shi, E.; Li, Y.; Marando, C. A.; Covington, M. B.; Yang, G.; Liu, X.; Pan, M.; Fridman, J. S.; Scherle, P.; Friedman, S.; Metcalf, B. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 159.
14. Sousa, H. A.; Crespo, J. G.; Afonso, C. A. M. *Tetrahedron: Asymmetry* **2000**, *11*, 929.