Identification of Sulfamoylbenzamide derivatives as selective Cathepsin D inhibitors

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Abstract: Aspartic proteases play very important role in post translational processing of proteins and several of them are essential for organism's viability. Here we present the enzyme inhibition activities of different Sulfamoylbenzamide derivatives against two aspartic proteases cathepsin D and plasmepsin II. Cathepsin D is an aspartic protease that degrades proteins at acidic pH in the lysosomes, or extracellular matrix. It is overexpressed by epithelial breast cancer cells and hence hyper-secreted. On the other hand plasmepsin II is an essential enzyme of *Plasmodium falciperum.* Cathepsin D and Plasmepsin II are pivotal drug targets for treatment of breast cancer and malaria respectively. Virtual screening of Sulfamoylbenzamide compounds followed by enzyme inhibition assays revealed these compounds as selective Cathepsin D inhibitors while inactive against Plasmepsin-II. IC_{50} values of five Sulfamoylbenzamide compounds tested are in range of 1.25-2.0 μM. N-(3-chlorophenyl)-2-sulfamoylbenzamide is identified as the most potent of all tested Sulfamoylbenzamide compounds with IC_{50} 1.25 μM. It was also noted that the docking score of theses compounds was better in case of Cathepsin D as compared to Plasmepsin-II. Docking score ranges from -29.9±1.16 to -35.1±0.13 in case of Cathepsin D, while from -24.0±0.10 to -29.5±0.10 in case of Plasmepsin-II.

Keywords: Aspartic proteases, Cathepsin D, Plasmepsin-II, enzyme inhibition.

INTRODUCTION

Proteases control several key functions of cells such as extravasation, invasion, motility, proliferation and metastasis (Smith and Marshall 2010; Roy *et al.,* 2009; Palermo and Joyce 2008). Among these, cathepsin proteases play main regulatory role as the factor-binding proteins and growth factors and their receptor activities have a major biological function in development of human colorectal cancer (Sakkiah *et al*., 2012., Masson *et al.*, 2010; Rawlings and Barrett, 1993). Human cathepsin D is an aspartic protease which functions at acidic pH extracellularly in the matrix or lysosomes. It have been demonstrated by several studies that Cathepsin D affects a range of steps in tumor metastasis and progression. It stimulates cancer cell growth by tumor angiogenesis stimulating fibroblast growth factors (Benes *et al.,* 2008; Masson *et al.*, 2010). Endosomes and lysosomes retain Cathepsin D in normal cells but in breast cancer cells it is over expressed and hyper-secreted by epithelial breast cells (Liaudet-Coopman *et al.*, 2006, Azim *et al.,* 2008; Gasparini 1998). Consequently degradative capacities of these cells are changed along with accumulation of Cathepsin D in the tumor microenvironment. Lysosomal Cathepsin D is secreted into the cytosol during apoptosis, where it may interact with and/or cleave nuclear anti-apoptotic or pro-apoptotic proteins. During the resolution of inflammatory responses, neutrophils rapidly undergo apoptosis. A direct and fast activation of caspase-8 by cathepsin D has been shown to be crucial in the initial steps of neutrophil apoptosis (Conus *et al.,* 2012).

The plasmepsin family of Plasmodium aspartic proteases is involved in haemoglobin degradation during the intra-erythrocyte phase of malarial infection (Ersmark *et al.,* 2006). Plasmepsins have been considered as promising target for new anti-malarial drugs (Ahmad *et al.,* 2010).

The bilobal structure of Cathepsin D and plasmepsin II proteases contain predominantly b-sheets with small a-helical segments. The active site is located at the interface between the two domains encloses two invariant aspartates. Despite the structural similarities among the members of aspartic proteases they have for less sequence similarities. For example, Cathepsin D exhibits about 35% sequence homology to Plasmepsins (Schechter and Berger 1967; Silva *et al*., 1996).

Benzenesulfonamide derivatives have a great potential for medicinal applications (Ashraf *et al.,* 2012; Siddiqui *et al.,* 2012, Siddiqui *et al.,* 2010, Siddiqui *et al.,* 2008, Siddiqui *et al.,* 2007). The sulfamoylbenzamide compounds are agonists and modulating ligands of cannabinoid receptors. These compounds are considered

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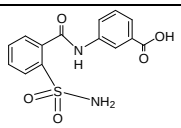
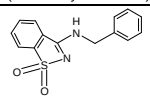
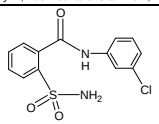
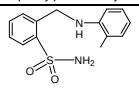
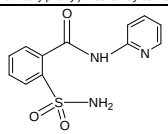
Table 1: Docking scores and inhibition data of selected Sulfamoylbenzamide compounds

Compd. ID	Structure	Docking in CD (FlexX score)	Inhibition of CD (IC_{50} in µM)	Docking in PII (FlexX score)	Inhibtion of PII (IC_{50} in µM)
W-5	 3-(2-sulfamoylbenzamido)benzoic acid	-32.5±0.20	2.0±0.10	-27.9±0.10	Not active
W-7	 N-benzyl-1,2-benzothiazol-3-amine 1,1-dioxide	-35.1±0.13	2.0±0.13	-29.5±0.10	Not active
W-24	 *N*-(3-chlorophenyl)-2-sulfamoylbenzamide	-30.0±0.10	1.25±0.08	-26.1±0.15	Not active
W-28	 *N*-(2,6-dimethylphenyl)-2-sulfamoylbenzamide	-30.9±0.13	1.5±0.08	-24.0±0.10	Not active
W-30	 *N*-(pyridin-2-yl)-2-sulfamoylbenzamide	-29.9±1.16	1.5±0.05	-26.9±0.10	Not active

CD= human cathepsin D; PII= Plasmepsin II

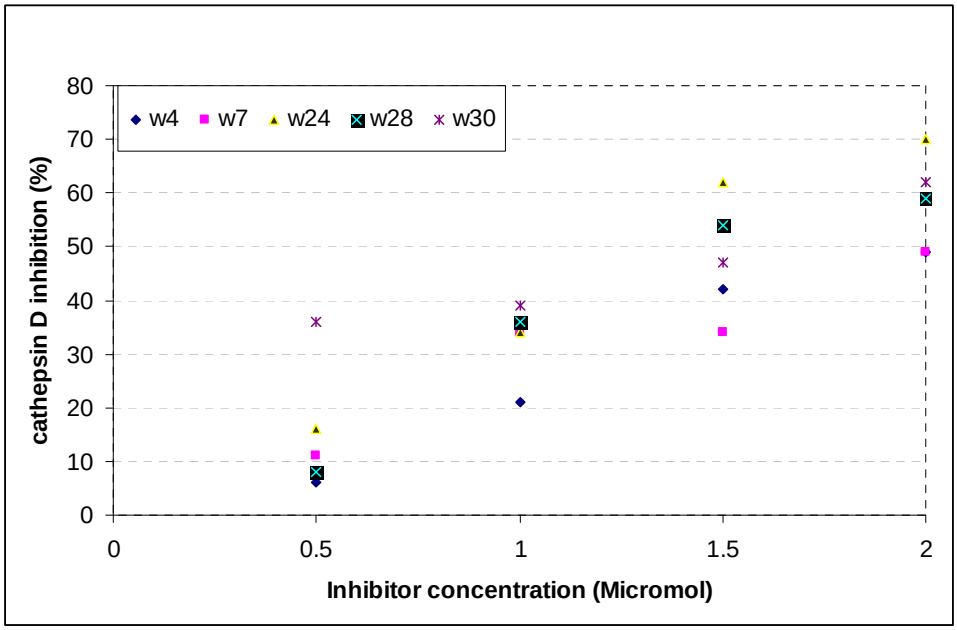


Fig. 1: Human cathepsin D inhibition plots of Sulfamoylbenzamide compounds.

useful for gastrointestinal disorders, inflammation, auto-immune diseases, ischemic conditions, immune-related disorders, hyper-tension and neurological disorders. During the course of present studies, ligand docking of several Sulfamoylbenzamide derivatives into the substrate-binding clefts of Plasmepsin-II from *Plasmodium falciparum* and human cathepsin D has been carried out. Enzyme inhibition assays were also performed using top scoring compounds from virtual screening. Interestingly, enzyme inhibition results showed that Sulfa-moylbenzamide compounds are selective inhibitors of human cathepsin D and not active against Plasmepsin II.

MATERIALS AND METHODS

In this study, virtual screening cum enzyme inhibition assay was carried out to find the potent inhibitors of Plasmepsin II and Cathepsin D.

Molecular docking

FlexX ligand docking software (version 2.0) (Rarey *et al.* 1996) was utilized for docking using Pepstatin-A complexed crystal coordinates of cathepsin D and plasmepsin-II (Silva et al, 1996) (Baldwin *et al.* 1993; Metcalf and Fusek 1993) (PDB id; 1M43 and 1LYB respectively). FlexX ligand docking method is based on incremental construction of ligand molecules from smaller fragments in the cavity of a protein. The free binding energy (ΔG) of protein-ligand complex is utilized to rank the generated docking results using a scoring function similar to that developed by Bohm (Bohm 1994). After completion of each docking experiment, top ten docking solutions were saved and utilized for in-depth analysis.

Enzyme inhibition assay

The enzyme activities of plasmepsin-II and cathepsin D were measured as described earlier (Haque et al, 1999) using fluorescence resonance energy transfer (FRET) based assay with the fluorogenic substrate DABCYL-Glu-Arg-Nle-Phe-Leu-Ser-Phe-Pro-EDANS (malaria FRET-1; AnaSpec Inc., USA). Purified Plasmodium falciparum plasmepsin-II was provided by Daniel E. Goldberg, Howard Hughes Medical Institute, St. Louis, Missouri, USA. Recombinant human liver cathepsin D was purchased from Biodesign International, USA. The assay was performed with plasmepsin-II/cathepsin D (1.2 nM) and substrate (malaria FRET-1; 1.0 μM) in 0.1 M Sodium acetate buffer pH 5.0, containing 0.01% Tween 20 and 10% Glycerol. Before the addition of substrate the DMSO solution of Sulfamoylbenzamide derivatives were added in the reaction mixture. The assays were performed with 5.0% final concentration of DMSO. The enzyme inhibition experiments were performed (in triplicates) in 96 well plate format and readings were obtained on a Perkin Elmer LS55 Fluorescence spectrometer with an excitation and emission wavelengths of 336 and 490 nm respectively. IC_{50} values were calculated by nonlinear regression analysis from plots of percentage inhibition versus inhibitor concentrations. The enzyme assays using 'standard inhibitor' Pepstatin-A (Sigma Inc, USA) was performed in the same experimental manner as for Sulfamoylbenzamide derivatives.

Antiplasmodial assays

The antiplasmodial assays were carried out in the laboratory of Prof. Daniel E. Goldberg at Howard Hughes Medical Institute, St. Louis, Missouri, USA. All testing has been done in HB-3 *Plasmodium falciparum* clone. EC50 values of the tested compounds were determined essentially as described by Francis and coworkers (Francis et al, 1994). 200 microliter aliquots of late ring stage cultures at 0.5% parasitemia were incubated with various concentrations of either pepstatinA (Roche Applied Sciences) or *trans*-epoxysuccinyl-L-leucylamino (4-guanidine)-butane (E64) in hypoxanthine-free rich medium for 42 h. 0.5 μCi of [3H]hypoxanthine (178.7 Ci/mmol; PerkinElmer) was added to the culture and the incubation continued for 24 h. Cultures were harvested on glass fiber paper, immersed in UltimaGold scintillation counting mixture (PerkinElmer, USA) and counted in a scintillation counter. The percentage of the inhibition of [3H] hypoxanthine uptake was plotted against the drug concentration and the curve was fitted using the modified dose-response logistic equation in KaleidaGraph software.

RESULTS

IC_{50} values of five Sulfamoylbenzamide compounds tested are in range of 1.25-2.0 μM. Cathepsin D inhibition plot of Sulfamoylbenzamide compounds is demonstrated in figure 1. IC_{50} value of N-(3-chlorophenyl)-2-sulfamoyl-benzamide is 1.25 μM, so it is identified as the most potent of all tested Sulfamoylbenzamide compounds. In case of Cathepsin D docking score was -29.9±1.16 to -35.1±0.13 while from -24.0±0.10 to -29.5±0.10 in case of Plasmepsin-II. Chemical structures, codes and inhibition data of these compounds are given table 1. Anti plasmodial activity of N-(3-chlorophenyl)-2-sulfamoyl-benzamide was also carried out to evaluate its antiplasmodial behavior as the representative molecule of the series. The IC_{50} was ≥3 μM which is insignificant.

DISCUSSION

Aspartic proteases play vital role in different cellular processes. Regulation and inhibition of their activities are of important consequences. Cathepsin D is overexpressed in breast cancer and considered as a possible target for anti-cancer drugs. Virtual screening of hundreds of compounds pointed out Sulfamoylbenzamide derivatives as potential Cathepsin D and/or Plasmepsin II inhibitors. Subsequent enzyme inhibition assay of selected Sulfamoylbenzamide derivatives revealed these compounds as selective Cathepsin D inhibitors. It was also noted that the docking score of theses compounds was better in case of Cathepsin D as compared to Plasmepsin-II. These findings supports the results of plasmepsin II inhibition assay as sulfamoylbenzamide derivatives are inactive against plasmepsin II.

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