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Design and identification of selective HER-2 sheddase inhibitors via P1′ manipulation and unconventional P2′ perturbations to induce a molecular metamorphosis

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Abstract—In an effort to obtain a MMP selective and potent inhibitor of HER-2 sheddase (ADAM-10), the P1′ group of a novel class of (6*S*,7*S*)-7-[(hydroxyamino)carbonyl]-6-carboxamide-5-azaspiro[2.5]octane-5-carboxylates was attenuated and the structure–activity relationships (SAR) will be discussed. In addition, it was discovered that unconventional perturbation of the P2′ moiety could confer MMP selectivity, which was hypothesized to be a manifestation of the P2′ group effecting global conformational changes.

The human epidermal growth factor receptor-2 (HER-2 or ErbB-2) is a tyrosine kinase receptor that is activated by ligand induced homo- and heterodimerization of the extracellular domain (ECD) between two members of the HER family or by proteolytic cleavage (shedding) of the 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 (NSCLC), ovarian cancer, and breast cancer patients.[1a] In addition, elevated plasma levels of HER-2ECD have been associated with metastasis 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 shedding is therapeutically desirable for treating cancer patients that overexpress HER-2.

We recently identified ADAM-10 as a major source of HER-2 ectodomain sheddase activity in HER-2 overexpressing breast cancer cells[4a] and reported the design, synthesis, evaluation, and identification of a novel class of (6*S*,7*S*)-7-[(hydroxyamino)carbonyl]-6-carboxamide-5-azaspiro[2.5]octane-5-carboxylates as the first potent and selective inhibitors of HER-2 sheddase.[4b] Now, we wish to report the results of a more comprehensive investigation of the structure–activity relationships with regard to the P1′ and P2′ substituents (see Scheme 1).

A series of hydroxamic acids of general formula **3** were prepared using sequential BOP reagent mediated amide coupling reactions starting from the designed polyfunctionalized chiral building block **1**, which was prepared by the methods that were previously disclosed starting from L-aspartic acid β-*tert*-butyl ester and 3-chloro-2-(chloromethyl)-1-propene.[4b,5]

Previously we reported that 4-phenyl–piperazine **4**, tetrahydropyridine **5**, and piperidine **6** hydroxamic acids

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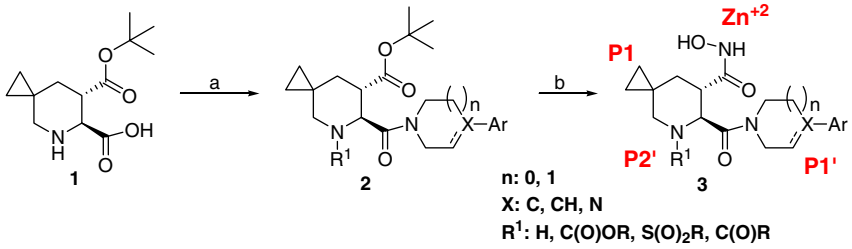
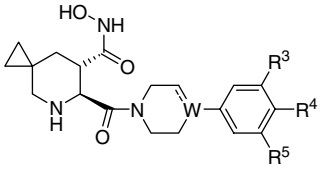


Scheme 1. Reagents: (a) i—BOP, amine, DIEA, DMF; ii—*i*-Pr_2EtN, R^1-Cl; (b) i—TFA, DCM; ii—NH_2OH, BOP, DIEA, DMF.

exhibited excellent cellular inhibition of HER-2 shedding (<140 nM) with excellent selectivity against MMP-1 and -3, modest to excellent selectivity against MMP-9, and negligible selectivity against MMP-2 (Table 1).[4b] Achieving selectivity toward MMP-2 and -9 proved to be an arduous task, particularly toward MMP-2, due to the homology of these metalloproteases within the active site. We found that selectivity against MMP-2 and -9 could be accomplished by slightly perturbing the torsion angle between the P1′ aryl ring and the heterocyclic ring to which it is attached.[4b] We speculated that the observed selectivity was due to a subtle difference at position 223 within the narrow and deep loop 3 region of the S1′ pocket, which for MMP-2, -3, and -9 is a tyrosine residue and for ADAM-10 is an alanine. For the current study, we hypothesized that the additional space that is created due to the smaller Ala residue at 223 of ADAM-10 might be exploited by substituting the P1′ phenyl ring at the 3-position to obtain MMP selectivity. Thus, a series of 3-substituted-aryl hydroxamic acids were prepared and the results are outlined in Table 1. Selectivity was assessed by comparing the enzymatic binding data of ADAM-10 to MMP-1, -2, -3, and -9.

The 3-methyl-, 3-ethyl-, and 3-methoxy-phenyl-tetrahydropyridine compounds **7**, **8**, and **9** did not show any indication of enhanced selectivity and were found to be 2- to 13-fold less potent against HER-2 sheddase in comparison to the unsubstituted phenyl-tetrahydropyridine **5** (Table 1). The 3-isopropyl-phenyl-tetrahydropyridine analog **10** displayed a similar loss in potency that was compensated for by a profound improvement in the selectivity against MMP-2 (>15-fold), MMP-9 (10-fold), and MMP-3 (2-fold). Exchanging the 3-isopropyl group of **10** for a cyclopropyl group completely

Table 1. Investigation of various substituents in the 3-position of the P1′ aryl ring



Compound	Bond	W	R^3	R^4	R^5	HER-2[a] (nM)	Enzyme Binding (nM)				
							ADAM-10	MMP-1	MMP-2	MMP-3	MMP-9
4	Single	N	H	H	H	52	118	>5000	195	>5000	650
5	Double	C	H	H	H	18	33	3878	5	1237	113
6	Single	CH	H	H	H	137	324	>5000	446	>5000	>5000
7	Double	C	Me	H	H	42	25	1892	13	359	111
8	Double	C	Et	H	H	240	33	>5000	21	—	190
9	Double	C	OMe	H	H	194	40	>5000	50	—	110
10	Double	C	*i*-Pr	H	H	229	73	>5000	1110	4999	2500
11	Double	C	Cyclopropyl	H	H	162	48	—	24	—	—
12	Double	C	Me	H	Me	86	85	>5000	433	>5000	>5000
13	Double	C	*i*-Pr	CN	H	902	154	—	2500	—	—
14	Double	C	Me	CN	Me	129	28	>5000	159	3500	>5000
15	Double	C	F	H	F	24	15	>5000	14	>5000	916
16	Double	C	Cl	H	Cl	NA[b]	99	—	4999	—	—
17	Single	N	Me	H	H	184	187	>5000	63	>1000	1268
18	Single	N	Me	CN	H	594	38	—	<5	—	—
19	Single	N	Cl	H	H	42	14	—	32	—	—
20	Single	N	*i*-Pr	H	H	NA	1102	—	>5000	—	—
21	Single	CH	Me	H	H	689	194	>5000	391	>5000	604
22	Single	CH	*i*-Pr	H	H	139	16	—	1387	>5000	—

[a] Values are obtained from a BT-474 cellular HER-2 ECD shedding assay.
[b] (NA, not active).

abated the MMP-2 selectivity. The 3,5-dimethyl derivative **12** exhibited a substantial gain in selectivity toward MMP-2 (>5-fold) and MMP-9 (17-fold) in comparison to the unsubstituted parent phenyl compound **5**; albeit with a concomitant loss in HER-2 sheddase potency.

Our previously disclosed exploratory studies indicated that placement of an electron withdrawing group (EWG) in the 4-position of the P1′ aromatic ring may lead to a cogent increase in potency.[4b] Therefore, a cyano group was placed in the 4-position of the MMP-selective 3-isopropyl analog **10** and the 3,5-dimethyl-phenyl tetrahydropyridine analog **12** in an effort to boost the potency while maintaining the selectivity. The 4-cyano-3-isopropyl-phenyl analog **13** was found to be less potent than **10** in both the enzymatic ADAM-10 and cellular HER-2 assays. In contrast, the 4-cyano-3,5-dimethyl-phenyl analog **13** was 3-fold more potent than **12** in the enzymatic ADAM-10 binding assay and retained the excellent selectivity against other MMPs; despite a slight loss in cellular HER-2 potency.

Alternatively, the aryl ring of the 3,5-dimethyl-phenyl tetrahydropyridine analog **12** was made electron deficient by directly substituting the methyl groups for EWGs. Thus, the 3,5-difluoro-derivative **15** was prepared and found to have the desired increase in potency in comparison to **12**; however, the selectivity against MMP-2 was abrogated. The slightly larger 3,5-dichloro-phenyl derivative **16** showed diminutive MMP-2 binding and HER-2 activity, possibly indicating that the outer limits of the spatial diameter within the S1′ pocket were reached. Overall, these results imply that the electronic disposition of the aromatic ring does not play as crucial a role in determining the binding properties for the tetrahydropyridine series as for the previously published piperazine series.[4b] This is in agreement with the initial proposed hypothesis that the role of the EWG in the piperazine series is to promote coplanarity between the piperazine and phenyl rings by facilitating electron donation from the piperazine nitrogen lone pair to the electron deficient aromatic π-system.[4b] For the tetrahydropyridine series, conjugative factors predispose the P1′ heterocycle and aryl ring to be coplanar; therefore, steric interactions appear to be the main determinant for the observed metalloprotease binding affinities for this series.

This hypothesis was further explored by preparing a series of 3-aryl substituted piperazine compounds (Table 1, compounds **17**–**20**). The 3-methyl aryl piperazine analog **17** had a higher binding affinity toward MMP-2 than HER-2 sheddase. Efforts to modify the torsion angle between the phenyl and piperazine rings in a favorable manner, as previously discussed, by the addition of a cyano group in the 4-position to afford analog **18** afforded another potent MMP-2 inhibitor. Replacement of the methyl group of **17** with an EWG to afford the 3-Cl analog **19** resulted in a notable gain in HER-2 potency, which was inconsequential due to a lack of MMP-2 selectivity. Based on the encouraging MMP-2 selectivity of the 4-(3-iso-propyl-phenyl)tetrahydro-pyridine compound **10**, the corresponding piperazine analog **20** was prepared and was found to be inactive against both HER-2 sheddase and MMP-2, which may imply that the tetrahydropyridine and piperazine ring systems have subtle conformational differences that must orient the attached phenyl ring and thus the 3-isopropyl group slightly differently within the narrow S1′ pocket.

To further explore the role of the P1′ heterocycle in orienting the aryl ring within the S1′ pocket, the 3-methyl and 3-isopropyl-phenyl piperidine compounds **21** and **22** were prepared. The 3-methyl analog **21** was found to be only slightly more selective than the unsubstituted parent phenyl piperidine compound **6** against MMP-2 and was 5-fold less selective against MMP-9. In contrast, the 3-isopropyl analog **22** displayed an 80-fold improvement in selectivity toward MMP-2 in comparison to **6** and a 19-fold accession in ADAM-10 potency. Comparison of the piperidine compound **22** to the tetrahydropyridine analog **10** indicates a 4-fold increase in ADAM-10 potency and a 6-fold increase in selectivity toward MMP-2. The improved binding profile of **22** may be a manifestation of a distortion in the dihedral angle between the P1′ heterocyclic ring and the aromatic ring, as previously mentioned.

In summary, excellent selectivity toward MMP-2 and -9 can be achieved by the introduction of a small substituent at the 3-postion of the P1′ phenyl ring. The effect that the 3-aryl substituent has on the metalloprotease binding profile is contingent on the P1′ heterocycle, since this predetermines the spatial orientation of the P1′ aryl ring and the appended 3-substituent. Based on the data, it is plausible that the P1′ 3-aryl substituent is occupying the space created by the Ala 223 of ADAM-10 and is interacting unfavorably with Tyr 223 of MMP-2 and -9, as originally proposed. The 3-isopropyl moiety appears to be the optimal group for this small lipophilic pocket rendering analogs **10** and **22** with excellent MMP-2 and -9 selectivity. The piperidine ring emerges as the optimal P1′ heterocycle for orienting the 3-isopropyl phenyl moiety within the fastidious S1′ pocket. Furthermore, the data suggests that the diameter of the S1′ pocket constricts with the following hierarchy: HER-2 > MMP-2 > MMP-9.

Previously, it was found that the metalloprotease binding properties could be attenuated by substitution of the core scaffold piperidine N–H.[4b] It was determined that simple N-methylation of the core nitrogen of compounds **4** and **5** resulted in a 2-fold loss in potency in the enzymatic ADAM-10 assay and a 4- and 9-fold loss in HER-2 cellular potency, respectively. Installation of a methyl carbamate to the core nitrogen of **5** had a similar effect on the binding profile; however, the pharmacokinetic properties of the carbamate were superior to the parent N–H compound **5**. In an effort to further explore the SAR of the P2′ group, a series of scaffold N-substituted analogs were prepared and the results are depicted in Table 2.

Comparison of the phenyl-tetrahydropyridine *N*-carbamates **23**, **24**, and **25** to their parent N–H compound **5** re-

Table 2. Investigation of the P2′ and P1′ substituents

Compound	R^1	R^2	HER-2[a] (nM)	Enzyme binding[a] (nM)				
				ADAM-10	MMP-1	MMP-2	MMP-3	MMP-9
23	CO_2Me	4-Ph-Tetrahydropyridine	68	59	>5000	39	4000	428
24	CO_2Bu-*i*	4-Ph-Tetrahydropyridine	150	19	>5000	35	2601	451
25	Cbz	4-Ph-Tetrahydropyridine	431	34	>5000	37	1669	309
26	C(O)Me	4-Ph-Tetrahydropyridine	72	40	>5000	50	>5000	160
27	SO_2Me	4-Ph-Tetrahydropyridine	93	51	>5000	53	>5000	677
28	C(O)OMe	4-Ph-Piperidine	169	19	—	472	—	—
29	CO_2THP[b]	4-Ph-Piperazine	26	37	>5000	106	4000	2623
30	SO_2Me	4-Ph-Piperazine	45	27	>5000	86	>5000	1499
31	H	3-Ph-Pyrrolidine	92	97	>5000	960	>3000	>3000
32	C(O)OMe	3-Ph-Pyrrolidine	289	81	—	>5000	—	—
33	H	3-Ph-Pyrrolid-3-ene	13	23	2500	13	450	77
34	C(O)OMe	3-Ph-Pyrrolid-3-ene	34	26	>5000	488	>5000	3048

[a] Values are obtained from a BT-474 cellular HER-2 ECD shedding assay.
[b] 4-Tetrahydropyran (THP).

veals that the selectivity toward MMP-3 and -9 is markedly enhanced, while the ADAM-10 and MMP-2 binding affinities remain impervious. The observed loss in cellular HER-2 potency as the alkoxy group distended may be attributed to a change in physiochemical properties. The phenyl-tetrahydropyridine *N*-acetyl and methylsulfonamide compounds **26** and **27** possessed a binding profile that closely resembled that of the corresponding methyl carbamate **23**. The phenyl–piperidine *N*-methyl-carbamate **28** had a 19-fold increase in MMP-2 selectivity and a 17-fold boost in ADAM-10 binding in comparison to the parent N–H compound **6**. The 4-phenylpiperazine carbamate **29** exhibited a considerable improvement in potency and an ~2-fold gain in selectivity against MMP-2 and a 13-fold improvement in selectivity toward MMP-9 in comparison to the parent N–H compound **4**. The methylsulfonamide analog **30** had a metalloprotease binding profile similar to the carbamate **29**. The 3-phenylpyrolidine methyl carbamate analog **32** had a 5-fold increase in MMP-2 selectivity in comparison to the parent N–H compound **31**. The 3-phenylpyrolid-3-ene methyl carbamate derivative **34** had an even more pronounced amelioration in selectivity toward MMP-2 (37-fold), MMP-9 (40-fold), MMP-3 (11-fold), and MMP-1 (2-fold) in comparison to the parent N–H compound **33**. For both of the methyl carbamate analogs **32** and **34**, the ADAM-10 affinity was similar to their corresponding parent N–H compounds **31** and **33**; however, both analogs suffered a ~3-fold loss in cellular HER-2 sheddase potency. This discrepancy can be explained by the alteration in physiochemical properties.

Overall, it appears that substitution of the scaffold piperidine N–H with an EWG can result in increased selectivity, especially with regard to MMP-3 and -9, often with either negligible or positive repercussions to the desired HER-2 potency. It is speculated that the observed influence that the P2′ group has on the metalloprotease binding affinity is not due to direct interactions between the P2′ substituent and the S2′ subsite, since the S2′ subsite is believed to be primarily solvent exposed. Instead, the variation in binding affinity may be an indirect manifestation of the P2′ group inducing global conformational changes due to $A^{(1,3)}$ strain, thus shifting the orientation of the P1′ group within the highly sensitive S1′ pocket. The data presented in Table 2 support this hypothesis indicating that the influence conferred by the P2′ substituent on the enzyme binding profile is interconnected to the P1′ group and that the nature of the P2′ group, so long as it is an EWG, is insignificant. It is also reasonable that the P2′ substituent may have an allosteric affect on the HER-2 sheddase active site, thus altering the disposition of the S1′ pocket and the concomitant interactions with the P1′ group.

In conclusion, the metalloprotease binding profile can be attenuated to selectively inhibit HER-2 sheddase by installing a substituent at the 3-position of the P1′ aro-

Table 3. PK properties of **23** and **34**

Compound	h-PB (% free)	Mouse PK[a] (po)		$T_{1/2}$ (h)
		$AUC_{(0-24\,h)}$ (nM h)	C_{max} (nM)	
23	30	3900	7700	1.9
34	34	2800	3000	1.8

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

matic ring or an EWG, such as a carbamate, on the scaffold piperidine nitrogen. In both cases the heterocycle to which the P1′ aromatic ring is attached plays a critical role in determining the effect that the substituent will have on the metalloprotease binding profile. Future SAR studies will examine the corollaries between modifying both of these variables simultaneously on the same compound.

Several lead compounds were identified that possessed superior selectivity against MMP-2 and -9 in comparison to the formerly reported lead compound **23** (INCB3619).[4b] Most notably compound **34** exhibited a 2-fold improvement in potency accompanied by a 19-fold and a 16-fold enhancement in selectivity toward MMP-2 and -9, respectively. Discrete oral pharmacokinetic murine studies indicated that **23** had a slightly higher plasma exposure than **34**, but based on the exceptional potency and selectivity exhibited by **34** further pharmacokinetic and pharmacodynamic studies are warranted and impending (Table 3).

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