Characterization of Selective Inhibitors of Matrix Metalloproteinase 13 That Prevent Articular Cartilage Degradation *In Vitro*

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SUPPLEMENTAL DATA

Table 1. Enzymes and Substrates used in protease profiling of MMP-13 inhibitors.

Enzyme name	Peptide substrate	Enzyme Source/Form	Enzyme Purity	Supplier	Cat #
ACE	_RPFNYLAK_	Recombinant Human ACE/CD143, Leu30-Leu126 with a C-terminal His-tag, expressed in mammalian cell line	>95%, by SDS-PAGE	R&D Systems	929-ZN
ACE2	_VYPSP_	Recombinant Human ACE2, Gln18-Ser740, with a Cterminal His-tag, expressed in mammalian cell line	>90%, by SDS-PAGE	R&D Systems	933-ZN
ADAM10	_DFVRAARR_	Recombinant Human ADAM10, Thr214 Glu672, with a C-terminal His-tag, expressed in baculoviral system	>90%, by SDS-PAGE	R&D Systems	936-AD
BACE1	_SEVNLDAEFRKKRR_	Recombinant Human beta-Secretase/BACE, full-length, expressed in baculoviral system	ND	Invitrogen	P2947
CASPASE 1	_WEHDGPKR_	Recombinant Human Asn120-His-404, expressed in E.Coli	>90%, by SDS-PAGE	Biomol	SE-168
CASPASE 2	_DEVDAA_	Recombinant Human Asn-150-Thr-435, expressed in E.Coli	>95%, by SDS-PAGE	Biomol	SE-175
CASPASE 3	_DEVDAA_	Recombinant Human Full-length, expressed in E.Coli	>95%, by SDS-PAGE	Biomol	SE-169
CASPASE 5	_LEHDGP_	Recombinant Human Gln-122 to Asn-418, expressed in E.Coli	>95%, by SDS-PAGE	Biomol	SE-171
CASPASE 6	_VEHDGG_	Recombinant Human Ala-24 to Asn-293, expressed in E.Coli	>80%, by SDS-PAGE	Biomol	SE-170
CASPASE 7	_DEVDAA_	Recombinant Human Ala-24 to Gln-303, expressed in E.Coli	>95%, by SDS-PAGE	Biomol	SE-177
CATHEPSIN D	_EVNLDAEFRK_	Native Human	>98%, by SDS-PAGE	Calbiochem	219394
CATHEPSIN K	_HHQKLVFFAE_	Recombinant Human, Full length, with C-terminal His-tag, expressed in E.Coli	>95%, by SDS-PAGE	Calbiochem	219461
CATHEPSIN L	_GGALRAG_	Recombinant Human, Full length, with C-terminal His-tag, expressed in P.Pastoris	>95%, by SDS-PAGE	Calbiochem	219382
CATHEPSIN S	_RTLTAK_	Recombinant Human, Full length, expressed in E.Coli	>90%, by SDS-PAGE	Calbiochem	219343
FACTOR XA	_ALPRTMFIQ_	Native Human	100% by SDS-PAAGE	Calbiochem	233526
FURIN	_RRVKRSLD_	Recombinant Human Furin, Asp108 Glu715, with a C-terminal His-tag, expressed in mammalian cell line	>95%, by SDS-PAGE	R&D Systems	1503-SE
IDE	_RPPGFSAF_	Recombinant Human Insulysin/IDE, Met42 Leu1019, with N-terminal His-tag, expressed in baculoviral system	>95%, by SDS-PAGE	R&D Systems	2496-ZN
MMP1	_GRIGFL_	Recombinant Human, Phe100-Gln268, Expressed in E.Coli	>95%, by SDS-PAGE	Biomol	SE-180
MMP12	_GRIGFL_	Recombinant Human, Phe99-Leu271, Expressed in E.Coli	>95%, by SDS-PAGE	Biomol	SE-138
MMP13	_GRIGFL_	Recombinant Human, Tyr104-Asn274, Expressed in E.Coli	>95%, by SDS-PAGE	Biomol	SE-246
MMP14	_GRIGFL_	Recombinant Human, Tyr112-Arg298, Expressed in E.Coli	>95%, by SDS-PAGE	Biomol	SE-259
MMP2	_GRIGFL_	Recombinant Human, Tyr110-Asp452, Expressed in E.Coli	>95%, by SDS-PAGE	Biomol	SE-237
MMP3	_GRIGFL_	Recombinant Human, Phe100-Thr272, Expressed in E.Coli	>95%, by SDS-PAGE	Biomol	SE-109
MMP7	_GRIGFL_	Recombinant Human, Tyr95-Lys267, Expressed in E.Coli	>95%, by SDS-PAGE	Biomol	SE-181
MMP8	_GRIGFL_	Recombinant Human, Phe99-Gln269, Expressed in E.Coli	>90%, by SDS-PAGE	Biomol	SE-255
MMP9	_GRIGFL_	Recombinant Human, Phe107-Pro449, Expressed in E.Coli	>95%, by SDS-PAGE	Biomol	SE-244
NEPRILYSIN	_TSSVEPY_	Recombinant Human Neprilysin/CD10, Tyr52-Trp750, with N-terminal His-tag, expressed in mammalian cell line	>95%, by SDS-PAGE	R&D Systems	1182-ZNC
TACE	_PLAQAVRSSSR_	Recombinant Human TACE/ADAM17, Arg215-Asn671, with a C-terminal His-tag, expressed in baculoviral system	>90%, by SDS-PAGE	R&D Systems	930-ADB
THROMBIN	_PRTLT_	Recombinant Human Coagulation Factor II/Thrombin, Met1-Glu622, with a C-terminal His-tag, expressed in mammalian cell line	>95%, by SDS-PAGE	R&D Systems	1473-SE
UPA	_GRTSSVEP_	Recombinant Human uPlasminogen activator/Urokinase, Met1-Leu432, with a C-terminal His-tag, expressed in mammalian cell line	>95%, by SDS-PAGE	R&D Systems	1310-SE

Table 2. CYP450 Inhibition assays conditions.

CYP450 Isoform	Substrate	[S], µM	[HLM], mg/mL	Incubation Time, min	Metabolite (mass transition), amu	Internal standard (mass transition), amu
CYP1A2	Tacrine	1	0.2	10	1-hydroxytacrine (215→182)	Bucetin (224→136)
CYP2B6	Bupropion	80	0.2	20	OH-Bupropion (256→139)	OH-Bupropion-[D_6] (262→244)
CYP2C8	Amodiaquine	1.5	0.02	5	Desethylamodiaquine (330→285)	Desethylamodiaquine-[D_3] (333→285)
CYP2C9	Diclofenac	5	0.05	5	4´-OH Diclofenac (312→268)	4´-OH Diclofenac-[$^{13}C_6$] (316→272)
CYP2C19	(S)-Mephenytoin	40	0.3	10	4´-OH (*S*)-Mephenytoin (235→150)	4´-OH (*S*)-Mephenytoin-[D_3] (238→150)
CYP2D6	Dextromethorphan	5	0.1	5	Dextrorphan (258→157)	Dextrorphan-[D_3] (261→157)
CYP3A4	Midazolam	3	0.02	5	1´-OH Midazolam (342→203)	1´-OH Midazolam-[$^{13}C_3$] (347→208)
CYP3A4	Testosterone	50	0.05	10	6ß-OH Testosterone (305→269)	6ß-OH Testosterone-[D_7] (312→276)

Table 3: X-ray data collection and protein structure refinement statistics.

Data collection	
Space group	*C*2
a (Å)	135.3
b (Å)	36.0
c (Å)	95.8
β (°)	130.2
Resolution range (Å)[a]	47.8-1.66 (1.75-1.66)
Wavelength (Å)	0.97950
Redundancy	3.7 (3.8)
Completeness (%)	98.5 (99.0)
$I/\sigma I$	15.2 (2.0)
R_{sym} (%)[b]	5.2 (59.0)
Wilson value	26.3
Refinement	
Number of monomers/AU	2
R_{cryst} (%)[c]	13.4
R_{free} (%)[d]	19.9
Rmsd bonds (Å)	0.005
Rmsd angles (°)	0.939
Ramachandran outliers (%)[e]	0.6
No. protein atoms	2,694
No. solvent atoms	268
No. ligand atoms	99
Avg. protein B-factors (Å^2)	32.3
Avg. solvent B-factors (Å^2)	45.5
Avg. ligand B-factors (Å^2)	48.8

[a]The number in parentheses is for the highest resolution bin. [b]$R_{sym} = \sum_{hkl}|I - \langle I\rangle|/\sum_{hkl}\langle I\rangle$ where I is the observed intensity and $\langle I\rangle$ is the average intensity of multiple symmetry-related observations of that reflection. [c]$R_{cryst} = \sum_{hkl}\big||F_{obs}| - |F_{calc}|\big|/\sum_{hkl}|F_{obs}|$. [d]$R_{free} = \sum_{hkl}\big||F_{obs,t}| - |F_{calc}|\big|/\sum_{hkl}|F_{obs,t}|$ where $|F_{obs,t}|$ is from a test set not used in the structural refinement. [e]Ramachandran plots were calculated using COOT.

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Supplemental Figures.

Supplemental Figure 1.

Supplemental Figure 1. (A) MMP-12 dose response results for the compound Q series. (B) Cathepsin K dose response results for compound Q series.