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

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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

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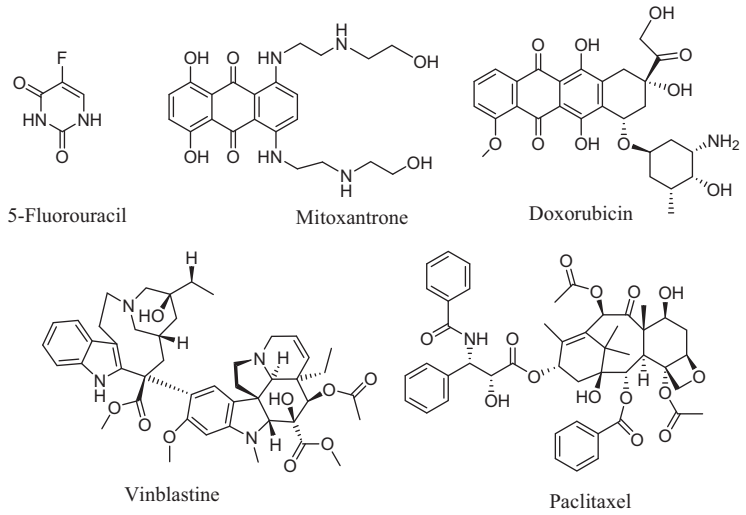
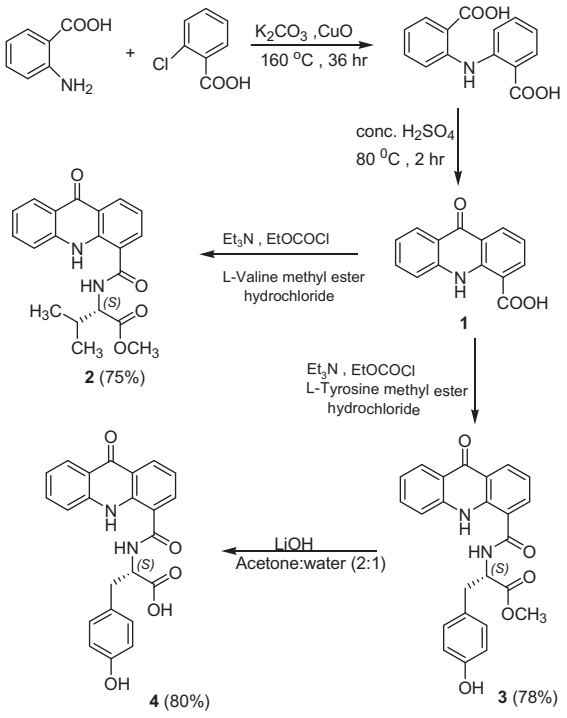


Chart 1.



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_α 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