Bioorganic & Medicinal Chemistry Letters xxx (2015) xxx–xxx

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

Structure-guided discovery of 2-aryl/pyridin-2-yl-1*H*-indole derivatives as potent and selective hepsin inhibitors

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ARTICLE INFO

Article history:
Received 7 July 2015
Revised 15 September 2015
Accepted 17 September 2015
Available online xxxx

Keywords:
Hepsin
Indole
Molecular docking
Crystallography
Cancer

ABSTRACT

Hepsin, a type II transmembrane serine protease, is upregulated in prostate cancer and known to be involved in the progression of metastasis. Here we report a structure-guided approach, which resulted in the discovery of 2-aryl/pyridin-2-yl-1*H*-indole derivatives as potent and selective inhibitors of hepsin. Potent and selective inhibition of hepsin by compound **8** is likely due to interactions of the amidine group at the S1 site with the cyclohexyl ring from the 2-aryl group projecting towards the S1′ site and the *tert*-hydroxyl group interacting with His57 side-chain as revealed by X-ray crystallography. Compounds **8** and **10**, showed K_i of 0.1 μM for hepsin, and exhibited inhibition of invasion and migration of hepsin-overexpressing cell line. Compounds described here could serve as useful tool reagents to investigate the role of hepsin as a potential therapeutic target in cancer.

Prostate cancer is one of the most common cancers with >233,000 newly diagnosed cases and nearly 30,000 prostate cancer related deaths in USA last year.[1] Most prostate cancer–related deaths are due to advanced disease, which results from metastasis to distant organs and is partly mediated by extracellular proteases belonging to matrix metalloprotease and serine protease family. Among the extracellular proteases, expression of hepsin, a type II transmembrane serine protease, is found to be upregulated more than 10-fold in prostate tumors,[2] and reported to be involved during initiation and progression of prostate cancer mestastasis.[3] Artificial expression of hepsin in mouse prostate epithelium has been demonstrated to contribute to matrix degradation, invasion and metastatic progression of prostate cancer.[4] Hepsin is a potential prognostic marker of prostate cancer for predicting the clinical outcome of the disease.[5] Studies with Probasin–hepsin/Large Probasin-T antigen (PB-hepsin/LPB-Tag) bigenic mouse model of prostate cancer showed that hepsin along with MYC impact on progression of prostate adenocarcinoma.[6] Recent findings[7] suggest that hepsin promotes prostate cancer metastasis mainly to the bone marrow. Biological function of hepsin supports its targeting for cancer therapy. Hepsin knockout mice exhibits non-lethal phenotype and therefore inhibition of hepsin could be an attractive option as a targeted therapy with few adverse effects.[8] Overexpression of hepsin in metastatic prostate cancer as compared to normal prostate could translate to specifically targeting malignant cells. Taken together, the available data suggest that hepsin represents a potential target for the treatment of prostate cancer.

The three dimensional X-ray structure of soluble human hepsin containing the scavenger receptor-like cysteine-rich domain with the protease domain exhibits structural similarity to enteropeptidase and matriptase/MT-SP1 belonging to chymotrypsin-like type II transmembrane serine proteases.[9] Hepsin consists of a cysteine rich scavenger receptor domain and protease domain linked by disulfide bond.[9] Laminin 332,[10] pro-urokinase,[11] pro-hepatocyte growth factor and pro-MSP[12] are reported to be the substrates for Hepsin. Kunitz-type serine protease inhibitors, HAI-1 and HAI-2 regulate HGF activity through inhibition of hepsin action.[12]

During the last decade, only a limited number of published reports on small molecule hepsin inhibitors became available.[7,13,14] Recently, a 3-amidino-phenylalanine sulfonamide based hepsin inhibitor with $K_i \sim 50$ nM and >90-fold selectivity against matriptase and HGFA was reported.[14] As part of our ongoing endeavor in discovering serine protease inhibitors[15,16] for treatment of cancer,

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http://dx.doi.org/10.1016/j.bmcl.2015.09.042
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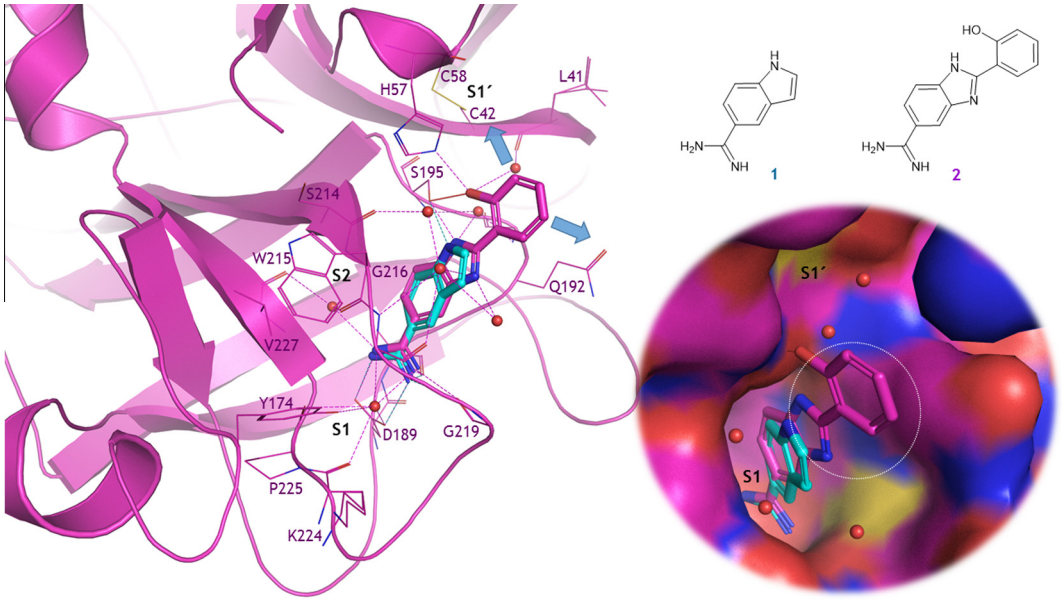
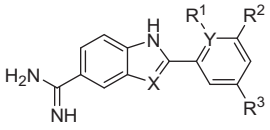
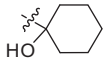
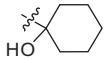


Figure 1. Docked model of **1** overlaid against the crystallographic binding mode of **2** (a 2-aryl substituted 5-amidino benzimidazole compound) in hepsin CD (PDB ID: 1P57). K_i of **2** for hepsin = 40 μM.[9]

Table 1
SAR of 2-aryl/pyridin-2-yl-1*H*-indole derivatives



ID	X	Y	R^1	R^2	R^3	K_i[a] in μM or (% inhibition at 5 μM)[b]						
						Hepsin	Matriptase	uPA	Factor Xa	Thrombin	Plasmin	Trypsin
2	N	C	OH	H	H	40.0[c]	NA	5.5[c]	14.0[c]	55.0[c]	46.0[c]	23.0[c]
3	C	C	OH	H	H	35.0	60.0	2.4[d]	2.7[d]	13.0[d]	12.0[d]	8.0[d]
4	C	C	OH		H	4.9	4.4	*(2)*	1.0	1.9	*(5)*	0.8
5	C	C	OH		Cl	4.8	4.8	*(12)*	NA	NA	NA	NA
6	C	C	OH		H	1.4	0.5	*(9)*	NA	NA	NA	NA
7	C	N	—		H	0.6	1.5	2.3	NA	NA	NA	NA
8	C	N	—		H	0.1	2.1	1.3	1.4	2.1	*(24)*	0.4
9	C	N	—		H	0.4	0.8	2.7	0.7	3.6	*(11)*	0.5
10	C	N	O		H	0.1	1.1	7.8	8.2	8.6	*(11)*	0.8

'NA' indicates that the compound was not assayed against the mentioned enzyme.
[a] Average of two values obtained from two independent dose response studies.
[b] % Inhibition: K_i was not determined for compounds which showed ⩽30% inhibition at 5 μM concentration.
[c] Values from Ref. 9.
[d] Values from Ref. 20.

high throughput screening of commercially available amidine-substituted fragments resulted in identification of sub mM hits, as exemplified by **1** which exhibited a $K_i \sim 500$ μM towards hepsin catalytic domain (CD). Compound **1** is a non-specific compound, known to weakly inhibit serine proteases like plasmin, urokinase, thrombin and trypsin.[17] The present article describes the