Discovery of a Potent, Selective, and Orally Active Human Epidermal Growth Factor Receptor-2 Sheddase Inhibitor for the Treatment of Cancer†

Wenqing Yao,* Jincong Zhuo, David M. Burns, Meizhong Xu, Colin Zhang, Yun-Long Li, Ding-Quan Qian, Chunhong He, Lingkai Weng, Eric Shi, Qiyan Lin, Costas Agrios, Timothy C. Burn, Eian Caulder, Maryanne B. Covington, Jordan S. Fridman, Steven Friedman, Kamna Katiyar, Gregory Hollis, Yanlong Li, Changnian Liu, Xiangdong Liu, Cindy A. Marando, Robert Newton, Max Pan, Peggy Scherle, Nancy Taylor, Kris Vaddi, Zelda R. Wasserman, Richard Wynn, Swamy Yeleswaram, Ravi Jalluri, Michael Bower, Bing-Bing Zhou, and Brian Metcalf

Incyte Corporation, Experimental Station, Wilmington, Delaware 19880

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Abstract: The design, synthesis, evaluation, and identification of a novel class of (6*S*,7*S*)-*N*-hydroxy-6-carboxamide-5-azaspiro[2.5]octane-7-carboxamides as the first potent and selective inhibitors of human epidermal growth factor receptor-2 (HER-2) sheddase is described. Several compounds were identified that possess excellent pharmacodynamic and pharmacokinetic properties and were shown to decrease tumor size, cleaved HER-2 extracellular domain plasma levels, and potentiate the effects of the humanized anti-HER-2 monoclonal antibody (trastuzumab) in vivo in a HER-2 overexpressing cancer murine xenograft model.

The human epidermal growth factor receptor-2 (HER-2[a] or ErbB-2) is a tyrosine kinase receptor that is activated upon homo-and heterodimerization with another member of the HER family or by proteolytic cleavage (shedding) of the extracellular domain (ECD).[1] Once activated, intracellular signal transduction pathways are initiated that mediate a diverse range of essential cellular activities such as cell proliferation, differentiation, motility, adhesion, and survival.[2] Overexpression of the oncogene HER-2/*neu* has been associated with aggressive pathogenesis, poor prognosis, and decreased responsiveness to conventional chemotherapeutic and hormonal treatment regimes in non-small cell lung cancer, ovarian cancer, and breast cancer patients.[1a] In addition, elevated plasma levels of HER-2 ECD have been associated with an increased metastatic potential and a decrease in disease-free and overall survival in patients with breast cancer.[1b,3] Therefore, inhibition of the protease responsible for HER-2 ECD shedding may be therapeutically desirable for treating cancer patients that overexpress HER-2, particularly breast cancer patients.

At the onset of the project, the protease that is responsible for HER-2 shedding had not been identified, although there was strong evidence suggesting that protein ectodomain shedding is mediated by zinc-dependent metalloproteases.[1c] Metalloproteases are comprised of the a disintegrin and metalloprotease (ADAM) and matrix metallo-protease (MMP) family members, which are highly conserved within the active site. Baselga et al. have demonstrated that HER-2 shedding can be induced by MMP activators and inhibited by broad spectrum MMP inhibitors, such as (2*R*,3*S*)-*N*(1)-[(1*S*)-1-benzyl-2-(methylamino)-2-oxoethyl]-*N*(4)-hydroxy-2-iso-butyl-3-[(2-thienylthio)methyl] succinamide (batimastat) and naturally occurring tissue inhibitor of metalloproteinase 1 (TIMP-1).[4] Based on the latter result, Baselga et al. concluded that ADAM-17, which has been widely implicated in the shedding of numerous proteins, was not the protease responsible for HER-2 ECD shedding, because ADAM-17 is not inhibited by TIMP-1.[4d,5] Conversely, ADAM-10 is inhibited by batimastat[6] and TIMP-1,[7] and Blobel et al. recently identified ADAM-10 as a major sheddase for two crucial EGFR ligands in mouse cells.[6] We therefore postulated that ADAM-10 may play a critical role in HER-2 ECD shedding, which was recently confirmed by Burn et al. of Incyte.[8] Irrespective of the exact nature of the protease, it is well-known that metalloproteases and sheddase activity can be inhibited by hydroxamic acid-derived compounds.[1c,9]

A number of hydroxamic acid-derived broad spectrum MMP inhibitors have been described in the literature. However, early clinical studies indicate that these compounds exhibit dose-limiting toxicities, such as fibroplasia and tendonitis (frozen shoulder syndrome).[10] Therefore, it is desirable to design a potent HER-2 sheddase inhibitor that is selective against MMPs to avoid deleterious physiological side effects. We envisioned that this could be achieved by exploiting subtle differences within the S_1' subsite of the otherwise highly conserved active sites within the metalloprotease family.[9]

At the inception of the project, Yoshiizumi et al. reported a series of 5,6,7,8-tetrahydropyrido[3,4-*b*] pyrazine-based hydroxamic acids **1** that were designed to inhibit the shedding of heparin-binding epidermal growth factor (HB-EGF) by inhibiting an unknown ADAM (Figure 1).[11] Our hypothesis was that a similar enzyme may play a role in HER-2 shedding and lead to the expedient synthesis and evaluation of pipecolic acid-derived hydroxamates. Compounds of this class, such as **2**, displayed moderate cellular inhibition of HER-2 shedding (0.67−1.9 μM), suggesting that the protease responsible for HER-2 and HB-EGF shedding might be similar. However, these compounds were found to be nonselective broad spectrum MMP inhibitors.

Xue et al. reported using cyclic succinates to inhibit ADAM-17.[12] Although, we knew that ADAM-17 was not the sheddase of interest based on Baselga's results,[4d] we believed that the *trans*-6-membered succinate might be a viable lead that could be attenuated to target the HER-2 sheddase by modifying the P_1' substituent and thus exploiting potential differences within the S_1' subsite of ADAM-17 and the HER-2 sheddase, as discussed above.

Thus, a series of cyclic succinates, such as compound **3**, were prepared and were found to have moderate HER-2 sheddase activity with an IC_{50} of 560 nM accompanied with a high affinity for MMPs (Figure 2).

In an effort to enhance the binding profile, the conformationally flexible piperidine scaffold ring was rigidified by the installation of a cyclopropyl group at the C-5 position. It was hypothesized that by rigidifying the piperidine core the spatial arrangement of the P_1' substituent would be more defined, which

† Dedicated to Professor Ralph F. Hirschmann on the occasion of his 85th birthday.

* To whom correspondence should be addressed. Tel.: 302-498-6707. Fax: 302-425-2704. E-mail: wyao@incyte.com.

[a] Abbreviations: HER-2, human epidermal growth factor receptor-2; ECD, extracellular domain; ADAM, a disintegrin and metalloprotease; MMP, matrix metalloprotease; TIMP-1, tissue inhibitor of metalloproteinase-1.

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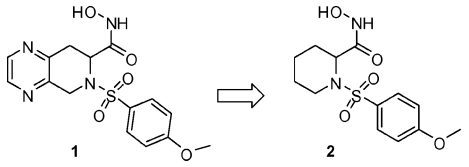


Figure 1. HB-EGF inhibitor structural leads.

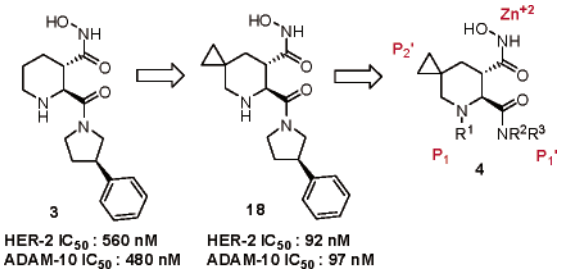
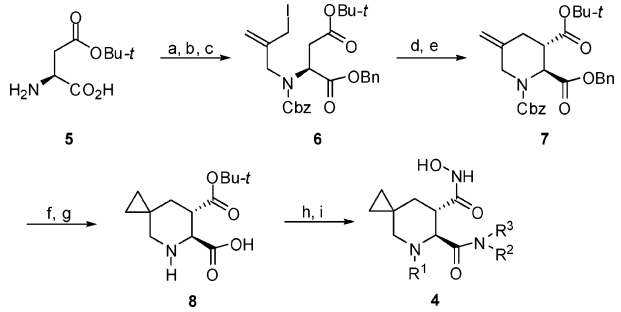


Figure 2. Design of spiro-pipecolic acid scaffold.

Scheme 1. Synthesis of Spiro-pipecolic Acid Analogs[a]



[a] Reagents and conditions: (a) BnBr, DBU, PhMe, 46%; (b) $CH_2{=}C(CH_2Cl)_2$, K_2CO_3, NaI, ACN, 70%; (c) NaI, acetone, 96%; (d) LiHMDS, THF, −78 to −30 °C, 72%; (e) CbzCl, 93%; (f) CH_2N_2, $Pd(OAc)_2$, 98%; (g) H_2, $Pd/BaSO_4$, MeOH, 98%; (h) (i) BOP, HNR^2R^3, DIEA, DMF; (ii) R^1X, DIEA, DCM; (i) (i) TFA, DCM; (ii) NH_2OH, BOP, DIEA, DMF.

would be beneficial in ascertaining information regarding the bioactive conformation from SAR studies. This hypothesis was supported by the 5-fold increase in potency observed for the C-5 cyclopropyl analog **18**. It was also speculated that the decrease in conformational flexibility may also enhance the oral bioavailability.[13]

Based on the above rational, a series of compounds with the general formula **4** were prepared from the designed polyfunctionalized chiral building block **8**, which was prepared from L-aspartic acid *β-tert*-butyl ester **5** and 3-chloro-2-(chloromethyl)-1-propene as depicted in Scheme 1.[13]

A series of secondary amides such as **9** were prepared and found to be inactive against the HER-2 sheddase. In contrast, 4-phenyl-piperazine **10** and 4-phenyl tetrahydropyridine **11** analogs exhibited excellent cellular activity against HER-2 sheddase (<60 nM, Table 1). The high degree of selectivity of these two compounds against MMP-1, which has a shallow S_1' pocket, and overall modest selectivity against MMP-2 and -9, which have a narrow and deep S_1' pocket, suggests that the S_1' pocket of the HER-2 sheddase might be similar to that of MMP-2 and -9. Replacing the tetrahydropyridine of compound **11** with a piperidine group resulted in an 8-fold loss in potency against HER-2 sheddase, but was compensated for by a substantial gain in selectivity against MMP-2 and -9, implying that there are subtle differences in the S_1' pocket between MMP-2/9 and the HER-2 sheddase.

4-Phenyl-tetrahydropyridine and 4-phenyl-piperazine groups prefer to adopt a coplanar conformation due to the π-system of the aromatic ring being in conjugation with the π-system of the tetrahydropyridine double bond or the lone pair of electrons on the piperazine nitrogen.[14] Conversely, the two ring systems of the 4-phenyl piperidine analog **12** prefer an orthogonal conformation with respect to one another. It was speculated that this difference in the orientation of the phenyl ring within the narrow and deep loop-3 region of the S_1' pocket may be exposing a subtle difference at residue 223, which is tyrosine for MMP-2, -3, and -9 and alanine for ADAM-10.

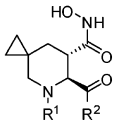
To explore this hypothesis, a series of compounds were prepared and evaluated that had varying torsion angles with respect to the P_1' aryl ring and the heterocycle that it is attached. Twisting the two ring systems out of coplanarity was achieved by either substituting the 3-position of the heterocycle ring, such as **13**, or the *ortho*-position of the aryl ring, such as **14**. Both of these designed compounds were found to be inactive in the HER-2 sheddase assay. However, introduction of a nitro group at the *para*-position of compound **14** to provide the 4-(2-methyl-4-nitrophenyl)piperazine analog **15** resulted in a dramatic increase in sheddase potency with excellent selectivity against other MMPs. In comparison to the unsubstituted 4-phenylpiperazine analog **10**, compound **15** displayed a slight loss in potency that was offset by the increase in selectivity against MMP-2 and -9. The corresponding 4-(2-methyl-4-nitrophenyl)-tetrahydropyridine compound **16** exhibited an increase in selectivity by more than 55-fold against MMP-2 and 3-fold against MMP-9 in comparison to the unsubstituted 4-phenyl-tetrahydropyridine analog **11**, albeit with a concomitant loss in potency. One explanation for this dramatic observed substituent effect is that the strong electron withdrawing properties of the nitro group render the aromatic π-system electron deficient and predisposed to electron donation, thus amplifying the conjugative effects and mitigating the steric strain of the *ortho*-methyl group. The counterbalancing effect of these two groups results in the aryl ring having an optimal torsion angle to achieve the desired selectivity.

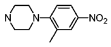
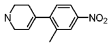
In an effort to mimic this optimal twisted coplanar conformation exhibited by the 4-(2-methyl-4-nitro-phenyl)piperazine analog **15**, the cyclic compound **17** was prepared (Table 1). Despite the slight loss in potency and selectivity against MMP-2 in comparison to compound **15**, there was a significant gain in potency in comparison to the unsubstituted 4-(2-methyl-phenyl)-piperazine analog **14**. These results support the hypothesis that the torsion angle between the heterocycle and appended aromatic ring is critical in determining the metalloprotease binding profile. An alternative approach to exploit the amino acid difference at position 223 was to change the directionality of the aryl ring by reducing the size of the heterocycle ring to which it was attached from a six- to a five-membered ring. 3-Phenylpyrrolidine **18** exhibited both exceptional potency against HER-2 sheddase and selectivity against MMPs (Table 1). The corresponding 3-phenylpyrrolid-3-ene **19** was 7-fold more potent, however, the selectivity against MMP-9 was diminished by 5-fold and the selectivity against MMP-2 was completely abrogated.

In summary, the above results suggest that the S_1' pocket of the HER-2 sheddase, like other metalloproteases, is extremely sensitive to minor spatial modifications of the P_1' substituent. This is manifested by the corollary that the orientation of the aryl group in the S_1' pocket can have dramatic effects on the binding properties, particularly with regard to the selectivity profile.

It was hypothesized that the scaffold piperidine N—H and the P_1' amide C═O could participate in hydrogen bonding and thus direct the orientation of the P_1' substituent. Substitution of

Table 1. In Vitro Data for P_1' and P_2' Substituents



			IC_{50} (nM)	Enzymatic Binding IC_{50} (nM)				
compd	R^1	R^2	HER-2[a]	ADAM-10[b]	MMP-1[c]	MMP-2[d]	MMP-3[e]	MMP-9[d]
9	Me	NH(*s*-Bu)	NA[f]		>5000	>1000	>1000	>5000
10	H		52	120	>5000	200	>5000	650
11	H		18	33	3900	5	1200	113
12	H		140	320	>5000	450	>5000	>5000
13	H		1000	720	>5000	>5000	>5000	>5000
14	H		NA	>5000	>5000	>5000	>5000	>5000
15	H		160	110	>5000	>1000	>1000	>5000
16	H		300	130	>5000	>5000	>5000	>5000
17	H		280	330	>5000	350	>5000	>5000
18	H		92	97	>5000	960	>3000	>3000
19	H		13	23	2500	13	450	77
20	Me		230	310	>5000	150	>5000	>5000
21	Me		160	77	>5000	64	>1000	>5000
22	CO_2Me		68	59	>5000	39	4000	428

[a] BT-474 cellular proliferation assay, see reference 8. [b] See reference 15. [c] See reference 16. [d] See reference 17. [e] See reference 18. [f] NA = not active.

the scaffold piperidine nitrogen would alleviate this interaction and twist the P_1' amide C═O to avoid steric and torsional strain, resulting in a different orientation for the P_1' substituent. In addition, substitution of the scaffold piperidine nitrogen may result in a change in the conformational distribution of the core piperidine ring. This global conformational change would also alter the orientation of the P_1' substituent and would modify the binding properties.

Methylation of the scaffold piperidine nitrogen resulted in a modest loss in potency and overall selectivity in comparison to the corresponding N—H compounds (Table 1, compounds **20** and **21**). However, there was a 2- to 5-fold gain in selectivity against MMP-9 for the piperazine **20** and tetrahydropyridine **21** N—Me analogs in comparison to their corresponding free N—H compounds **10** and **11**, respectively.

The introduction of a methyl carbamate to the spiro-piperidine core of compound **11**, which afforded compound **22**, resulted in a 4-fold decrease in HER-2 sheddase activity while maintaining a similar selectivity profile. However, comparison of the pharmacokinetic properties of compounds **11** and **22** indicated that compound **22** had a superior murine plasma exposure with an AUC of 3900 nM·h and a C_{max} of 7700 nM, following oral administration (Table 2).

In an effort to assess the working hypothesis, the pharmacodynamic properties of compound **22** were evaluated. In vivo, **22** (INCB3619)[8a] was shown to decrease HER-2 ECD plasma levels and tumor size in the HER-2 overexpressing human breast cancer mouse xenograft model BT-474-SC1 (Figure 3).[8] Compound **22** was also shown to potentiate the effects of humanized anti-HER-2 monoclonal antibody (trastuzumab) with regards to tumor size reduction in this model. These results support the

Table 2. PK Properties of **11** and **22**

			mouse PK (po)[a]	
cmpd	proj. Cl (L/h/kg)	PB (% free)	$AUC_{(0-24h)}$ (nM·h)	C_{max} (nM)
11	0.9	60	350	580
22	0.4	30	3900	7700

[a] Average of three animals administered at 10 mg/Kg as a suspension in 10% DMAC in 0.5% methylcellulose.

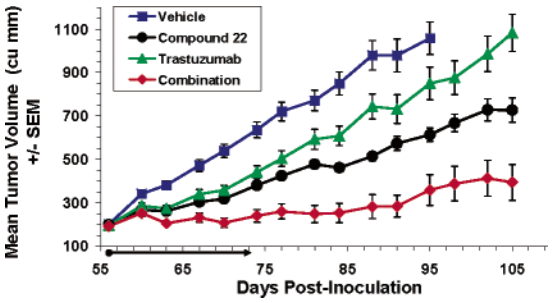


Figure 3. Combination effect of **22** and trastuzumab in a HER-2 overexpressing human breast cancer mouse xenograft model BT-474-SC1. Compound **22** was administered subcutaneously using ALZET osmotic pumps at 30 mg/kg/d for 28 d, as indicated by the black arrow below the *x*-axis. Tumor volumes are shown as mean values with error bars (SEM; $n = 7$).[8]

working hypothesis that inhibition of HER-2 shedding in cancer cell lines that overexpress HER-2 can be therapeutically beneficial and may potentiate the anti-cancer effects of existing chemotherapeutics in a clinical setting.

In conclusion, a novel class of conformationally restricted azaspiro-hydroxamic acids has been developed to inhibit the protease that is responsible for HER-2 shedding, which has been implicated in the pathogenesis of various cancers. The potency and selectivity profile was optimized by exploiting the subtle differences in the S_1' pocket of the MMPs and the HER-2 sheddase by attenuating the P_1' substituent, in particular the orientation of the aryl group. The encouraging in vitro and in vivo data prompted comprehensive SAR, pharmacodynamic, and pharmacokinetic studies, and the results are impending.

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Supporting Information Available: Analytical data and experimental protocols described in this letter. This material is available free of charge via the Internet at http://pubs.acs.org.

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