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Discovery of novel selective HER-2 sheddase inhibitors through optimization of P1 moiety

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ABSTRACT

A novel series of carbamates was discovered as potent and selective HER-2 sheddase inhibitors. Significant enhancement in potency and selectivity was achieved through attenuating the P1 moiety, which was conventionally believed to be exposed to solvent.

The ErbB family of tyrosine kinase receptors is composed of four members, epidermal growth factor (EGF) receptor (EGFR; HER-1 or ErbB1), HER-2 (neu or ErbB2), HER-3 (ErbB3), and HER-4 (ErbB4). Following ligand binding, EGF receptor family members form homo- or heterodimers, autophosphorylate tyrosine residues in their cytoplasmic tails and thereby trigger intracellular signaling cascades which lead to cell growth, survival, or differentiation.[1] Although HER-2 lacks a functional ligand binding domain, it is the preferred partner for heterodimerization with other family members.[2] Moreover, when overexpressed, HER-2 homodimers with signaling activity form spontaneously. Non-mutated HER2 is overexpressed in a significant fraction of human breast (~25%) as well as ovarian, gastric, and non-small cell lung tumors. In fact, the presence of high HER-2 levels is associated with aggressive clinical progression of breast and ovarian cancer.[3] A humanized monoclonal antibody, trastuzumab (Herceptin®), directed against the extracellular domain (ECD) of HER-2 has proven to be an effective therapy for breast cancer patients who overexpress HER-2.[4]

In HER-2 overexpressing cancer cells, the ECD of HER-2 is cleaved by the ADAM (a disintegrin and metalloproteinase) family of zinc-dependent, membrane-associated metalloprotease. The ectodomain shed of HER-2 renders its remaining transmembrane portion, p95, a constitutively active, phosphorylated tyrosine kinase.[5] In vitro studies indicate that the p95 fragment of HER-2 is 10–100 times more oncogenic than the full length receptor.[6] In the clinic, the presence of the HER-2 ECD in the serum of cancer patients has been linked to a poor prognosis,[7] with decreases in serum ECD levels during treatment being a predictor of response to trastuzumab therapy.[8] Accordingly, inhibition of the protease responsible for HER-2 ECD shedding and p95 production may have potential therapeutic benefit in HER-2 positive patients.

Many of the existing broad-spectrum metalloprotease inhibitors, such as marimastat, inhibit not only MMPs (matrix metalloproteinases) but also ADAMs in vitro and thereby prevent the processing of multiple ErbB ligands.[9,10] However, their clinical development has been hampered by side effects, many of which are likely due to MMP inhibition.[11] Recently we have identified ADAM-10 as a major source of HER-2 ectodomain sheddase activity in breast cancer cells that overexpress HER-2.[12] We have also shown that selective inhibitors of HER-2 sheddase decrease tumor size, decrease cleaved HER-2 ECD plasma levels, and potentiate the effects of trastuzumab in vivo.[13,14] The selectivity profile of these novel azaspiro-hydroxamic acids was optimized by exploiting the subtle differences between the S1' pocket of the MMPs and the HER-2 sheddase by attenuating the P1′ substituent.

Herein we report that the potency and selectivity profile of sheddase inhibitors can also be attenuated by modification of the P1 substituent. In the following discussion HER-2 potency describes the cellular inhibition of HER-2 sheddase and ADAM-10 potency denotes the enzymatic inhibition of HER-2 sheddase. The

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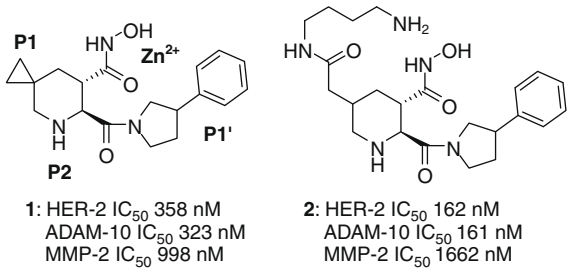


Figure 1. Starting point of P1 optimization.

ADAM-10 data is used to evaluate the MMP selectivity, since it is more comparable with the MMP affinity data.

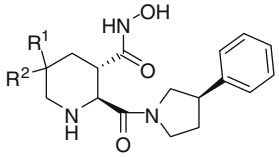
Conventional SAR studies of metalloproteases have focused primarily and often entirely on the S1′ pocket to achieve both potency and selectivity, since it was believed that the P1 substituent was primarily solvent exposed.[15–17] Recently, Condon et al disclosed the optimization of a series of TACE inhibitors through modification of the P1 moiety.[18] Our investigation into the SAR of the P1 moiety was triggered by the finding that compound **2** designed as a protein probe was two fold more potent than parent compound **1** (Fig. 1). When the more drug-like compound **4** was prepared (Table 1), we were delighted to find that it was 16 times more potent in the HER-2 cellular assay and almost seven times more potent in the ADAM-10 enzymatic assay than compound **3** (the single diastereoisomer of 1). While the cellular activity is complicated by protein binding and permeability, the ADAM-10 affinity assay results are experimentally significant. More impressively, compound **4** was 2.5 times more selective against MMP-2 than compound **3**.

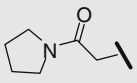
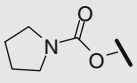
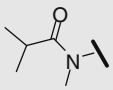
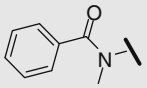
Other possible P1 moiety linkages were also investigated, as shown in Table 1. The oxygen linked compound **5** has comparable potency to **3** in the ADAM-10 assay, but is more than 2.4 times selective against MMP-2 than compound **3**. The nitrogen linked compounds **6** and **7** also inhibit ADAM-10 with potency similar to **3**, but their selectivity against MMP-2 was found to be decreased.

To further characterize the P1 moiety, a series of compounds with different P1 were prepared in the non-selective,[13] 4-phenyl-1,2,3,6-tetrahydropyridin-1-yl P1′ series as shown in Table 2. While all the analogs were found to be generally more potent than spiro-cyclopropyl compound **8**, the oxygen linked compound **10** was outstanding with its 10-fold selectivity against MMP-2. This result suggested some specific unfavorable interactions between the oxygen linked carbamate and the MMP-2 S1 cliff. On the other hand, the selectivity profile of the carbon linked amides (**4** vs **9**) apparently depended on the P1′ moiety.

At this point, the configuration at C-5 position of carbamate **10** was determined to be axial. Its equatorial counterpart **14** (Fig. 2) was found to be less active for HER-2 and ADAM-10 and not selective against MMP-2, resembling the profile of the spiro-cyclopropyl

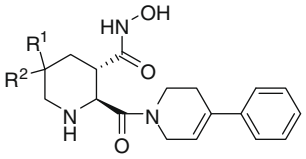
Table 1
P1 SAR in phenyl pyrrolidinyl series[21]

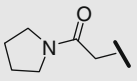
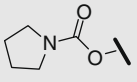
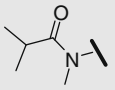
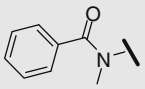
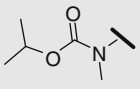


Compound	R^1 and R^2	HER-2 IC_{50} (nM)	ADAM-10 IC_{50} (nM)	MMP-2 IC_{50} (nM)
3	Spiro-cyclopropyl	92	97	960
4		5.6	14	365
5		28	83	>2000
6		52	138	502
7		29	84	210

Note: The stereochemistry at C-5 position was not determined. Data shown is for the more active isomer in each case.

Table 2
P1 SAR in phenyl tetrahydrapyridinyl series[21]



Compound	R^1 and R^2	HER-2 IC_{50} (nM)	ADAM-10 IC_{50} (nM)	MMP-2 IC_{50} (nM)
8	Spiro-cyclopropyl	18	33	<5
9		4.3	17	<5
10		5.6	9	102
11		15.5	23	11
12		10	12	12
13		8.5	11	<5

Note: The stereochemistry at C-5 position was not determined. Data shown is for the more active isomer in each case.

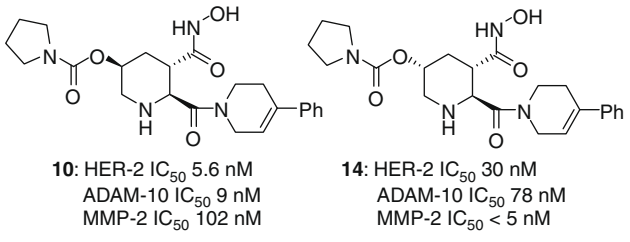


Figure 2. Diastereomers **10** and **14**.

compound **8**. Although the diastereomers of **9**, **11**, **12**, and **13** differ in potency, the selectivity profile was found to be similar (data not shown). The difference in selectivity observed between **10** and **14** suggested that an appropriately directed P1 moiety may interact with a S1 pocket, in addition to the possible interactions between the carbamate oxygen and S1 cliff.

Comparing different P1′ moieties in the carbamate series as shown in Table 3, saturated compound **15** is less active, but more selective than **10**. The P1′ moieties of compound **16** and **10** would be expected to have similar coplanar conformations (due to conjugation between the π-system of the aromatic ring and the lone pair of electrons on the piperazine ring)[19] and possess similar activity. However, **16** is more selective than **10**.

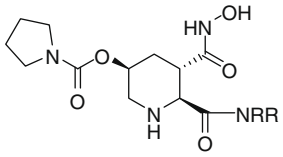
With small P1′ inner ring, the pyrrolidinyl analogs **17**, **18**, and **19** are less active for HER-2 and ADAM-10. When the compounds with different P1′ moieties were evaluated in the inhibition of other MMPs, the phenyl piperazinyl compound **16** showed better selectivity profile than **10**.

Substitution on piperidine nitrogen of **16** was hypothesized to alleviate potential hydrogen bonding between the scaffold N–H and P1′ carbonyl and twist the P1′ amide C=O to avoid steric and torsional strain. It could also change the conformational distribution of the scaffold piperidine ring.[13] Therefore, substitution on nitrogen was expected to alter the orientation of the P1′ moiety and thus modify the binding profiles. In the carbamate series, piperidine core nitrogen substitution resulted in a modest loss of potency for HER-2 and ADAM-10, but a slight increase in selectivity against MMP-2, as demonstrated in Table 4.

Looking into different carbamates, the inhibition potency for HER-2 and ADAM-10 were found to increase when the amino group was changed from azetidinyl to pyrrolidinyl and to piperidinyl. Continued increase in the ring size of the amino group to azepanyl and azocanyl then resulted in decreased potency as shown in Table 5. The selectivity for MMP-2 was also found to increase as the amino ring size enlarged up to the six-membered ring. Partial unsaturated pyrrolidinyl compound **24** was less active for ADAM-10, but more active for HER-2 than saturated analog **20**. Bis-fluoro substituted analog **25** was less potent than **24** in both HER-2 and ADAM-10 assays. All compounds tested in MMP-1, MMP-3, and MMP-9 did not show any inhibitory activities when tested at 5 μM.

The caco-2 permeability of compound **26** was determined to be 0.9×10^{-6} cm/s. Its projected clearance from human microsome incubation is 0.58 L/h/kg. When dosed 5 mg/kg po as a suspension

Table 3
In vitro profile of different P1′ analogs[21]



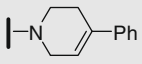
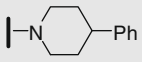
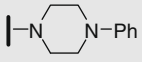
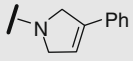
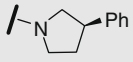
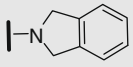
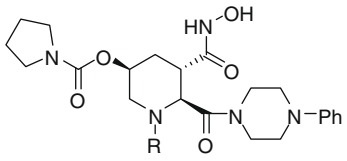
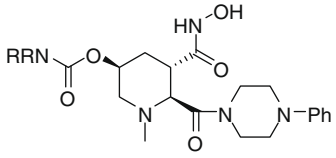
Compound	NRR	HER-2 IC_{50} (nM)	ADAM-10 IC_{50} (nM)	MMP-1 IC_{50} (nM)	MMP-2 IC_{50} (nM)	MMP-3 IC_{50} (nM)	MMP-9 IC_{50} (nM)
10		5.6	9	>5000	102	>5000	498
15		42	99	>5000	>2000	>5000	>5000
16		3.8	11	>5000	235	>5000	>5000
17		11	60	>5000	506	>5000	>5000
18		28	83	>5000	>2000	>5000	>5000
19		70	186	2500	>2000	>5000	>5000

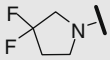
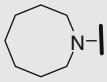
Table 4
The effects of piperidine core substitution[21]



Compound	R	HER-2 IC_{50} (nM)	ADAM-10 IC_{50} (nM)	MMP-1 IC_{50} (nM)	MMP-2 IC_{50} (nM)	MMP-3 IC_{50} (nM)	MMP-9 IC_{50} (nM)
16	H	3.8	11	>5000	235	>5000	>5000
20	Me	11.5	18	>5000	471	>5000	>5000
21	CO_2Me	8.1	20	>5000	613	>5000	>5000

Table 5
SAR of P1 moiety in phenyl piperazine series[21]



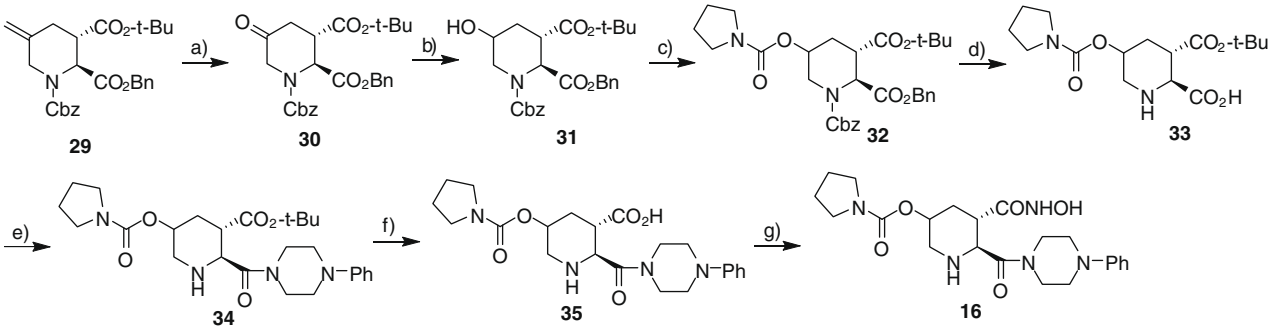
Compound	NRR	HER-2 IC_{50} (nM)	ADAM-10 IC_{50} (nM)	MMP-1 IC_{50} (nM)	MMP-2 IC_{50} (nM)	MMP-3 IC_{50} (nM)	MMP-9 IC_{50} (nM)
22	NMe_2	31	58	>5000	730	>5000	>5000
23		44.5	20	>5000	432	>5000	>5000
20		11.5	18	>5000	471	>5000	>5000
24		3.8	30	>5000	581	>5000	>5000
25		50.5	125	ND	>2000	ND	ND
26		7.3	9.6	>5000	344	>5000	>5000
27		4.4	35	>5000	843	>5000	>5000
28		27.5	26	>5000	1475	>5000	>5000

ND = not done.

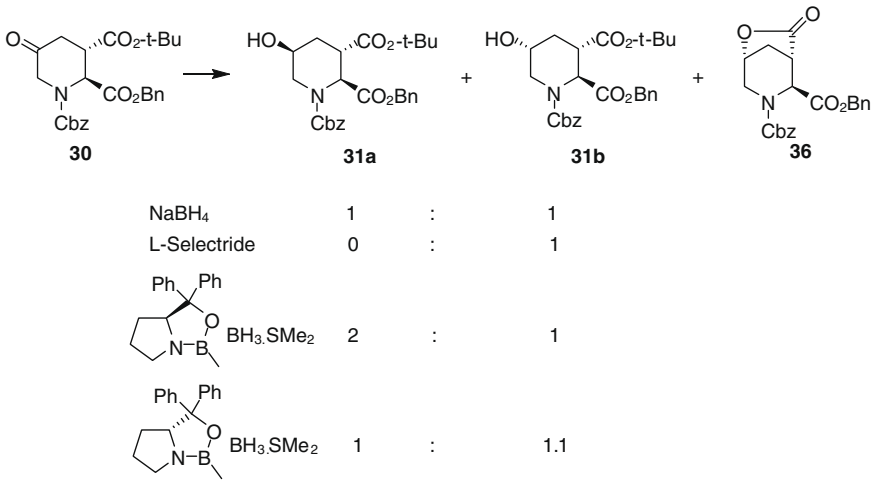
in 10% DMAC in 0.5% methylcellulose in an n-in-1 study, compound **28** exhibited an AUC of 323 nM/L h and $T_{1/2}$ of 0.88 h in mice.

The novel HER-2 sheddase inhibitors were synthesized from known compound **29**[13] as exemplified by the preparation of compound **16** (Scheme 1). Ozonolysis of **29** gave ketone **30** in 90% yield. Reduction of **30** with sodium borohydride provided alcohol **31** as an inseparable mixture of axial and equatorial isomers in 86% yield. Treatment of **31** with carbonyl diimidazole followed by pyrrolidine yielded carbamate intermediate **32** in a yield of 92%. After hydrogenation, the acid **33** was coupled with phenylpiperazine in the presence of (benzotriazol-1-yloxy)tris(dimethylamino)phosphonium hexafluoro-phosphate (BOP) to generate amide **34**. The later was treated with trifluoroacetic acid to remove the *tert*-butyl protective group. The resulting acid **35** was then subjected to BOP catalyzed coupling with hydroxylamine hydrochloride salt in the presence of diisopropylethylamine to afford analog **16**. The axial- and equatorial isomers were separated by reversed phase HPLC in a ratio of 1:1.

The reduction of ketone **30** with sodium borohydride afforded an alcohol **31** and 5% of lactone **36,** presumably generated by in situ cyclization. The axial- and equatorial isomers could be separated by chiral HPLC and the ratio was determined to be 1:1 (shown in Scheme 2). When the reduction was carried out with L-Selectride in THF, the equatorial isomer **31b** was generated exclusively. With (*S*)-oxazaborolidine-borane complex as catalyst,[20] the reduction of **30** in dichloromethane produced the two isomers in a ratio of 2:1. When the (*R*)-oxazaborolidine-borane



Scheme 1. Synthesis of compound **16**. Reagents and conditions: (a) O_3, CH_2Cl_2, −78 °C, 90%; (b) $NaBH_4$, MeOH, 86%; (c) carbonyl diimidazole, CH_2Cl_2, rt, then pyrrolidine, 92%; (d) H_2, 10% Pd/C, MeOH, 98%; (e) 1-phenylpiperazine, BOP, *i*-Pr_2NEt, DMF, rt, 78%; (f) TFA, CH_2Cl_2, 100%; (g) $NH_2OH{\cdot}HCl$, BOP, *i*-Pr_2NEt, DMF, 60%.



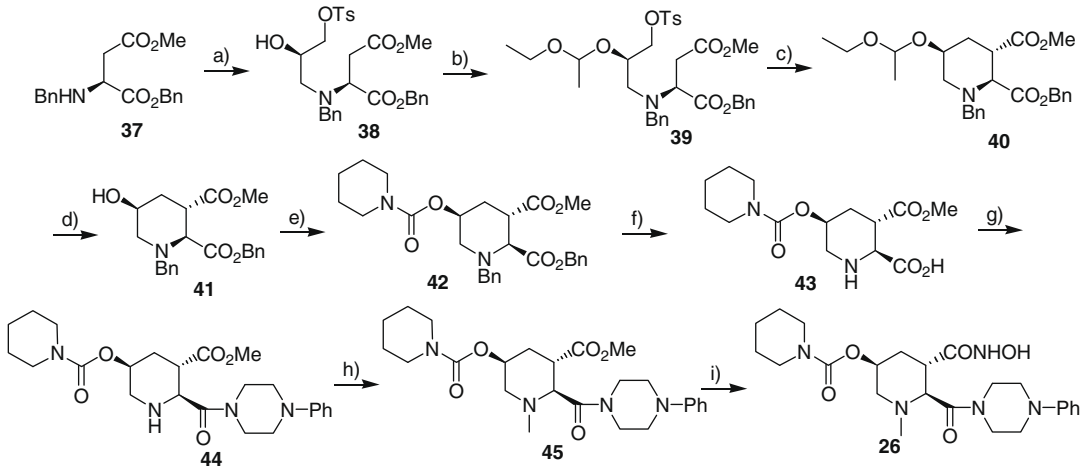
Scheme 2. Reduction of ketone **30**.

complex was used, alcohols **31a** and **31b** were afforded in 1:1.1 ratio.

Since the selectivity of ketone reduction was less than satisfactory and the resulting alcohol mixtures were inseparable by flash chromatography, a novel synthesis was devised to accommodate the imperative need of biologic evaluation, as shown in Scheme 3. Reaction of secondary amine **37** with commercially available (*S*)-(+)-glycidyl tosylate in the presence of trimethylaluminum gave alkylated product **38** in 91% yield. The use of trimethylaluminum as catalyst has been proven to be superior, since other Lewis acids have been less effective. For instance, when lithium perchlorate was used as catalyst, there was no reaction; while the ytterbium(III) triflate catalyzed reaction in acetonitrile takes 4 days to achieve 80% conversion. When dichloromethane was used as solvent, the ytterbium(III) triflate catalyzed reaction was fast, but gave a mixture of starting material, the desired alkylated product, and a cyclized lactone in a ratio of 1:1:1.

The alcohol **38** was then protected as its ethoxy ethyl ether in a yield of 98%. Subjection of protected **39** to the intra-molecular alkylation conditions provided piperidine **40** in 74% yield. The C-2 and C-3 cis-isomer was not detected. The C-3 configuration was established through chemical correlation. After deprotection, alcohol **41** was subjected to trimethylaluminum in dichloromethane. While 2-oxa-5-azabicyclo[2.2.2]octan-3-one was detected, no 6-oxa-3-azabicyclo[3.2.1]octan-7-one was identified. Since the C-5 stereochemistry was carried from chiral starting material, the formation of 2-oxa-5-azabicyclo[2.2.2]octan-3-one but not the 5-membered lactone suggested C-3 configuration was trans to C-2 and C-5. Reaction of alcohol **41** with carbonyl diimidazole followed by piperidine afforded carbamate **42**. Hydrogenation of the latter followed by BOP promoted coupling with phenyl piperazine yielded amide **44**. Reductive amination of **44** with formaldehyde then produced piperidine N-methylated compound **45** in a yield of 86%. Treatment of **45** with freshly prepared hydroxylamine methanol solution successfully gave the desired product **26** in 71% yield.

In summary, a novel series of carbamates has been identified as potent and selective HER-2 sheddase inhibitors. Although it was believed that the P1 substituent was primarily solvent exposed, we were able to demonstrate that significant enhancement in potency and selectivity could be achieved by attenuating the P1 moiety. While the increase in binding potency maybe the result of solvent-repulsion, non-specific lipophilic interaction at the solvent exposed S1 pocket, the astonishing increase in the selectivity against MMP-2 clearly indicates a specific interaction between the P1 moiety and S1 cliff and pocket. Since the dose-limiting



Scheme 3. Stereoselective synthesis of compound **26**. Reagents and conditions: (a) (*S*)-(+)-glycidyl tosylate, $AlMe_3$, CH_2Cl_2, −78 °C, 91%; (b) ethyl vinyl ether, PPTS, CH_2Cl_2, 98%; (c) LiHMDS, PhMe–THF (8:1), −78 °C to 0 °C, 74%; (d) 0.5 N HCl, THF, 97%; (e) carbonyl diimidazole, CH_2Cl_2, rt, then piperidine, 94%; (f) H_2, 10% Pd/C, MeOH, 98%; (g) 1-phenylpiperazine, BOP, *i*-Pr_2NEt, DMF, rt, 80%; (h) 36% HCHO, $NaBH(OAc)_3$, THF–AcCN, 86%; (i) 1.64 M NH_2OH in MeOH, 71%.

toxicities (fibroplasias and tendonitis, i.e., frozen shoulder syndrome) of broad-spectrum MMP inhibitors in early clinical trials are attributed to their non-selective inhibition of multiple MMPs,[11] it is desirable to design potent HER-2 sheddase inhibitors that are selective against MMPs to avoid deleterious physiological side effects. This discovery opened a new area which could be explored to obtain a desired selectivity profile for improved HER-2 sheddase inhibitors.

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