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

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

# Identification of amino acid appended acridines as potential leads to anti-cancer drugs

Palwinder Singh [a,*], Arun Kumar [a], Anuradha Sharma [b], Gurcharan Kaur [b]

[a] Department of Chemistry, Guru Nanak Dev University, Amritsar, India
[b] Department of Biotechnology, Guru Nanak Dev University, Amritsar, India

ARTICLE INFO

*Article history:*
Received 13 June 2015
Revised 8 July 2015
Accepted 18 July 2015
Available online xxxx

*Keywords:*
Acridines
Antiproliferative
C6 glioma cells
α-Tubulin, GFAP, Mortalin and HSP-70 markers

ABSTRACT

In order to develop the amino acid appended acridines as potential leads for anticancer drugs, they were subjected to preliminary investigations. Screening through MTT assay as well as the phase contrast micrographs and Confocal images of immunostained C6 Glioma cells for markers such as α-tubulin, GFAP, mortalin and HSP-70 cells indicated that the compounds possess significant antiproliferative activity. The compounds also arrested cells in $G_0/G_1$ phase of the cell cycle as indicated by flow cytometry results. Moreover, the upregulation of the senescence markers such as mortalin and HSP70 in the presence of compounds **8**, **9** and **12** indicate their senescence inducing potential.



The understanding of the molecular biochemistry of initiation/propagation of cancer and the role of various biological entities in the progression of this disease has helped in identifying the cellular target/s of the anti-cancer drugs. Primarily, caused by the breaking and mutation in the DNA (gene mutation),[1] various types of infections, radiations, genetic inheritance and environmental pollutants are responsible for the initiation of cancer.[2] In the form of natural remedial measures, the alkaloids like vinblastine, vincristine, vindesine and terpenoids including paclitaxel and docetaxel are known to act as anti-cancer drugs[3] through the inhibition of mitotic phase amongst the G1 (growth phase), S (synthesis phase), G2 (growth phase) and M (mitosis) phases of the cell division. Furthermore, since the enzymes thymidylate synthase (TS), thymidylate phosphorylase (TP), ribonucleotide reductase (RR), topoisomerase II (topoII), tubulin, aminoacyltransferase, dihydrofolate reductase do take part at different stages of cell division;[4] they are made the primary targets during the process of chemotherapy of cancer. Therefore, in addition to the remedial approaches like genetic engineering, radiotherapy and surgery for counteracting the initiation/propagation of cancer,[5] chemotherapy is a primary treatment approach for the pre- and post-surgical cases. Hence, the development of tailor-made chemical entities for the treatment of cancer is continuously drawing the attention of the scientific community. 5-Fluorouracil, mitoxantrone, doxorubicin and some other synthetic drugs[6] (Chart 1) are proving highly beneficial to the cancer patients. However, a number of bottlenecks including moderate efficacy, side effects, low patient tolerance, cost factor, etc., which are associated with the use of anti-cancer drugs, necessitate the search for new molecules for making the chemotherapeutic approach more effective.

Amongst the structurally diverse categories of chemotherapeutic agents, the acridine based drugs have shown profound effect on the inhibition of propagation of cancerous cells and thereby controlling the tumor growth.[7] Besides the other counteractive features, this class of anti-cancer drugs works through DNA intercalation. Reports are also available about the anti-proliferative properties of the amino acid derivatives of acridines.[8] On the basis of the natural selectivity and affinity of the amino acids for the biological system; here we report amino acid/peptide appended acridines as the potential anti-cancer agent.

Acridone (9-oxo-9,10-dihydroacridine-4-carboxylic acid) (**1**) was prepared by the reaction of anthranilic acid with 2-chlorobenzoic acid. Treatment of acridone **1** with L-valine methyl ester hydrochloride in the presence of triethyl amine and ethyl chloroformate provided compound **2**. Similarly, treatment of acridone **1** with L-tyrosine methyl ester hydrochloride provided compound **3**. Compound **3** was treated with LiOH for hydrolyzing its ester unit and thereby compound **4** was obtained (Scheme 1).

Not randomly but keeping in mind the hydrophilicity/hydrophobicity factor (Lipinski's rule of '5', Table S1), amino acid

* Corresponding author. Tel.: +91 183 2258802x3495; fax: +91 183 2258819.
*E-mail address:* palwinder_singh_2000@yahoo.com (P. Singh).

http://dx.doi.org/10.1016/j.bmcl.2015.07.059
0960-894X/

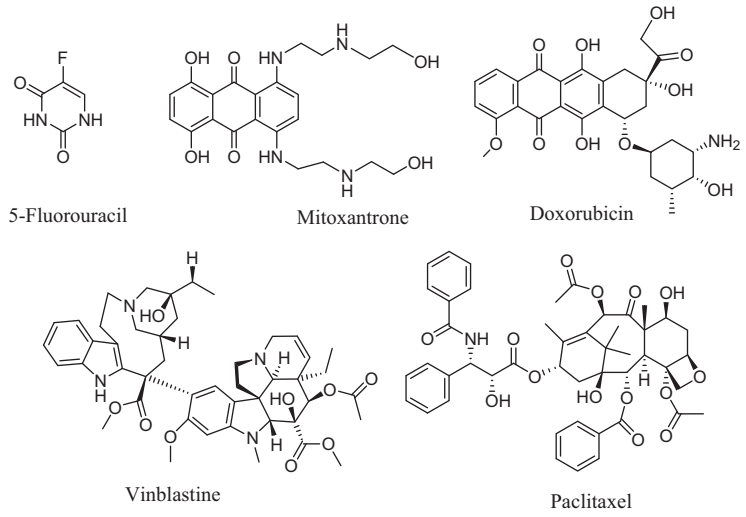
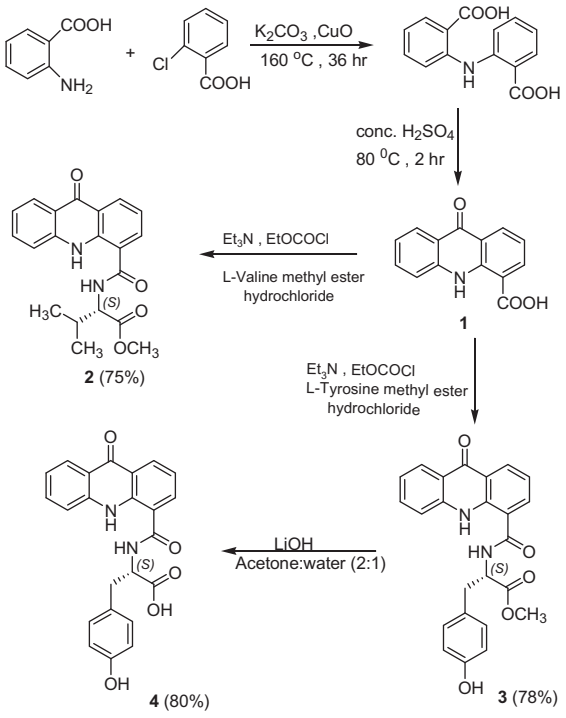



**Chart 1.**




**Scheme 1.**

chain of compound **4** was further extended by introducing proline and glycine. As depicted in Scheme 2, compound **4** was treated with L-proline methyl ester hydrochloride to procure compound **5**. Ester hydrolysis of compound **5** and subsequent treatment with glycine methyl ester hydrochloride provided compound **7** which on treatment with LiOH gave compound **8**. Likewise, the sequential incorporation of glycine and proline in compound **4** provided compound **12**.

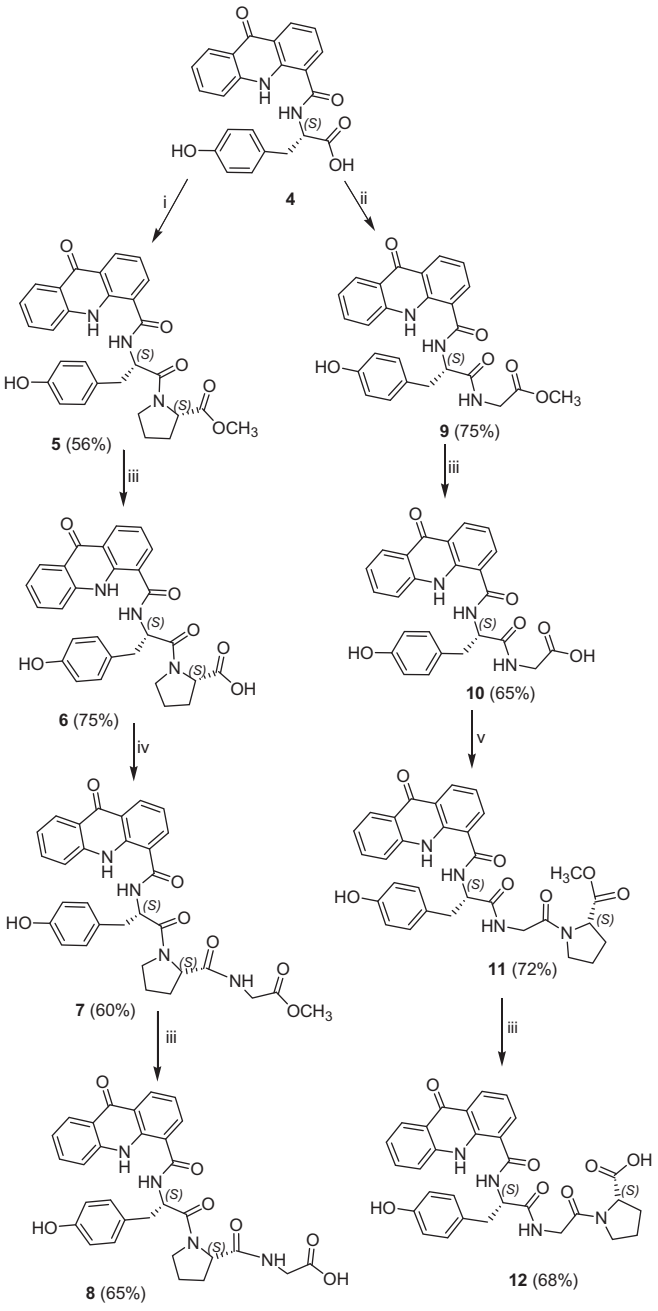
Mechanistically; for the amide bond formation step, ethyl chloroformate activated the carboxyl group of acridone **1** through the formation of mixed anhydride and therefore the coupling with L-valine methyl ester hydrochloride was achieved in 15–20 min at 0 °C (Scheme 3). Same reaction procedure was employed for the other amide/peptide bond formation reactions in Schemes 1 and 2. Triethyl amine has probably acted as hydrochloride acceptor and did not promote enolization at the $C_\alpha$ of amino acid,[9] hence, keeping the possibility of isomerization of the products **5**, **7**, **9** and **11** at the minimum. Non-epimerization during the synthesis of these compounds was also ascertained by preparing compound **3** using D-Tyr methyl ester hydrochloride and DL-Tyr methyl ester hydrochloride and comparing the HPLC chromatograms of the corresponding compounds **3** (Figs. S25–S27). Similarly, the other isomer of compound **5** was also prepared by the reaction of compound **4** with D-Pro methyl ester hydrochloride and DL-Pro methyl ester hydrochloride (Fig. S29). HPLC of all the products was performed by using Chirobiotic® T 10 μm chiral HPLC column (25 cm × 4.6 mm) and no epimerization was observed in any of the peptide bond formation reactions (Figs. S28–S34). All the compounds were characterized with NMR, Mass and IR spectral techniques.

Using MTT assay, the cells were incubated with compounds **2**, **3**, **4**, **8**, **9** and **12** for 72 h and were tested for their antiproliferative activity using concentrations ranging from 0.5 μM to 50 μM. The $IC_{50}$ of these compounds was in the range of 14–20 μM (Fig. 1). Cell cycle analysis has clearly shown that the compounds under investigation arrest C6 cells in $G_0/G_1$ phase of the cell cycle (Fig. 2). In comparison to the other compounds; **8**, **9** and **12** resulted in statistically significant arrest of the cells in $G_0/G_1$ phase of the cell cycle and hence these three compounds were subjected to further investigation.

Phase contrast imaging of cells indicated that concentrations above 10 μM were toxic (Figs. S35 and S37) which resulted in distorted cell morphology, so 10 μM concentration was selected for further studies. Cells treated with 10 μM concentration of **8**, **9** and **12** showed significant decrease in rate of proliferation of C6 cells as compared to control cultures (Fig. S36A).

Flow cytometry based detailed cell cycle analysis showed significant decrease in the cell number in $G_2$/M and S phase after treatment with compounds **8**, **9** and **12** in comparison to the control (Fig. S36C), thus showing reduction in their proliferation. On the other hand, a significant increase was observed in $G_0/G_1$ population of cells after exposing to test compounds. These observations suggest that the compounds **8**, **9** and **12** arrested the cancer cell population in the resting phase, that is, $G_0/G_1$ phase of cell cycle, like the normal cells, and thus may prove to be potential antiproliferative compounds.

α-Tubulin is a cytoskeletal marker protein and its immunostaining was carried out to observe the morphological changes in




**Scheme 2.** Reaction conditions: (i) $Et_3N$, EtOCOCl, L-proline methyl ester hydrochloride; (iii) LiOH, acetone/water (2:1); (ii) $Et_3N$, EtOCOCl, glycine methyl ester hydrochloride; (iv) $Et_3N$, EtOCOCl, glycine methyl ester hydrochloride; (v) $Et_3N$, EtOCOCl, L-proline methyl ester hydrochloride.

cells after treatment with **8**, **9** and **12** as it stains both the cell body as well as its processes. Phase contrast micrographs and confocal images of α-tubulin immunostained cells also showed reduced cell

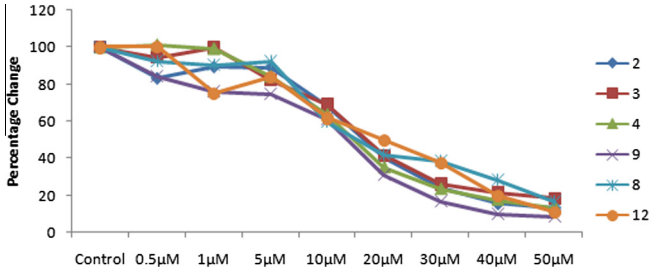



**Figure 1.** MTT assay showing decrease in cell number upon treatment with compounds **2**, **3**, **4**, **8**, **9** and **12**.

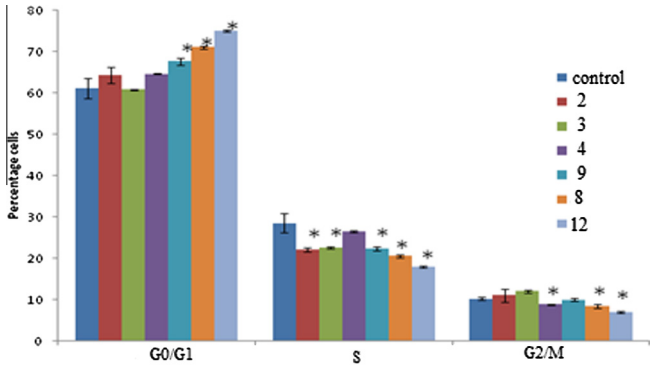
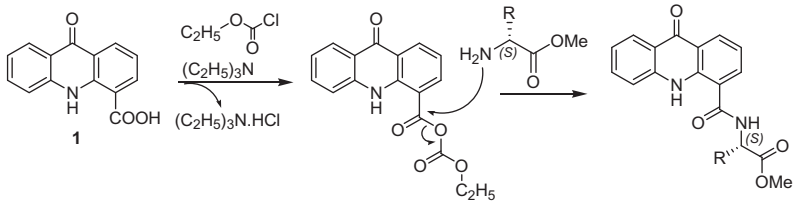



**Figure 2.** Histogram representing distribution of cells in $G_0/G_1$, S and $G_2/M$ phase of cell cycle analyzed by PI using flow cytometer. Values are presented as mean ± SEM of at least three independent experiments. *($P$ <0.05), represent statistical significant difference between control and treated groups.

number and well differentiated morphology with long stellate processes and small cell body in the treated cultures. GFAP is an astrocyte specific intermediate filament (IF) protein marker[10] and many CNS pathologies such as trauma and tumor have been shown to cause astrogliosis[11] which is characterized by enhanced GFAP expression and astrocytic proliferation.[12] The current data shows that treatment of C6 cells with **8**, **9** and **12** was associated with down regulation of GFAP expression (Figs. 3B and 5A). Based on these observations, it may be suggested that these test compounds have the potential to suppress the proliferation as well as the reactive astrogliosis seen in C6 glioma tumor cells.

To further elucidate the senescence inducing potential of these compounds, the expression of senescence markers such as heat shock family protein members-mortalin and HSP70 was studied (Fig. 4). Immortal cells are reported to have mortalin expression in perinuclear region,[13,14] whereas, normal cells show pancytoplasmic distribution of this protein.[14] In control C6 glioblastoma cells, perinuclear expression of mortalin was observed which got translocated to pancytoplasmic locations and nucleus after treatment with **8**, **9** and **12**. Interestingly, the nuclear expression of mortalin was also upregulated (Fig. 4A). This perinuclear to pancytoplasmic translocation is reported to be related induction of senescence, whereas, a recent Letter suggested that nuclear




**Scheme 3.**

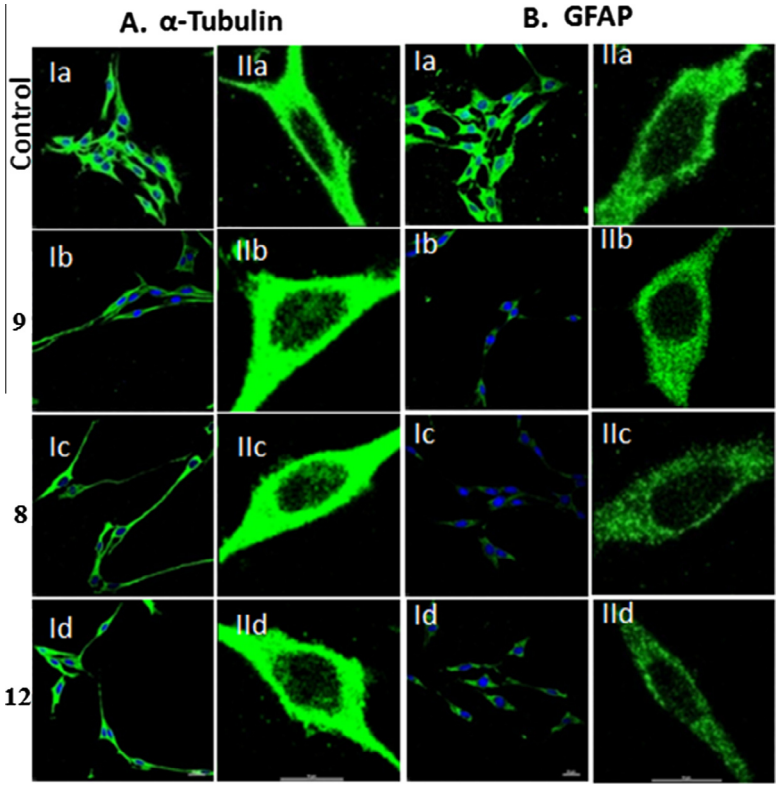



**Figure 3.** Confocal images of C6 glioma cells showing α-tubulin (A) and GFAP (B) expression in control (Ia), **9** (Ib), **8** (Ic) and **12** (Id) treated cells. Right panel of α-tubulin (A IIa–d) is enlarged view of single cell corresponding to images in left panel (A Ia–d). Similarly, the right panel of GFAP (B IIa–d) is the enlarged view of single cell corresponding to images in the left panel (B Ia–d).

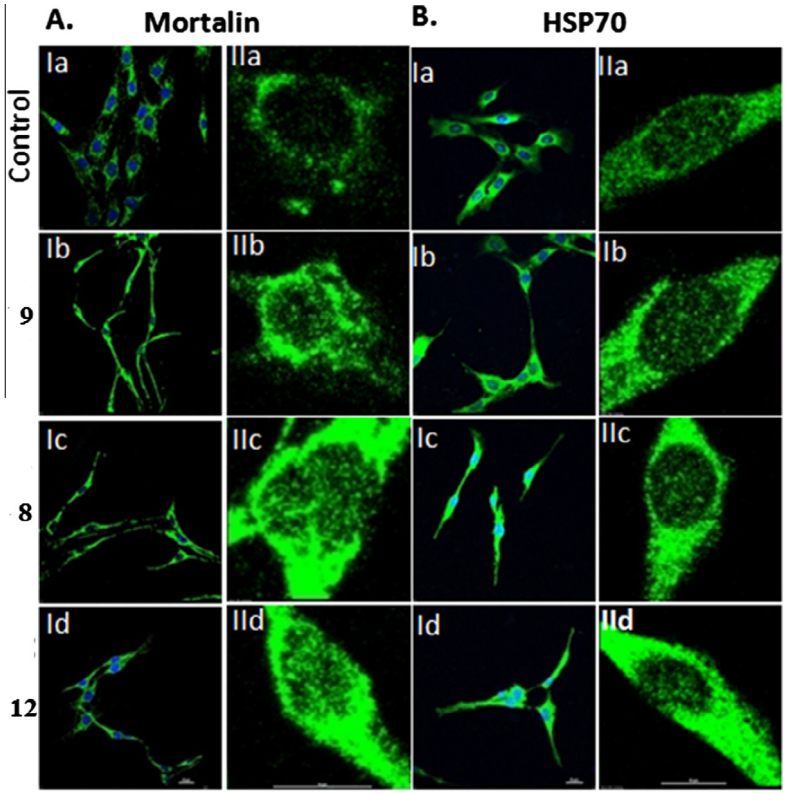



**Figure 4.** Confocal images of C6 glioma cells showing Mortalin (A) and HSP70 (B) expression in control (Ia), **9** (Ib), **8** (Ic) and **12** (Id) treated cells. Right panel of Mortalin (A IIa–d) is enlarged view of single cell corresponding to images in the left panel (A Ia–d). Similarly, the right panel of HSP70 (B IIa–d) is the enlarged view of single cell corresponding to images in the left panel (B Ia–d). All the images of Mortalin and HSP70 (Ia–d) were captured at same magnification. Difference in cell size observed in Ia as compared to Ib, Ic and Id is because of differentiation which these cells have undergone after treatment with test compounds (Ib, Ic and Id) as compared to control (Ia).

Please cite this article in press as: Singh, P.; et al. *Bioorg. Med. Chem. Lett.* (2015), http://dx.doi.org/10.1016/j.bmcl.2015.07.059

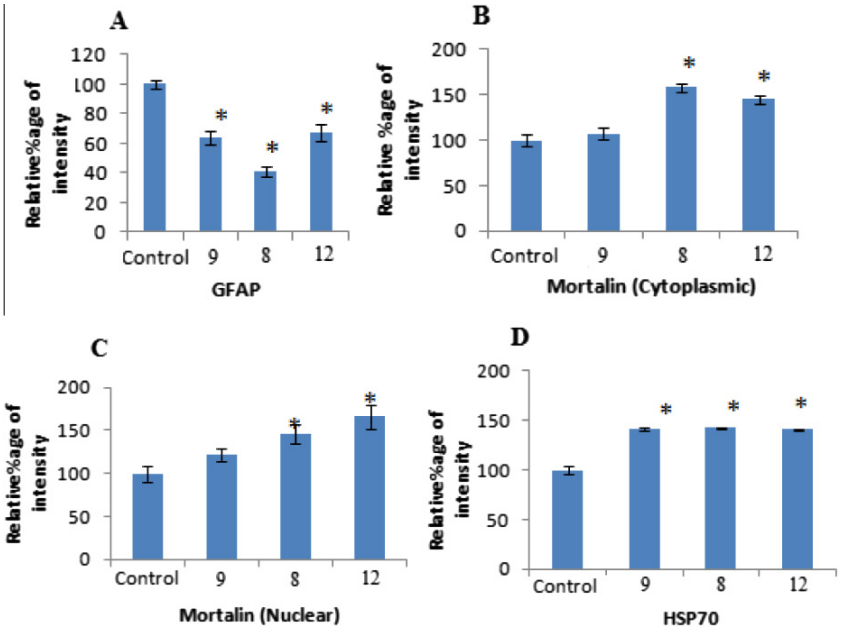



**Figure 5.** Histogram represents intensity analysis of GFAP (A), Mortalin (cytoplasmic) (B), Mortalin (Nuclear) (C), HSP70 (D) expression. Values are presented as mean ± SEM of at least three independent experiments. *($P$ <0.05), represent statistical significant difference between control and treated groups. Scale bar represents length of 20 μm (left panel) and 10 μm (right panel).

mortalin plays an important role in differentiation induction via retinoid receptor interactions.[15] These observations are also supported by previous Letters on anticancer and differentiation inducing activities of some natural products.[16–18]

This upregulated expression of mortalin (Figs. 4A, 5B and C) was also followed by elevated expression of mitochondrial stress response protein HSP70. HSP70 is an ATP-dependent housekeeping gene,[19] which is highly involved in glial cell differentiation and neurite outgrowth. There was significant increase in HSP70 expression in cells treated with 10 μg/ml concentration of **8**, **9** and **12** (Figs. 4B and 5D) which further supports the senescence and differentiation inducing potential of these compounds.[13,20]

In conclusion, the preliminary investigation of a series of amino acid appended acridines revealed considerable potential of these compounds as anticancer agents. Compounds exhibited significant antiproliferative activity against C6 glioblastoma cell line. This may be possibly attributed to arresting of cells in $G_0/G_1$ phase of the cell cycle. The compounds were also seen to upregulate mortalin and HSP70 expression thus indicating their senescence inducing potential. Synthesis of more compounds of this series and their further biological studies are underway.

## Acknowledgments

Financial assistance in the form of sponsoring research projects by DST, New Delhi and CSIR, New Delhi is gratefully acknowledged. A.K. acknowledges CSIR, New Delhi for fellowship. University Grants Commission, New Delhi is acknowledged for Grant under University with potential for Excellence to Guru Nanak Dev University.

## Supplementary data

Supplementary data (NMR, mass spectra, LC–MS of the compounds) associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2015.07.059.

## References and notes

1. Avendano, C.; Menendez, J. C. *Medicinal Chemistry of Anticancer Drugs*, 1st ed.; Elsevier, 2008. Chapter 1.
2. (a) Wang, L.; He, X.; Bi, Y.; Ma, Q. *Chem. Res. Toxicol.* **2012**, *25*, 1303; (b) Lundin, A.; Driscoll, B. *Cancer Lett.* **2013**, *338*, 89; (c) Lerebours, A.; Stentiford, G. D.; Lyons, B. P.; Bignell, J. P.; Derocles, S. A. P.; Rotchell, J. M. *Environ. Sci. Technol.* **2014**, *48*, 10448; (d) Friedenson, B. *Med. Hypotheses* **2011**, *77*, 791.
3. (a) Skwarczynski, M.; Hayashi, Y.; Kiso, Y. *J. Med. Chem.* **2006**, *49*, 7253; (b) Kingston, D. G. I. *J. Nat. Prod.* **2009**, *72*, 507; (c) Silvestri, R. *J. Med. Chem.* **2013**, *56*, 625.
4. (a) Singh, P.; Bhardwaj, A. *Mini Rev. Med. Chem.* **2008**, *8*, 388; (b) Bailly, C. *Chem. Rev.* **2012**, *112*, 3611.
5. Thurston, D. E. *Chemistry and Pharmacology of Anticancer Drugs*; Taylor & Francis Group, 2006. Chapter 1.
6. Avendano, C.; Menendez, J. C. *Medicinal Chemistry of Anticancer Drugs*, 1st ed.; Elsevier, 2008.
7. (a) Su, T. L.; Chen, C. H.; Huang, L. F.; Chen, C. H.; Basu, M. K.; Zhang, X. G.; Chou, T. C. *J. Med. Chem.* **1999**, *42*, 4741; (b) Kotakis, I. K.; Magiatis, P.; Pouli, N.; Marakos, P.; Skaltsounis, A. L.; Pratsinis, H.; Leonce, S.; Pierre, A. *J. Med. Chem.* **2002**, *45*, 2599; (c) Li, B.; Gao, C. M.; Sun, Q. S.; Li, L. L.; Tan, C. Y.; Liu, H. X.; Jiang, Y. Y. *Chin. Chem. Lett.* **2014**, *25*, 1021; (d) Fadeyi, O. O.; Adamson, S. T.; Myles, E. L.; Okoro, C. O. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 4172.
8. (a) Dzierzbicka, K.; Kolodziejczyk, A. M. *J. Med. Chem.* **2001**, *44*, 3606; (b) Dzierzbicka, K.; Kolodziejczyk, A. M. *J. Med. Chem.* **2003**, *46*, 183.
9. (a) Vaughan, J. R.; Osato, R. L. *J. Am. Chem. Soc.* **1951**, *73*, 5553; (b) El-Faham, E.; Albericio, F. *Chem. Rev.* **2011**, *111*, 6557.
10. Kamphuis, W.; Mamber, C.; Moeton, M.; Kooijman, L.; Sluijs, J. A.; Jansen, A. H. P.; Verveer, M.; Groot, L. R.; Smith, V. D.; Rangarajan, S.; Rodrıguez, J. J.; Orre, M.; Hol1, E. M. *PLoS One* **2012**, *7*, e42823.
11. Pekny, M.; Nilsson, M. *GLIA* **2005**, *50*, 427.
12. Niranjan, R.; Nagarajan, R.; Hanif, K.; Nath, C.; Shukla, R. *Neurol. Sci.* **2014**, *35*, 409.
13. Wadhwa, R.; Kaul, S. C.; Mitsui, Y.; Sugimoto, Y. *Exp. Cell Res.* **1993**, *207*, 442.
14. Wadhwa, R.; Taira, K.; Kaul, S. C. *Cell Stress Chaperones* **2002**, *7*, 309.
15. Shih, Y. Y.; Lee, H.; Nakagawara, A.; Juan, H. F.; Jeng, Y. M. *PLoS One* **2011**, *6*, e26236.
16. Mishra, R.; Kaur, G. *PLoS One* **2013**, *8*, e78764.
17. Mishra, R.; Kaur, G. *Mol. Neurobiol.* **2014**. http://dx.doi.org/10.1007/s12035-014-8892-5.
18. Kataria, H.; Wadhwa, R.; Kaul, S. C.; Kaur, G. *PLoS One* **2013**, *8*, e55316.
19. Mayer, M. P.; Bukau, B. *Cell. Mol. Life Sci.* **2005**, *62*, 670.
20. Yanga, J.; Ozaa, J.; Bridgesa, K.; Chenc, K. Y.; Liua, A. Y. C. *Brain Res.* **2008**, *1203*, 39.