Med Chem Res (2017) 26:1723–1734
DOI 10.1007/s00044-017-1826-9

MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

A comparative account of sar studies of semicarbazones and thiosemicarbazones on cathepsins H and L

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Received: 29 March 2015 / Accepted: 28 February 2017 / Published online: 5 April 2017

Abstract Cathepsins H and L, lysosomal cysteine proteases have been found in elevated levels in tumor invasion, metastasis, inflammation, atherosclerosis, and various other tissue degenerative diseases. In the past decade, work has largely been focused on evaluation of some non-peptidyl inhibitors of cathepsins as these have been considered as viable drug targets for major diseases. Semicarbazones and thiosemicarbazones, carbonyl derivatives are extensively studied for wide variety of biological activities such as anticonvulsant, anticancer, anti-inflammatory, antihypertensive, antimicrobial, and antiparasitic. These derivatives have also shown to possess parasiticidal activity against *Plasmodium falciparum, Plasmodium berghei, Trypanosoma cruzi, Trypanosoma brucei rhodesiense* and *Toxoplasma gondii*. With this background, the present work involved the inhibition and kinetic studies of substituted semicarbazones and thiosemicarbazones on cathepsin H and L. A comparative account of structure–activity relationship for inhibition exerted by synthesized semicarbazones and thiosemicarbazones with varied functional moieties on cathepsins H and L is presented.

Keywords Cathepsin H · Cathepsin L · Inhibitors · Semicarbazones · Thiosemicarbazones · Kinetic studies

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Introduction

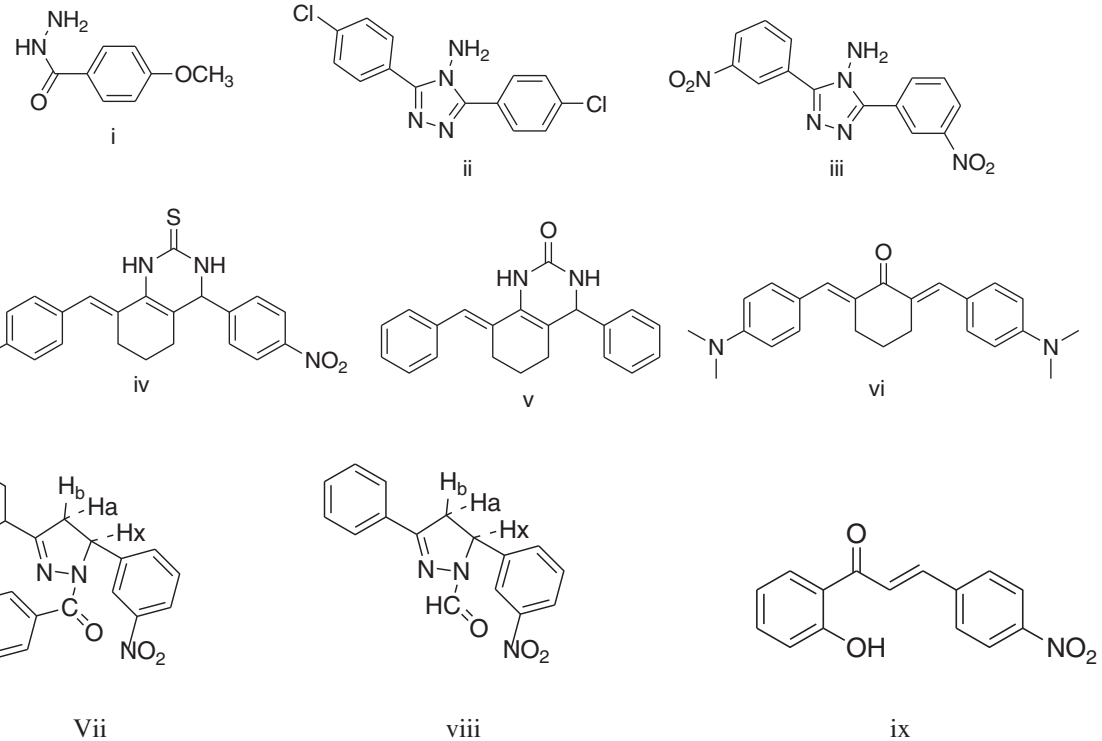
Cathepsins initially thought to be 'housekeeping' molecules for cell's garbage disposals because of their lysosomal localization are also known to play vital role in specific physiological processes, and are also linked to many severe genetic disorders (Wolters and Chapman 2000; Jedeszko and Sloane 2004; Toomes et al. 1999). Cathepsins B, H, and L belonging to Papain super family have been found to be key factors in the pathogenesis of cancer invasion, arthritis, osteoporosis, and microbial infections. Targeting these enzymes is therefore one of the strategies in the development of new chemotherapeutic agents for a number of diseases (Selzer et al. 1999). These enzymes are also involved in pathology of chronic inflammatory diseases of airways and joints such as asthma (Cimerman et al. 2001) and certain forms of arthritis (Maciewicz and Etherington 1988), periodontal disease (Lah et al. 1986), muscular dystrophy (Kamatsu et al. 1986), pancreatitis (Mort and Buttle 1997), and tumor growth and metastasis (Frohlich et al. 2001).

Implications of cathepsins B, H, and L in tumorigenesis (Mohamed and Sloane 2006; Joyce and Hanahan 2004; Lankelma et al. 2010; Henkin 1993; Van der Stappen et al. 1991), recognition of these as prognostic markers in several types of cancer, including breast, with increased primary tumor expression associated with poor outcome (Jain et al. 2010; Lah et al. 2000; Nouh et al. 2011) and correlation of their over expression with advancement of tumor (Chan et al. 1986; Podgorski and Sloane 2003; Ostensen et al. 1983; Kirschke 1977; Waghray et al. 2002; Linnerth et al. 2005; Schweiger et al. 2004; Turk et al. 2002; Liu et al. 2006) focus the development of selective inhibitors of these enzymes.

Semicarbazones and thiosemicarbazones, a class of small molecules that have been extensively studied for wide

variety of biological activities, have been evaluated as anticancer (Nutting et al. 2009), antihypertensive (Warren et al. 1977), anti-inflammatory (Swathi and Sarangapani 2014), antimicrobial (Nfor et al. 2011), and anticonvulsant agents (Puthucode et al. 1998). These derivatives have also shown to possess parasiticidal activity against *Plasmodium falciparum*, *Plasmodium berghei*, *Trypanosoma cruzi*, *Trypanosoma brucei* rhodesiense and *Toxoplasma gondii* (De-Oliveira et al. 2008; Aguiree et al. 2004; Fujii et al. 2005; Tenorio et al. 2005). Thiosemicarbazones have been demonstrated as potential inhibitors of cathepsin L (Kumar et al. 2010a, b) and are proposed to have potential application in the treatment of chagas disease, sleeping sickness and malaria probably due to their inhibitory potency on parasitic cysteine proteases.

Recent studies by our group have demonstrated the effectiveness of the various non-peptidyl inhibitors (i-ix) of cathepsin B and cathepsin H such as bischalcones based quinazoline-2(1H)-ones, quinazoline-2(1H)-thiones (Raghav and Singh 2014a), acyl hydrazides, triazoles (Raghav and Singh 2014b), hydrazones (Raghav and Singh 2014c), hydroxyl chalcones (Raghav and Garg 2014a) and their cyclized derivatives, formyl and benzoyl pyrazolines (Raghav and Garg 2014b).



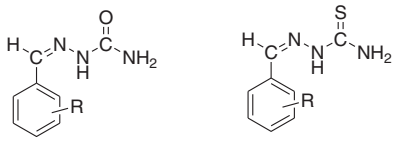
Previously, we have synthesized semicarbazones and thiosemicarbazones with different functionalities and evaluated these as inhibitors of cathepsin B (Raghav and Kaur 2014). In this direction to screen their affectivity toward other cathepsins, the present work deals with the inhibition studies of semicarbazones and thiosemicarbazones on cathepsins H and L. Supportive kinetic studies and in silico docking studies are also performed to compare the results.

Experimental

Materials and methods

All the chemicals were of analytical grade. Fast Garnet GBC (*o*-aminoazotoluene diazonium salt), Leu-βNA and ZPheArg-4mβNA were purchased from Bachem Feinchemikalien AG, Switzerland. The protein sample was concentrated using Amicon stirred cells with YM 10 membrane under nitrogen pressure of 4–5 psi. The source of enzyme was fresh goat liver obtained from local slaughter house.

Melting points were taken in open capillaries and are uncorrected. The progress of the reactions was monitored on silica gel G plates using iodine vapor as visualizing agent. Elisa plate reader was used for measuring absorbance in the visible range. The spectrofuge was used for centrifugation purpose. IR spectra were recorded on Horizon 300 MHz spectrometer. NMR spectra were recorded on Bruker 300 MHz instrument. The chemical shifts are expressed in ppm units from an internal TMS standard. All commercially available reagents were used as-received.



R= *o*-Cl, *m*-Cl, *p*-Cl, *o*-OCH_3, *m*-OCH_3, *p*-OCH_3, *o*-NO_2, *m*-NO_2, *p*-NO_2, H

Fig. 1 Synthesized semicarbazones and thiosemicarbazones

Synthesis

The synthesis and characterization of title compounds semicarbazones (**1a–1j**) and thiosemicarbazones (**2a–2j**) by IR, NMR were previously reported (Raghav and kaur 2014) (Fig. 1).

Pharmacology

Purification of cathepsins H and L

All the purification steps were carried out at 4 °C. Cathepsins H and L were extracted and purified from goat liver by the established procedure reported previously (Raghav et al. 2015) including the steps of acetone powder preparation, homogenization, acid autolysis, 30–70% $(NH_4)_2SO_4$ fractionation, molecular sieve chromatography on sephadex G-100 and ion exchange chromatographies on CM Sephadex C-50 and DEAE A-50 sephadex. The specific activities of the cathepsin H and L were equal to ~24.01 nmol/min/mg and ~16.78 nmol/min/mg respectively.

Enzyme inhibition studies

Cathepsin H activity was determined using Leu-βNA substrate at pH 7.0 whereas cathepsin L activity was determined using ZPheArg-4mβNA (Raghav et al. 2015) substrate at pH 6.0 respectively. Effect of synthesized semicarbazones (**1a–1j**) was observed on the activities of cathepsins H and L at 1×10^{-3} M, 1×10^{-8} M final concentration of each compound, respectively whereas effect of synthesized thiosemicarbazones (**2a–2j**) was observed on the activities of cathepsins H and L at 1×10^{-3} M, 1×10^{-7} M final concentration of each compound, respectively. First of all, enzyme was equilibrated in buffer of appropriate pH at 37 °C. Then 20 µl of individual compound was added in the reaction mixture separately to effect the final concentration of each compound as quoted before. After an incubation time of 30 min residual enzyme activity was estimated by the usual enzyme assay using the respective substrates. The experiments were performed in triplicate for each concentration and % activity has been calculated with respect to the control, where no compound was added but an equivalent amount of solvent was present (Table 1). Enzyme assays were similarly conducted at lower concentrations of each compound to observe the inhibitory effect of compounds at varying concentrations. The results are presented in Figs 2, 3 for cathepsins H and L respectively.

Enzyme kinetic studies

After establishing the inhibitory action of semicarbazones (**1a–1j**) and thiosemicarbazones (**2a–2j**) on cathepsins H and L experiments were designed to evaluate the type of inhibition and to determine the K_i value of these compounds on cathepsin H and L. For that, enzyme activity was evaluated at different substrate concentration in presence and absence of a fixed concentration of inhibitor. Line-weaver Burk plots were drawn between 1/[S] and 1/V (Figs 4, 5). The K_m value of cathepsins H and L for Leu βNA and ZPheArg-4mβNA was found to be 5.0×10^{-4} M and 0.5×10^{-4} M respectively. The K_i values have been summarized in Table 2.

Drug modeling studies

Docking studies were performed using iGemdock software. To conduct these, small molecular weight ligands were prepared using marvin sketch and were saved as MDL Mol File. Enzyme structure active site was retrieved from the Protein Data Bank (http://www.rcsb.org/) cav8PCH H_NAG.pdb and cav3BC3L_CSW (Guncar et al. 1998; Chowdhary et al. 2008). The prepared ligands and the binding site was loaded in the iGemdock program and docking was run by setting GA parameters for Standard Docking Accuracy Settings, Docking experiments show a decrease in energy when enzyme and ligands interact. The E_{total} resulting after H-bonding and van der Waals interactions are presented in Tables 3, 4. The docking poses of the most inhibitory compounds 1**b, 2g** for cathepsin H and **1g**, **2h** for cathepsin L are shown in Figs 6, 7.

Result and discussion

In drug discovery, one of the strategies is to design structural analogs of potent inhibitors of enzymes involved in physiological disorder. Preferential inhibition of one enzyme over the other can add to the drug potential with reduced toxicity where the otherwise unwanted enzyme inhibition can lead to severe side effects. In the present work we report the inhibition studies of semicarbazones and thiosemicarbazones already established as inhibitors of cathepsins B (Raghav and Kaur 2014) on cathepsins H and

Table 1 Effect of substituted semicarbazones (**1a–1j**) and thiosemicarbazones (**2a–2j**) on the activities of cathepsins H and L

Compounds	R	Cathepsin H		Cathepsin L	
		Mean ± S.M.D	% Residual activity (nmol/min/ml)	Mean ± S.M.D	% Residual activity (nmol/min/ml)
Control		5.237 ± 0.022	100.00	4.395 ± 0.091	100.00
1a	*o*-Cl	2.199 ± 0.061	42.00	3.736 ± 0.281	85.00
1b	*m*-Cl	0.015 ± 0.004	0.30	3.594 ± 0.044	82.00
1c	*p*-Cl	3.838 ± 0.091	73.30	1.758 ± 0.279	40.00
1d	*o*-OMe	3.450 ± 0.077	66.00	2.286 ± 0.140	52.00
1e	*m*-OMe	2.690 ± 0.447	51.40	0.879 ± 0.245	20.00
1f	*p*-OMe	2.916 ± 0.076	55.60	1.318 ± 0.078	30.00
1g	*o*-NO_2	2.875 ± 0.362	55.20	0.439 ± 0.029	10.00
1h	*m*-NO_2	3.224 ± 0.295	61.80	2.403 ± 0.274	55.00
1i	*p*-NO_2	4.888 ± 0.666	93.50	2.197 ± 0.240	50.00
1j	H	4.826 ± 0.332	92.50	2.021 ± 0.222	46.00
2a	*o*-Cl	0.000 ± 0.000	0.00	2.548 ± 0.221	58.00
2b	*m*-Cl	0.000 ± 0.000	0.00	4.210 ± 0.287	96.00
2c	*p*-Cl	1.088 ± 0.135	20.80	2.115 ± 0.084	48.00
2d	*o*-OMe	0.000 ± 0.000	0.00	3.516 ± 0.081	80.00
2e	*m*-OMe	0.000 ± 0.000	0.00	2.916 ± 0.208	66.00
2f	*p*-OMe	1.936 ± 0.093	37.00	3.594 ± 0.304	82.00
2g	*o*-NO_2	0.318 ± 0.046	6.10	1.318 ± 0.155	30.00
2h	*m*-NO_2	0.706 ± 0.238	13.50	0.439 ± 0.074	10.00
2i	*p*-NO_2	1.328 ± 0.354	25.40	0.659 ± 0.016	15.00
2j	H	0.764 ± 0.249	14.60	1.538 ± 0.404	35.00
Leupeptin		2.511 ± 0.082	47.95 (10^{-5} M)	0.474 ± 0.021	10.80 (10^{-9} M)
Leu-CH_2Cl		0.340 ± 0.055	6.50 (10^{-5} M)	4.329 ± 0.098	98.50 (10^{-5} M)

The results are the mean and S.M.D. of the experiment conducted in triplicate and is calculated as activity in nmoles/min/ml in enzyme preparation. The % residual activity is calculated w.r.t. control, where no compound was added but an equivalent amount of solvent was present. the concentration of compounds were 1×10^{-3} M for cathepsin H and 1×10^{-8} M (**1a–1j**) for cathepsin L whereas for compounds (**2a–2j**) in case of cathepsin L the concentration taken was 1×10^{-7} M. The specific activity of the cathepsins H and L were ~24.01 nmol/min/mg and ~16.78 nmol/min/mg respectively.

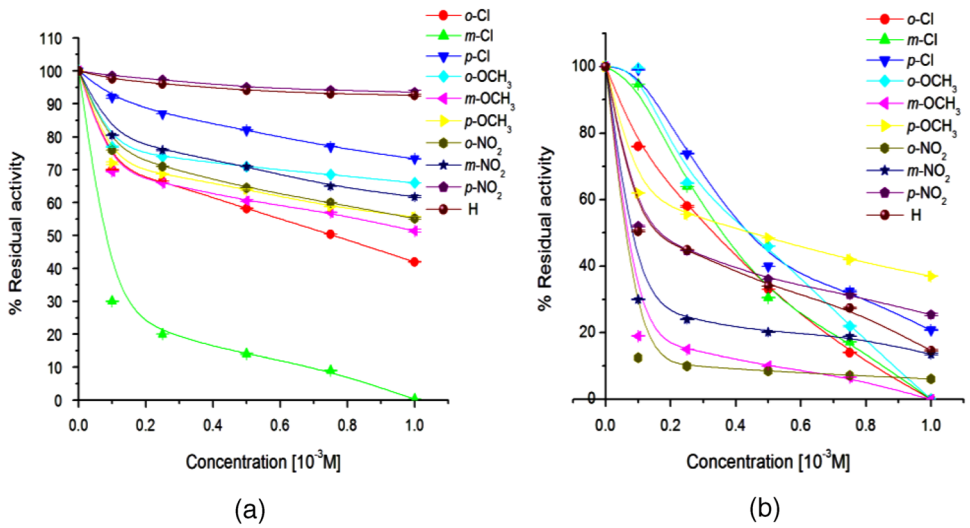


Fig. 2 Percentage (%) residual activities of cathepsin H in presence of different concentrations of various (**1a**-**1j**) semicarbazones (**a**) and (**2a–2j**) thiosemicarbazones (**b**)

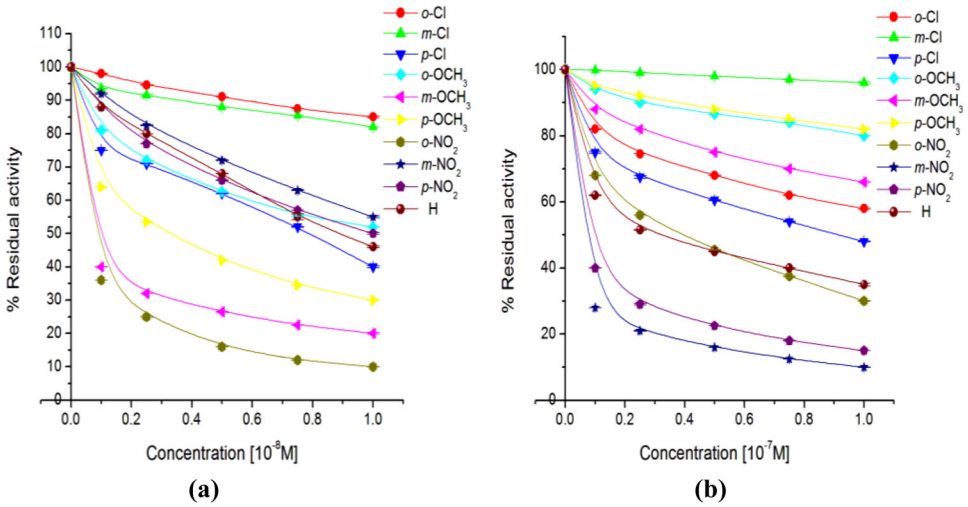


Fig. 3 Percentage (%) residual activities of cathepsin L in presence of different concentrations of various (**1a–1j**) semicarbazones (**a**) and (**2a–2j**) thiosemicarbazones (**b**)

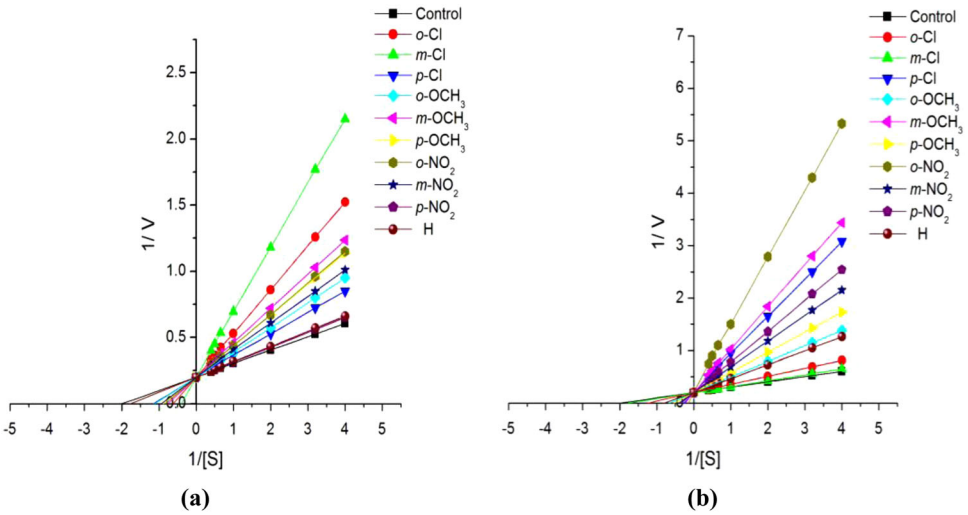


Fig. 4 Lineweaver–Burk plots for inhibition of various (**1a–1j**) semicarbazones (**a**) and (**2a–2j**) thiosemicarbazones (**b**) on cathepsin H at fixed concentration of inhibitor and varying substrate i.e. Leu-βNA concentration

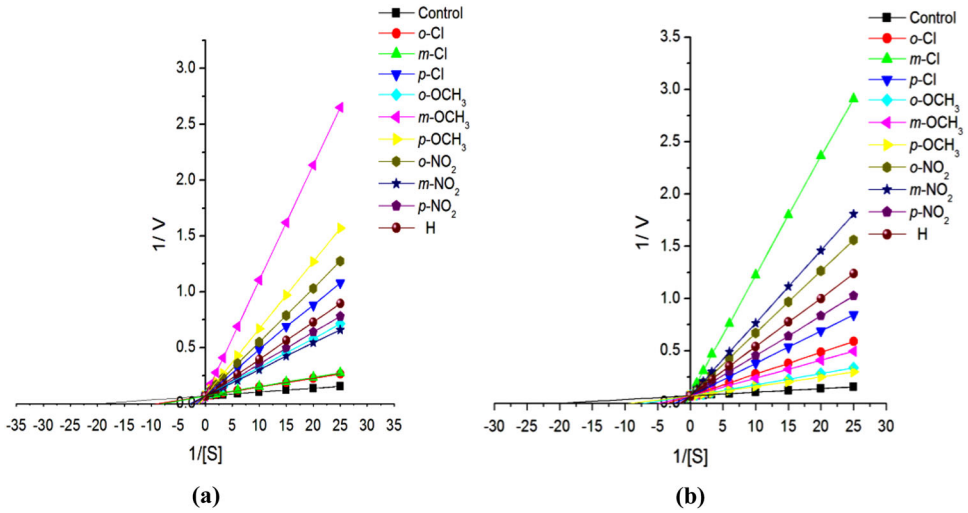


Fig. 5 Lineweaver–Burk plots for inhibition of various (**1a–1j**) semicarbazones (**a**) and (**2a–2j**) thiosemicarbazones (**b**) on cathepsin L at fixed concentration of inhibitor and varying substrate i.e. Z-Phe-Arg-4mβNA concentrations

Table 2 K_i values of various semicarbazones (**1a–1j**) and thiosemicarbazones (**2a–2j**) for cathepsins H and L

Compounds	Cathepsin H K_i [1×10^{-4} M]	Cathepsin L K_i [1×10^{-8} M]
1a	4.30	0.780
1b	0.27	0.750
1c	16.60	0.086
1d	10.80	0.156
1e	6.30	0.036
1f	7.10	0.063
1g	7.00	0.005
1h	10.00	0.170
1i	81.90	0.149
1j	70.40	0.105
2a	2.08	1.97
2b	7.04	18.14
2c	1.70	1.23
2d	2.80	4.95
2e	0.16	2.65
2f	3.50	5.81
2g	0.09	0.63
2h	0.25	0.05
2i	2.00	0.10
2j	0.60	0.86

Cathepsin H assays were conducted at 1.0×10^{-3} M concentrations of various semicarbazones (**1a–1j**) and thiosemicarbazones (**2c**, **2f**, **2g**, **2h**, **2i**, and **2j**); however, for compounds **2a**, **2b**, **2d**, and **2e** the inhibitor concentration taken was 1.0×10^{-4} M. For cathepsin L inhibition constant was evaluated at 1.0×10^{-8} M and 1.0×10^{-7} concentrations of various semicarbazones (**1a–1j**) and thiosemicarbazones (**2a–2j**), respectively.

The results are calculated using line weaver-burk equation for competitive inhibitors

L, two other significant and related cysteine proteases. Enzyme kinetics and structure–function relationship has been studied, which is vital to understand the mode of action of drug molecule and is fundamental to the modern design of pharmaceuticals in industries (Sami and Shakoor 2011).

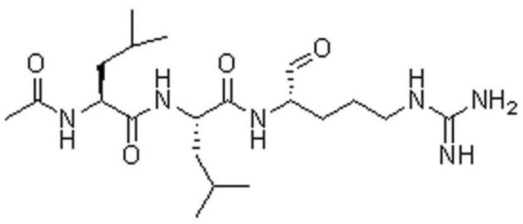
In the past decade, work was focused on the identification and development of cysteine protease inhibitors and their potential use as anti parasitic agents (Du et al. 2002; Greenbaum et al. 2004; Romeiro et al. 2009; Brak et al. 2010). A large work has been accomplished on peptidyl or peptidyl analogs as inhibitors to cysteine proteases (Otto and Schirmeister 1997; Steverding 2011). However, these inhibitors are not considered to be viable drug candidates for treating diseases like cancer, and apoptosis, because of the possibility of immunogenic reactions or gastric instability. Besides peptidyl inhibitors there were also triumph for non-peptidyl inhibitors of cysteine proteases (Dana et al. 2013; Schenker et al. 2008). Therefore our attempt is to find out such non-peptidyl inhibitors of cathepsins B, H, and L that can lead to drug research and development toward these enzymes. The work on cathepsin B has already been published (Raghav and Kaur 2014). In the present work we report the inhibitory effect of semicarbazones and thiosemicarbazones on cathepsins H and L, two other pharmacologically significant lysosomal cysteine proteases.

Effect of synthesized compounds on the activity of cathepsins H and L

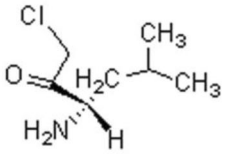
The effect of differently substituted semicarbazones (**1a–1j**) and thiosemicarbazones (**2a–2j**) on the activity of cathepsins H and L at varying concentrations is shown in Figs 2, 3. From these plots of % residual activities vs. the concentrations of different compounds, it can be observed that at a particular concentration all the synthesized compounds inhibited cathepsin L activity more than cathepsin H.

The inhibition type and K_i values

The type of inhibition caused by various compounds was determined through Lineweaver–Burk double reciprocal plot. In order to establish inhibition ability of the under consideration compounds, results were compared with potent inhibitors of cathepsin L, e.g., Leupeptin and cathepsin H e.g. Leu-CH_2Cl, respectively. As reported in literature, K_i value for human liver cathepsin H was reported to be 9.2×10^{-6} M (Azaryan and Galoyan 1987) and K_i value for goat brain cathepsin L was reported to be 1.45×10^{-9} M (Kamboj et al. 1993).



Leupeptin



Leu-CH_2-Cl

Table 3 Docking studies of cathepsin H in presence of semicarbazones (**1a–1j**) and thiosemicarbazones (**2a–2j**)

#Ligand	Total energy	VDW	HBond	Elec
cav8PCH H_NAG-1a-0.pdb	−72.1301	−47.7092	−24.4208	0
cav8PCH H_NAG-1b-2.pdb	−71.7535	−48.4915	−23.262	0
cav8PCH H_NAG-1c-0.pdb	−73.0731	−52.5686	−20.5045	0
cav8PCH H_NAG-1d-2.pdb	−75.454	−51.9753	−23.4787	0
cav8PCH H_NAG-1e-2.pdb	−84.7457	−54.3544	−30.3914	0
cav8PCH H_NAG-1f-0.pdb	−79.0581	−51.8714	−27.1867	0
cav8PCH H_NAG-1g-2.pdb	−94.5788	−53.652	−40.9268	0
cav8PCH H_NAG-1h-1.pdb	−83.3711	−52.4449	−30.9262	0
cav8PCH H_NAG-1i-2.pdb	−82.5434	−51.8368	−30.7065	0
cav8PCH H_NAG-1j-1.pdb	−70.4079	−45.6228	−24.7851	0
cav8PCH H_NAG-2a-2.pdb	−69.4764	−49.5247	−19.9517	0
cav8PCH H_NAG-2b-0.pdb	−70.5064	−56.0896	−14.4168	0
cav8PCH H_NAG-2c-1.pdb	−69.523	−49.8405	−19.6825	0
cav8PCH H_NAG-2d-1.pdb	−71.89	−54.5053	−17.3847	0
cav8PCH H_NAG-2e-0.pdb	−79.8713	−53.1062	−26.7651	0
cav8PCH H_NAG-2f-0.pdb	−76.1204	−51.3316	−24.7888	0
cav8PCH H_NAG-2g-2.pdb	−89.0642	−56.4203	−32.2849	−0.35898
cav8PCH H_NAG-2h-0.pdb	−85.3976	−54.8087	−30.5889	0
cav8PCH H_NAG-2i-1.pdb	−79.3537	−46.3588	−32.9949	0
cav8PCH H_NAG-2j-1.pdb	−63.6656	−47.5275	−16.1381	0
cav8PCH H_NAG-Leu-βNA-0.pdb	−75.60	−66.10	−9.50	0
cav8PCH H_NAG-Leu-CH_2Cl-1.pdb	−59.09	−42.59	−16.50	0

The results are one of the docking experiments run using iGemdock. The ligands were prepared in marvin sketch and the active site was extracted from the structure of cathepsin H retrieved from protein data bank (http://www.rcsb.org/) as cav8pcH H_NAG.pdb. After loading the prepared ligands and the binding site docking was started at standard docking accuracy settings

For evaluating the type of inhibition caused by different semicarbazones (**1a–1j**) and thiosemicarbazones (**2a–2j**) cathepsins H and L activity was measured at varying substrate i.e., Leu βNA and ZPheArg-4mβNA concentration in presence and absence of a fixed concentration of compound. The plots of 1/V and 1/[S] were straight lines intersecting at the *Y*-axis and shows that value of V_{max} remains constant in all the compounds, whereas the value of $K_{m'}$ change with each compound. These studies suggested that semicarbazones (**1a–1j**) and thiosemicarbazones (**2a–2j**) are competitive inhibitors to cathepsins H and L. Using the Lineweaver–Burk equation of competitive inhibition the K_i values were calculated, which has been presented in Table 2.

$$K_{m'} = K_m(1+[I]/K_i)$$

Lineweaver–Burk plots of different semicarbazones (**1a–1j**) and thiosemicarbazones (**2a–2j**) for cathepsins H and L are shown in Figs 4, 5.

Structure–activity relationship

Out of various synthesized semicarbazones (**1a–1j**) and thiosemicarbazones (**2a–2j**), *m*-chlorobenzaldehyde semicarbazone, (**1b**) and *o*-nitrobenzaldehyde thiosemicarbazone, (2g) with K_i values of 2.7×10^{-5} M and 0.9×10^{-5} M showed maximum inhibition on cathepsin H, whereas in case of cathepsin L, *o*-nitrobenzaldehyde semicarbazone, (1g) and *m*-nitrobenzaldehyde thiosemicarbazone, (2h) with K_i values of 0.5×10^{-10} M and 0.5×10^{-9} M showed maximum inhibition. Followed by these results it was concluded that the synthesized compounds showed more inhibition on activity of cathepsin L than on cathepsins H. By comparing the results obtained from the previous study (Raghav and kaur 2014) it is concluded that the semicarbazones (**1a–1j**) and thiosemicarbazones (**2a–2j**) were less inhibitory to the activity of cathepsin B than cathepsin L but more inhibitory than cathepsin H. The inhibitory trend obtained on these three lysosomal cathepsins, the synthesized compounds semicarbazones (**1a–1j**) and thiosemicarbazones (**2a–2j**) showed maximum inhibition towards cathepsin L followed by cathepsin B and then cathepsin H. Also by comparing the synthesized compounds on the activity of individual cathepsin it was accomplished that thiosemicarbazones (**2a–2j**) showed maximum inhibition than semicarbazones (**1a–1j**) towards cathepsin B and H whereas in case of

Table 4 Docking studies of cathepsin L in presence of semicarbazones (**1a–1j**) and thiosemicarbazones (**2a–2j**)

#Ligand	Total energy	VDW	HBond	Elec
cav3BC3 L_CSW-1a-0.pdb	−78.1342	−57.6766	−20.4576	0
cav3BC3 L_CSW-1b-0.pdb	−79.2604	−64.452	−14.8084	0
cav3BC3 L_CSW-1c-0.pdb	−75.9692	−59.764	−16.2052	0
cav3BC3 L_CSW-1d-0.pdb	−83.8302	−60.9906	−22.8396	0
cav3BC3 L_CSW-1e-0.pdb	−96.7133	−68.3815	−28.3318	0
cav3BC3 L_CSW-1f-2.pdb	−107.017	−72.4441	−34.5731	0
cav3BC3 L_CSW-1g-1.pdb	−98.9952	−63.3022	−36.4318	0.738759
cav3BC3 L_CSW-1h-2.pdb	−99.0098	−69.7876	−29.4971	0.274873
cav3BC3 L_CSW-1i-0.pdb	−93.5538	−61.638	−32.6361	0.720295
cav3BC3 L_CSW-1j-2.pdb	−87.247	−66.1169	−21.1301	0
cav3BC3 L_CSW-2a-2.pdb	−75.0521	−64.5521	−10.5	0
cav3BC3 L_CSW-2b-2.pdb	−94.7276	−73.2098	−21.5178	0
cav3BC3 L_CSW-2c-0.pdb	−79.4708	−66.2551	−13.2157	0
cav3BC3 L_CSW-2d-2.pdb	−83.7684	−65.9792	−17.7892	0
cav3BC3 L_CSW-2e-0.pdb	−86.4389	−66.2037	−20.2351	0
cav3BC3 L_CSW-2f-0.pdb	−78.274	−60.4967	−17.7774	0
cav3BC3 L_CSW-2g-2.pdb	−94.7472	−58.2976	−36.8786	0.428985
cav3BC3 L_CSW-2h-0.pdb	−87.4328	−57.4597	−30.3885	0.41535
cav3BC3 L_CSW-2i-1.pdb	−84.5414	−62.6547	−22.9758	1.08918
cav3BC3 L_CSW-2j-2.pdb	−74.6524	−59.8668	−14.7856	0
cav3BC3L-CSW-Z-Phe-Arg-4mβNA-1.pdb	−139.6610	−107.287	−34.0340	−1.6600
cav3BC3L-CSW-Leupeptin-1.pdb	−116.9980	−93.4381	−23.3261	−0.2333

The results are one of the docking experiments run using iGemdock. The ligands were prepared in marvin sketch and the active site was extracted from the structure of cathepsin L retrieved from protein data bank (http://www.rcsb.org/) as cav3BC3L_CSW-pdb. After loading the prepared ligands and the binding site docking was started at standard docking accuracy settings

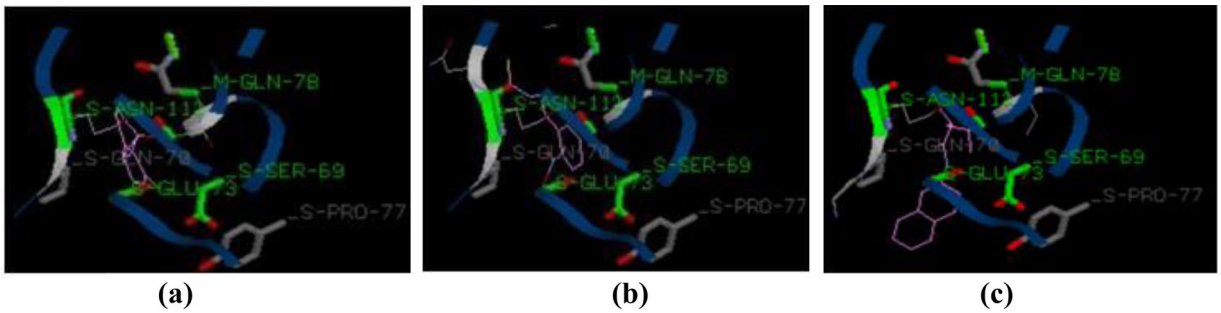


Fig. 6 Binding of most inhibitory 1b (**a**), 2 g (**b**) and Leu-βNA (**c**) into the binding site of cathepsin H (cav8PCHH_NAG)

cathepsin L thiosemicarbazones (**2a–2j**) showed less inhibition than semicarbazones (**1a–1j**). These results can lead to the development of selective inhibitors of cathepsins B, H, and L.

The structure–activity relationship study revealed that the maximum inhibitory compounds for cathepsins B, H, and L either possessed chlorine or nitro moiety. The effect of nitro substituent can be explained on the basis of the electronic effect induced by the substituent. Nitro being strongly electron withdrawing may affect the nucleophilic center in the molecule rendering it more susceptible to the attack of sulfhydryl group of target enzymes. The electronic effect of chloro can also be explained similarly. The chloro group can have an added advantage being more lipophilic in nature. A proposed mechanism of inhibition of cathepsin B by semicarbazones and thiosemicarbazones is also reported (Raghav and kaur 2014).

Semicarbazones containing peptidyl inhibitor have been previously reported as potential inhibitors of cathepsins B (Barrett 1986). The inhibitory capacity of these molecules

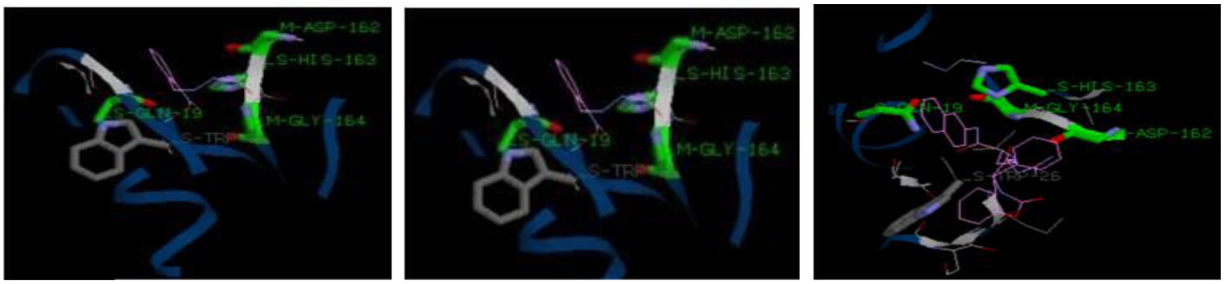


Fig. 7 Binding of most inhibitory compounds 1 g (**a**), 2 h (**b**) and Z-Phe-Arg-4mβNA (**c**) into the binding site of cathepsin L (cav3BC3L_CSW)

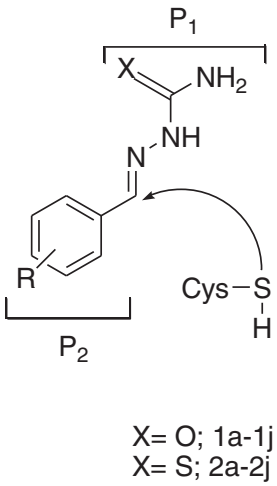


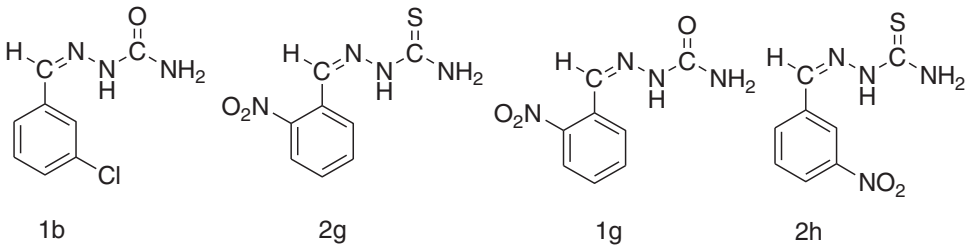
Fig. 8 Interaction of compounds's site P_1, P_2 with enzyme's subsite

has been attributed to the specific peptidyl binding of inhibitory compound with the enzyme binding sites. In the non-peptidyl semicarbazones and thiosemicarbazones the subsite P_2 is occupied by the aromatic ring orienting the $–CONH_2$, $–CSNH_2$ group toward the P_1 site and competes with the binding of substrate with the enzyme (Fig. 8). Hence, these have evaluated as competitive inhibitors (cf enzyme kinetic studies (Figs 4 and 5) and docking (Figs 6 and 7), respectively).

Docking studies

The docking approach was used to study the interaction of compounds with the active site of cathepsin B, H, and L to observe binding poses of individual compounds. Individual binding poses of each compound was assessed and their interactions in the active site of the enzyme were analyzed. The empirical scoring function of iGemDOCK is the estimated sum total of van der Waals, H-bonding and electrostatic energy. Figure 6 show the binding of most inhibitory compound **1b** and **2g** in the active site of cathepsin H. It is clearly observed that Ser-69 and Glu-73 residues present at the catalytic site of the enzyme are involved in the binding of compounds. In addition, Gln-78 and Asn-112 amino acids residues are also involved in the stabilization of compounds in binding site.

The binding energies of title compounds in the amino acyl binding site of cathepsin H (cav8PCHH_NAG) is presented in Table 3. Experimental results obtained can be correlated with the ligand–binding interactions. It is observed that for **1b** and **2g** the binding energies computed come out to be −71.75 and −89.06. In each series, these most inhibitory compounds show a decrease in binding energy toward higher side. The binding energies show effective interaction between the enzyme binding site and inhibitory compounds may be responsible for these inhibition patterns. These results are somewhat different than the



Small molecular weight representative molecules

results of in vitro studies, which clearly indicate that thiosemicarbazones are more effective inhibitors than semicarbazones (Table 3). Figure 6c shows the docking results of substrate Leu-βNA with the cathepsin H active site. The amino acids Ser-69, Glu-78, and Asn-122 which interact with the most inhibitory compounds **1b** (Fig. 6a) and **2g** (Fig. 6b) can be observed interacting with the substrate Leu-βNA. The results are in correlation with the enzyme kinetic studies, where the compounds have been evaluated as competitive inhibitors.

Figure 7 shows the binding of most inhibitory compounds **1g** and **2h** in the active site of cathepsin L. The results of the docking studies support the in vitro experimental studies conducted on goat liver cathepsin L, which shows that semicarbazones are better inhibitor than thiosemicarbazones and this is different in contrast with the results obtained in case of cathepsin H. The binding energies of title compounds in the amino acyl binding site of cathepsin L (cav3BC3L_CSW) is presented in Table 4. The binding energies of **1g** and **2h** were found to be −98.99 and −87.43, respectively.

Figure 7c shows the docking results of substrate Z-Phe-Arg-4mβNA with the cathepsin L active site. It can be observed that the results are in correlation with the enzyme kinetic studies, where the compounds have been evaluated as competitive inhibitors as the amino acids Asp-162, Gly-164, and His-163 interact with the most inhibitory compounds **1g** (Fig. 7a) and **2h** (Fig. 7b) can be observed interacting with the substrate Z-Phe-Arg-4mβNA.

Conclusion

The present work concluded that the synthesized title compounds have been evaluated as better inhibitors for cathepsin L than cathepsin H and previously reported cathepsin H. One more aspect of the present work is concluded that cathepsin L inhibited semicarbazones more than that of thiosemicarbazones, whereas in case of cathepsin H thiosemicarbazones show more inhibition than semicarbazones. Best inhibitor for cathepsin H has been evaluated as *m*-chlorobenzaldehyde semicarbazone, (**1b**) and *o*-nitrobenzaldehyde thiosemicarbazone, (**2g**) with K_i values of 0.27×10^{-4} M and 0.09×10^{-4} M, for cathepsin L *o*-nitrobenzaldehyde semicarbazone, (**1g**) and *m*-nitrobenzaldehyde thiosemicarbazone, (**2h**) showed maximum inhibition with K_i values of 0.005×10^{-8} M and 0.05×10^{-8} M.

Acknowledgement Among the authors, Ravinder Kaur acknowledges Kurukshetra University, Kurukshetra for providing URS and other lab facilities.

Compliance with ethical standards

Conflict of interest The authors have declared no conflict of interest.

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