Bioorganic & Medicinal Chemistry Letters 20 (2010) 3906–3910

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

Design, synthesis and anticancer activity of piperazine hydroxamates and their histone deacetylase (HDAC) inhibitory activity

Bhadaliya Chetan [a], Mahesh Bunha [a], Monika Jagrat [a], Barij Nayan Sinha [a], Philipp Saiko [a], Geraldine Graser [b], Thomas Szekeres [b], Ganapathy Raman [c], Praveen Rajendran [c], Dhatchana Moorthy [c], Arijit Basu [a], Venkatesan Jayaprakash [a,*]

[a] Department of Pharmaceutical Sciences, Birla Institute of Technology, Mesra, Ranchi, Jharkhand 835 215, India
[b] Department of Medical and Chemical Laboratory Diagnostics, General Hospital of Vienna—Medical University of Vienna, Waehringer Guertel 18-20, A-1090 Vienna, Austria
[c] Orchid Research Laboratories Ltd, Chennai 600 119, India

ARTICLE INFO

Article history:
Received 23 February 2010
Revised 20 April 2010
Accepted 8 May 2010
Available online 15 May 2010

Keywords:
Histone deacetylase inhibitors
Piperazine linker
Hydroxamates
RR pharmacophore

ABSTRACT

Six compounds were synthesized with piperazine in linker region and hydroxamate as Zinc Binding Group (ZBG). They were screened against three cancer cell-lines (NCIH460; HCT116; U251). Compounds **5c** and **5f** with GI_{50} value of 9.33 ± 1.3 μM and 12.03 ± 4 μM, respectively, were tested for their inhibitory potential on hHDAC8. Compound **5c** had IC_{50} of 33.67 μM. Compounds were also screened for their anticancer activity against HL60 human promyelocytic leukemia cell line due to the presence of pharmacophoric features of RR inhibitors in them. Compound **5c** had IC_{50} of 0.6 μM at 48 h.

HDAC activity is invariably increased in cancer cells and inhibitors of HDAC were proven to be novel class of antiproliferative agents.[1,2] HDAC inhibition has recently been clinically validated as a new therapeutic strategy for cancer treatment with the FDA approval of Suberoyl Anilide Hydroxamic Acid (SAHA) for the treatment of cutaneous T cell lymphoma.[3] Small molecules inhibitors of HDAC that are currently in clinical trials for cancer treatment are MS-275 and NVP-LAQ824 (Fig. 1).

Over-expression of class-I HDAC in certain cancer cells and the anticancer property of their inhibitors (Pan-HDAC inhibitors) has been reported by many groups.[4] Recently Pan-HDAC inhibitors have gained appreciation over isoform specific inhibitors for the treatment of cancer.[5] But high expression of only HDAC8 in neuroblastoma tumerogenesis demands selective HDAC8 inhibitors.[6] We have recently reported selective inhibitors of hHDAC8 with piperazine linker.[7] Here, we are reporting six novel hHDAC8 inhibitors with piperazine linker.

The compounds **5a–5f** were synthesized by the reactions outlined in the Scheme 1. The common intermediate *N*-hydroxypiperazine-1-carboxamide (**2**) was synthesized in two step using modified procedures of Grobner and Steinberg by replacing hydrazine with piperazine.[8] The Schiff's bases of methyl hydrazinecarbodithioate (**4a–4f**) were prepared by the condensation of methyl hydrazinecarbodithioate (**3**) with different aldehydes and ketones.[9] Finally, the *S*-methyl group of **4a–4f** upon displacement by the common intermediate **2** provided the final compound **5a–5f**. The intermediates were characterized by means of elemental analysis (for CHNS, experimental values are within ±0.4% variation with that of calculated values) and FT-IR spectral and the final compounds were characterized through ^{1}H NMR and FAB-MS spectral data. The structure and physicochemical and spectral characteristics of synthesized molecules were presented in Table 1. Experimental procedures were presented in Supplementary data.

All the six compounds synthesized were tested for anticancer activity in in vitro cancer cell-line assay based on cell viability using the dye, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT).[10] The Microculture Tetrazolium Assay is based on metabolic reduction of dye MTT to water insoluble blue formazan crystal by Mictochondrial dehydrogenase enzyme. The formation of formazan complex is directly proportional to number of viable cells. The compounds were tested against NCIH460, HCT116 and U251 cell lines[11] and the results are presented in Table 2. Except compound **5a** all the other five compounds were having mean IG_{50} value less than or equal to 40 μM concentration. Compound **5c** was the one which is having the best mean GI_{50} value of 9.33 ± 1.3 μM in the series, that was comparable to SAHA (5.03 μM). The **5f** and **5d** were the next two potent molecules in this series with 12.03 ± 4 μM and 18.33 ± 6.3 μM, respectively. Compounds **5a** and **5b** with bulkier

* Corresponding author. Tel.: +91 6512276247.
E-mail address: venkatesanj@bitmesra.ac.in (V. Jayaprakash).

0960-894X/$ - see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2010.05.020

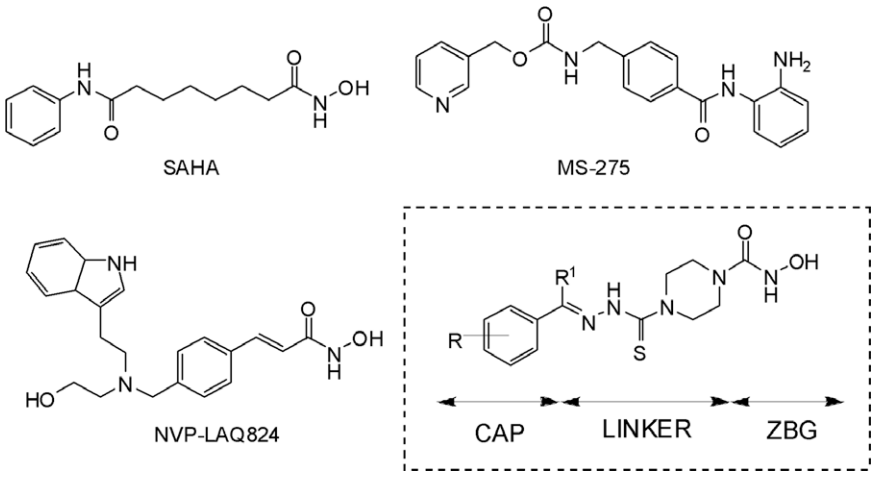
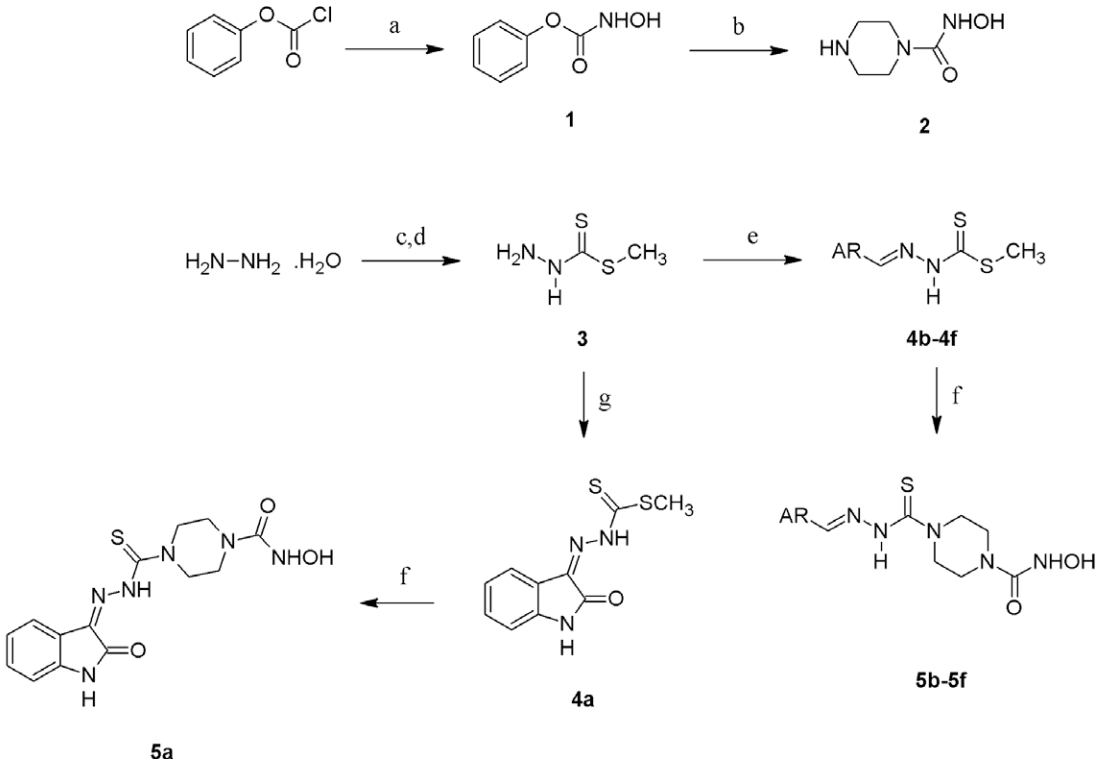


Figure 1. HDAC inhibitors in clinical trial. Pharmacophoric features of HDAC inhibitors in the title compounds (in box).

groups in the cap region did not exhibited favorable activity profile. Experimental procedures were presented in Supplementary data.

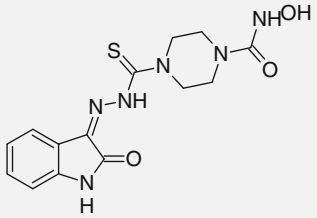
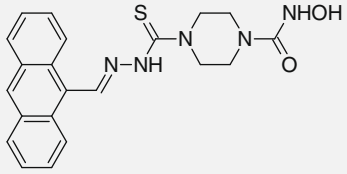
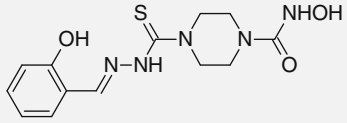
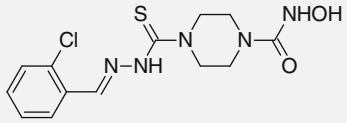
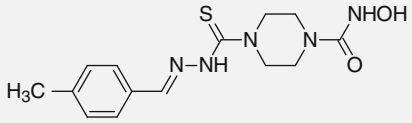
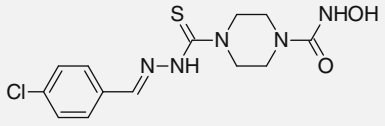
Compounds **5c** and **5f** were selected for in vitro enzyme assay against human HDAC1 and HDAC8 by means of Microplate HDAC Fluorometric assay.[12,13] The assay utilizes acetylated lysine as substrate, which upon deacetylation by HDAC liberates free lysine. Free lysine upon treatment with developer (Trypsin) produces a fluorophore, whose intensity can be measured at excitation wavelength of 360 nm and wavelength of 460 nm. Both the compounds (**5c** and **5f**) inactivated hHDAC8 preferentially over hHDAC1 and the results are presented in Table 2. Compound **5c**, potent one amongst two, inhibited hHDAC8 at IC_{50} 33.67 μM and **5f** at 43.16 μM. Compared with SAHA, compounds **5c** and **5f** were less potent in the magnitude of 748 and 959-fold, respectively, but preferentially inhibited hHDAC8. Experimental procedures were presented in Supplementary data.

Molecular docking studies were performed using the GLIDE program (version 5.0, Schrodinger, LLC, New York, 2008) to understand the interaction of **5c** with hHDAC8. The Maestro user interface (version 8.5, Schrodinger, LLC, New York, 2008) was employed to set up and execute the docking protocol and also for analysis of the docking results. Validation of docking protocol was done by redocking. Human HDAC8 bound to SAHA (PDB ID: 1T69) was selected for docking studies and was prepared for docking through protein preparation wizard, energy minimization has been carried out using OPLS2001 force field. Structures of **5a–5f** were sketched using built panel on



Scheme 1. Reagents and conditions: (a) hydroxylamine HCl, K_2CO_3, ether/water, 4–6 h stirring; (b) piperazine hexahydrate, ethanol, 12 h, reflux; (c) CS_2, KOH, water, isopropanol, 2–3 h stirring at <10 °C; (d) CH_3I, 2–3 h stirring at <10 °C; (e) aromatic aldehyde, methanol, 24 h, reflux; (f) intermediate 2 in MeOH or EtOH, 24 h, reflux; (g) isatin, methanol, 24 h, reflux.

Table 1
Physicochemical characteristics of synthesized molecules **5a–5f**

Code	Structure	MF	MW	MP (°C)	Yield (%)	FT-IR (cm^{-1})	^{1}H NMR (δ/ppm)	FAB-MS (m/z)
5a		$C_{14}H_{16}N_6O_3S$	348	220	43.5	3223 (ν –OH), 1197 (ν C=S), 3026 (ν –NH), 1562 (ν C=N), 1562 (ν C=O)	7.1–8.32 (m, 4H, Ar-H), 2.18–2.82 (m, 8H, piperazine –CH–), 8.42 (s, 1H, –CS–NH–), 8.63 (s, 1H, –N*H*–OH), 9.87 (s, 1H, –NH–O*H*), 10.12 (s, 1H, Isatin NH)	349 (M+1)
5b		$C_{21}H_{21}N_5O_2S$	407	198–200	61.5	3153 (ν –OH), 1097 (ν C=S), 3001 (ν –NH), 1552 (ν C=N), 1622 (ν C=O)	7.2–8.7 (m, 9H, Ar-H), 2.25–2.7 (m, 8H, piperazine –CH–), 9.31 (s, 1H, Ar-CH=), 8.97 (s, 1H, –CS–NH–), 9.69 (s, 1H, –N*H*–OH), 11.85 (s, 1H, –NH–O*H*)	408 (M+1)
5c		$C_{13}H_{17}N_5O_3S$	323	280–282	82.7	3238 (ν –OH),	6.9–7.47 (m, 4H, Ar-H), 2.78 (s, 8H, piperazine –CH–), 8.50 (s, 1H, Ar-CH=), 9.23 (s, 1H, –CS–NH–), 10.58 (s, 1H, –N*H*–OH), 10.67 (s, 1H, –NH–O*H*), 11.53 (s, 1H, Ar-OH)	324 (M+1)
5d		$C_{13}H_{16}ClN_5O_2S$	341	150	79	3236 (ν –OH), 1205 (ν C=S), 3008 (ν –NH), 1481 (ν C=N), 1765 (ν C=O)	7.2–7.4 (m, 4H, Ar-H), 2.67 (s, 8H, piperazine –CH–), 8.30 (s, 1H, Ar-CH=), 8.96 (s, 1H, –CS–NH–), 9.13 (s, 1H, –N*H*–OH), 10.25 (s, 1H, –NH–O*H*)	341(M+), 342 (M+1), 343 (M+2)
5e		$C_{14}H_{19}N_5O_2S$	321	170–172	52.3	3296 (ν –OH), 1253 (ν C=S), 3142 (ν –NH), 1473 (ν C=N), 1624 (ν C=O)	7.2–7.8 (m, 4H, Ar-H), 2.67 (s, 8H, piperazine –CH–), 8.29 (s, 1H, Ar-CH=), 8.77 (s, 1H, –CS–NH–), 9.33 (s, 1H, –N*H*–OH), 10.96 (s, 1H, –NH–O*H*), 2.39 (s, 3H, $-CH_3$)	322 (M+1)
5f		$C_{13}H_{16}ClN_5O_2S$	341	160	83	3230 (ν –OH), 1200 (ν C=S), 3026 (ν –NH), 1453 (ν C=N), 1762 (ν C=O)	7.2–7.8 (m, 4H, Ar-H), 2.67 (s, 8H, piperazine –CH–), 8.37 (s, 1H, Ar-CH=), 8.94 (s, 1H, –CS–NH–), 9.33 (s, 1H, –N*H*–OH), 10.30 (s, 1H, –NH–O*H*)	341(M+), 342 (M+1), 343 (M+2)

maestro and prepared for docking through Ligprep module (energy minimized using MMFF force field). GLIDE grid generation wizard has been used to define the docking space. Docking was performed using SP (Standard Precision mode) docking protocol. The molecular docking results are presented in Table 2. ZBG hydroxamate functional group of all the molecules were found to be close to Zn^{2+} atom in the active site, and establishes a hydrogen bond with TYR306, which shows the major and favorable interaction of the ligands with hHDAC8 (Fig. 2a). Amongst the six molecules docked, compound **5c** was the one with the best Glide and E model score of −7.67 and −94.5, respectively. It exhibited three hydrogen bonding interaction with HIS142, GLY206 and TYR306 (Fig. 2b). The hydroxamate group is placed near the Zn^{2+} atom with distance of 2.078 Å (hydroxamate carbonyl O to Zn) and 1.846 Å (hydroxamate hydroxyl O to Zn) which is close to the measures of SAHA (2.309 and 2.037 Å for crystallographic and 2.078 and 2.025 Å for redocked). Docking protocol was presented in Supplementary data.

Present work once again substantiated the suitability of piperazine linker for designing hHDAC selective inhibitors. Further cap group modification in this series may provide a potent and selective inhibitor of hHDAC8. Compared with compound **4c** reported earlier,[7] the compound **5c** has one atom less between hydroxamate

Table 2
In-vitro cell line assay, hHDAC8 enzyme assay and molecular docking data of compounds **5a–5f**

Code	NCIH460 (Lung) (μM)			HCT116 (Colon) (μM)			U251 (Glioma) (μM)			MEAN GI_{50} (μM)	HDAC8 (μM)	Molecular docking		HL60 human promyelocytic leukemia at 48 h (μM)
	GI_{50}	TGI	LC_{50}	GI_{50}	TGI	LC_{50}	GI_{50}	TGI	LC_{50}			Glide score	E model score	
5a	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	—	−7.67	−94.5	
5b	9.5	>100	>100	70	>100	>100	65	>100	>100	40.5 ± 16.5	—	−7.54	−90.4	
5c	5.15	>100	>100	8.6	>100	>100	14.25	>100	>100	9.33 ± 1.3	33.67	−7.32	−91.8	0.6
5d	30	>100	>100	11.5	62.5	>100	13.5	80	>100	18.33 ± 6.3	—	−7.13	−83.3	
5e	51	60	>100	31	85	>100	27.5	>100	>100	36.5 ± 15.8	—	−7.09	−83.2	
5f	16	60	>100	11.6	51	>100	8.5	100	>100	12.03 ± 4	43.16	−6.85	−83.2	
SAHA	8	100	>100	0.6	>100	>100	6.5	40	>100	5.03	0.045	−4.89	−83	

[*] Mean GI_{50} acceptable range (SAHA = 0.4–8.5 μM) from published and historical data.

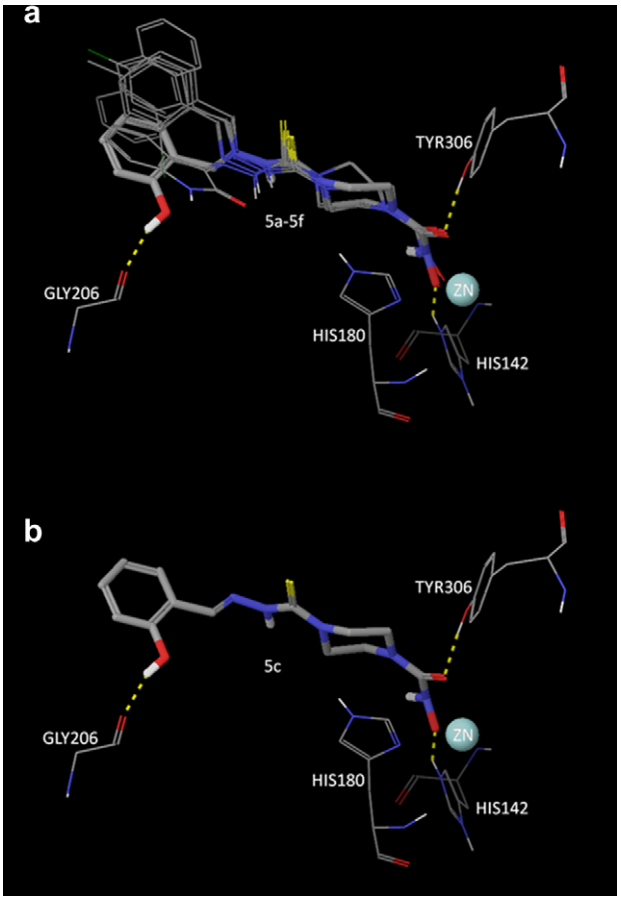


Figure 2. (a) Alignment of the molecules **5a–5f** in the active site, **5c** in tube representation, hydrogen bonds were shown as yellow broken lines; (b) Interaction of **5c** with active site residues in 1t69.

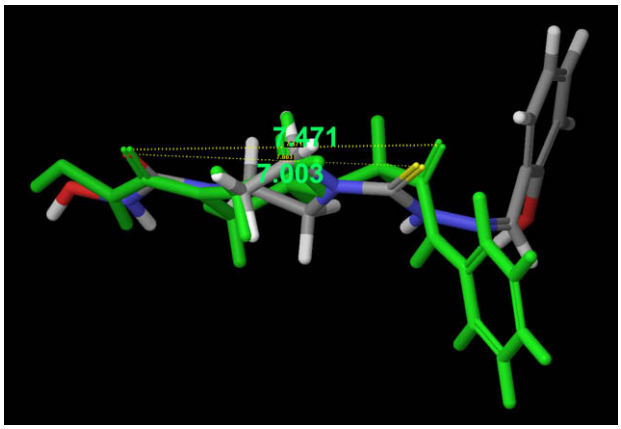


Figure 3a. The distance between two carbonyl groups in compounds **4c** (green, 7.471 Å) and **5c** (colored by atom type, 7.003 Å) are shown.

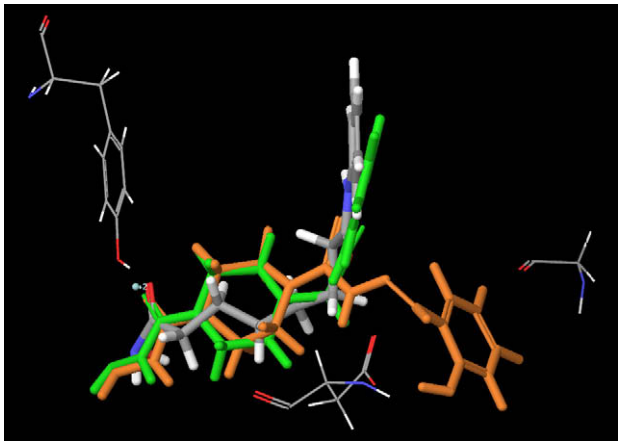


Figure 3b. Docked conformers of compounds **5c** (orange), **4c** (green) and SAHA (colored by atom type) were presented.

carbonyl and thiocarbamoyl group. The distance between two carbonyl groups in **4c** was 7.471 Å, while in **5c** was 7.003 Å (Fig. 3a). At the same time the absence of methylene group between piperazine N1 and acyl carbonyl group orient the cap group differently in the pocket (Fig. 3b). These factors together has kept thiocarbamoyl N–H at a distance not favouring hydrogen bonding interaction with ASP101, a favorable interaction shown by many HDAC inhibitors and **4c**.[7] This may led to the reduced potency of **5c** compared with **4c**.

Also it was observed, higher concentration of **5c** was required to inhibit hHDAC8 (IC_{50} = 33.67 μM) than that required to inhibit cancer cell lines (GI_{50} = 9.33 ± 1.3 μM). This suggested that compound **5c** may act on other targets too. Due to the presence of hydroxyurea and salicylaldehyde Schiff base pharmacophores in **5c** (Fig. 3), we suspect that these molecules may also inhibit Ribonucleotide reductase (RR) (Fig. 4). On this background, the compounds **5c** was screened against HL60 human promyelocytic leukemia cell line (ATCC, Manassas, VA, USA), in which overexpression of RR has been reported. HL60 cells incubated with increasing concentrations of **5c** at 37 °C, Cell counts and IC_{50} values were determined after 24, 48, and 72 h using the microcell counter

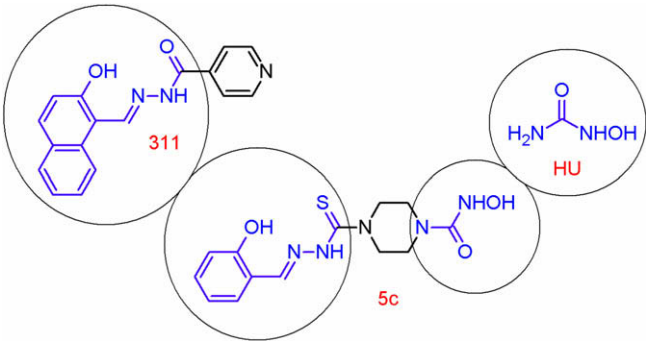


Figure 4. Pharmacophoric features of ribonucleotide reductase inhibitors in **5c**. HU: Hydroxyurea.

CC-108. Viability of cells was determined by trypan blue exclusion. Results were calculated as number of viable cells. Compound **5c** was found to have IC_{50} of 0.6 μM at 48 h (Table 2), that was comparable to that of compound **311** having IC_{50} 0.4 μM at 48 h.[14] This fact suggests the possible inhibition of Ribonucleotide reductase as suspected (experimental procedures were presented in Supplementary data). Compound **5c** requires further investigation in this regard for its Ribonucleotide Reductase inhibitory activity that may provide a novel dual inhibitor of HDAC/RR type.

Acknowledgments

The author was grateful to the Head, Sophisticated Analytical Instrument Facility (SAIF), CDRI Lucknow, India, for providing spectral data and the first author was thankful to UGC for Junior Research Fellowship.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2010.05.020.

References and notes

1. Paris, M.; Porcelloni, M.; Binaschi, M.; Fattori, D. *J. Med. Chem.* **2008**, *51*, 1505.
2. Park, J. H.; Jung, Y.; Kim, T. Y.; Kim, S. G.; Jong, H. S.; Lee, J. W.; Kim, D. K.; Lee, J. S.; Kim, N. K.; Kim, T. Y.; Bang, Y. J. *Clin. Cancer Res.* **2004**, *10*, 5271.
3. Duvic, M.; Vu, J. *Expert Opin. Invest. Drugs* **2007**, *16*, 1111.
4. Nakagawa, M.; Oda, Y.; Eguchi, T.; Aishima, S.; Yao, T.; Hosoi, F.; Basaki, Y.; Ono, M.; Kuwano, M.; Tanaka, M.; Tsuneyoshi, M. *Oncol. Rep.* **2007**, *18*, 769.
5. Witt, O.; Lindemann, R. *Cancer Lett.* **2009**, *280*, 123.
6. Oehme, I.; Deubzer, H. E.; Wegener, D.; Pickert, D.; Linke, J. P.; Hero, B.; Schneider, A. K.; Westermann, F.; Ulrich, S. M.; Deimling, A. V.; Fischer, M.; Witt, O. *Clin. Cancer Res.* **2009**, *15*, 91.
7. Chakrabarty, S.; Jasmine; Bhadaliya, C.; Sinha, B. N.; Mahesh, A.; Bai, H.; Blond, S. Y.; Venkatesan, J. *Arch. Pharm.* **2010**, *343*, 167.
8. Ren, S.; Wang, R.; Komatsu, K.; Bonaz-Krause, P.; Zyrianov, Y.; McKenna, C. E.; Csipke, C.; Tokes, Z. A.; Lien, E. J. *J. Med. Chem.* **2002**, *45*, 410.
9. Klayman, D. L.; Bartosevich, J. F.; Griffin, T. S.; Mason, C. J.; Scovill, J. P. *J. Med. Chem.* **1979**, *22*, 855.
10. Mosmann, T. *J. Immuno. Methods* **1983**, *65*, 55.
11. Monks, A.; Scudiero, D.; Skehan, P.; Shoemaker, R.; Paull, K.; Vistica, D.; Hose, C.; Langley, J.; Cronise, P.; Vaigro-Wolff, A.; Gray-Goodrich, M.; Campbell, H.; Mayo, J.; Boyd, M. *J. Natl. Cancer Inst.* **1991**, *83*, 757.
12. Wegener, D.; Hildmann, C.; Riester, D.; Schwienhorst, A. *Anal. Biochem.* **2003**, *321*, 202.
13. Wegener, D.; Wirsching, F.; Riester, D.; Schwienhorst, A. *Chem. Biol.* **2003**, *10*, 61.
14. Richardson, D. R.; Milnes, K. *Blood* **1997**, *89*, 3025.