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Synthesis and biological evaluation of novel 2-imino-4-thiazolidinone derivatives as potent anti-cancer agents

K. Appalanaidu [a,†,‡], Rajesh Kotcherlakota [b,†,‡], T. L. Dadmal [a,c], Vishnu Sravan Bollu [b,†], Ravindra M. Kumbhare [a,*,†], Chitta Ranjan Patra [b,*,†]

[a] Fluoroorganics Division, Indian Institute of Chemical Technology (CSIR-IICT), Tarnaka, Hyderabad 500007, India
[b] Biomaterials Group, Indian Institute of Chemical Technology (CSIR-IICT), Tarnaka, Hyderabad 500007, India
[c] Government of Maharashtra's Ismail Yusuf College, Jogeshwari (E), Mumbai 400060, India

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ABSTRACT

A new series of 2-imino-4-thiazolidinone derivatives (**7a**–**7t**) has been synthesised and screened for their cytotoxicity against three cancer cell lines (B16F10, A549, PANC-1) and normal cell line (CHO). Among the compounds tested, compounds **7k**, **7m**, **7n** showed potent cytotoxicity against B16F10 cell line with IC_{50} between 3.4 and 7 μM. Interestingly these three compounds are non toxic to non cancerous CHO cells and induced apoptosis in B16F10 cells observed by DNA damage analysis through PI/Hoechst double staining method. Compounds **7k** and **7n** induced G0/G1 cell cycle arrest while compound **7m** induced G2/M cell cycle arrest in B16F10 cells which confirms that these compounds have role in cancer cell cycle regulation. Additionally, compound **7m** showed generation of intracellular reactive oxygen species (ROS) in B16F10 cells that may contribute in the cell cycle arrest whereas compounds **7k** and **7n** show anti-cancer activity through independent of ROS formation. Induction of apoptosis, cell cycle arrest in B16F10 cells are found to be the anti-cancer mechanism of these three compounds. The results all together demonstrate the potent cytotoxic nature of these compounds in cancer cells could be considered as new class of chemotherapeutic agents in near future.

Cancer is one of the leading causes of death in the world.[1] Genotoxicity and cytotoxicity of anti-cancer drugs to the normal tissues is major problems in cancer therapy and produces the risk of inducing secondary malignancy as well as leads to many side effects. Among the various therapies such as surgery, chemotherapy, radiation and monoclonal antibody therapy,[2] chemotherapy is a most common treatment for all type of cancers. In clinics, new combination of chemotherapeutic drugs has been used for the better treatment of different cancers. However, cancer research always requires the discovery of new drugs that can kill the cancer cells or stop the growth of cancer cells more specifically and overcome the limitation of toxicity to normal tissue which leads to many side effects. In order to develop drugs with such capabilities, scientists have focused upon many different aspects of cancer biology during their research. In this framework, five-member ring, 4-thiazolidinone derivatives have attracted much attention over the years since it is a significant and versatile scaffold that has occupied a prominent position in medicinal chemistry.[3] The detailed literature survey of 4-Thiazolidinone and its derivatives demonstrated a wide broad spectrum pharmacological properties such as anti-microbial,[4a,b] anti-malarial,[5a,b] anti- HIV,[6a,b] anti-inflammatory,[7a–c] anti-oxidant,[8a,b] anti-tuberculostatic[9a,b] and COX-2 inhibitor activities.[10] Various researchers have documented the progress of this scaffold through chemical modifications.[11–17] Among the thiazolidinone derivatives, substituted 4-Thiazolidinone such as MKT-077, 1-ethyl-2-[[3-ethyl-5-(methylbenzothiazolin-2-ylidene)-4-oxothiazolidin-2-ylidene]methyl]pyridinium chloride) **I** (Fig. 1) (formerly known as FJ-776) know for anti-proliferative activity against cancer cell lines through its ability to inhibit members of the heat shock protein 70 (Hsp70) family of molecular chaperones. However, MKT-077 is rapidly metabolized, which limits its use as either a chemical probe or potential therapeutic. ALC 67 molecule **II** (Fig. 1), exhibit potent and selective in vitro antitumor properties in human cancer cell lines (example liver, colon, breast and endometrial cancer) which were also demonstrated to induce apoptosis by activating caspase-9.

* Corresponding authors. Tel.: +91 40 27191776; fax: +91 40 27193185 (R.M.K.); tel.: +91 40 27191480 (O); fax: +91 40 27160387 (C.R.P.).
E-mail addresses: kumbhare@iict.res.in (R.M. Kumbhare), crpatra@iict.res.in (C.R. Patra).
† Academy of Scientific and Innovative Research (AcSIR), Taramani, Chennai 600 113, India.
‡ Contributed equally.

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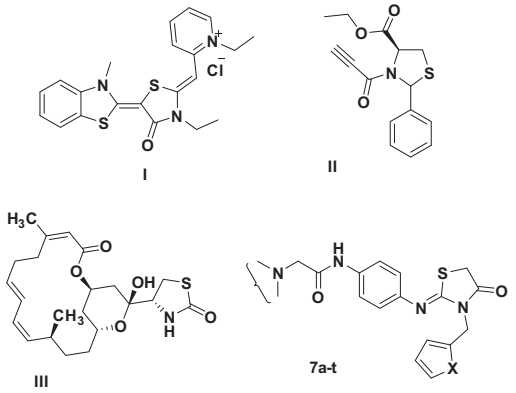


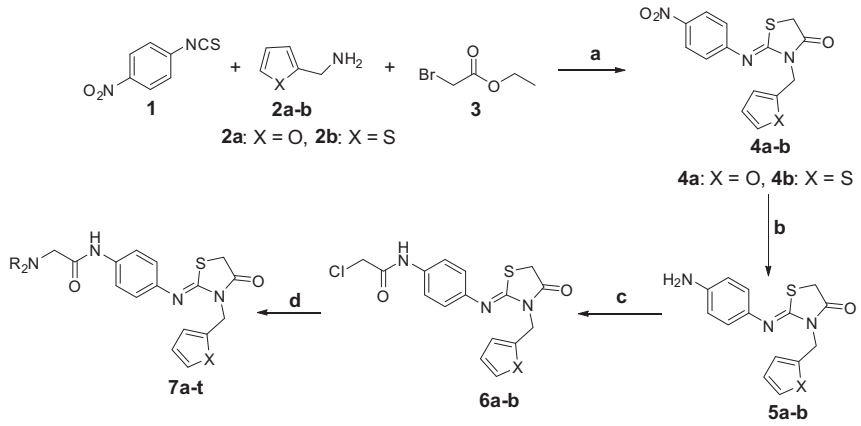
Figure 1. Chemical structures of biologically active compounds.

Latrunculin A **III** (Fig. 1) has a strong anti-cancer effect in a peritoneal dissemination model of human gastric cancer in mice. Furthermore, *N*-Alkyl amides (NAAs) are a promising group of bioactive compounds, which are exhibiting diverse applications in medicinal chemistry.[18–23]

Therefore, it was anticipated that chemical entities with amide functional 4-thiazolidinone scaffolds would result in improved biological activities. In view of these verdicts, we have attempted to incorporate amide functionality into 4-thiazolidinone, a restrained structure like titled compounds to evaluate its antitumor activity. Hence in continuation of our efforts to design and synthesizing a novel heterocyclic compounds and evaluating their anti-cancer activity,[24–26] here we are disclose the synthesis of amide functionalized 4-thiazolidinone scaffolds and their anti-tumor activity. A random screen of these analogues on a panel of three selected cancer cell lines displayed higher anti-cancer activity on B16F10 cell line, than others. These compounds caused DNA damage and ROS production that resulted in apoptosis by cell cycle arrest.

All the present 4-thiazolidinone derivatives (**7a**–**t**) were synthesized as shown in Scheme 1. Initially, we designed the compounds **4a** and **4b** as per our previously reported method[27] by the reaction of 4-nitrophenyl isothiocyanate **1**, ethyl bromoacetate **3** and appropriate amines **2a**–**b** in DMF as solvent. Reduction of **4a**–**b** with stannous dichloride dihydrate in ethanol,[28] provides (*Z*)-2-((4-aminophenyl)imino)-3-(furan-2-ylmethyl)thiazolidin-4-one **5a** and (*Z*)-2-((4-aminophenyl)imino)-3-(thiophen-2-ylmethyl) thiazolidin-4one **5b**. Further these amines were treated with chloroacetyl chloride to obtain **6a**–**b**. Finally, reaction of appropriate precursor's **6a**–**b** with corresponding primary and secondary amine in ethanol provides targeted compounds **7a**–**t** (spectral data incorporated in Supporting information) in good yield.

Initially, in vitro anti-cancer activity of the synthesized 4-thiazolidinone derivatives (**7a**–**7t**) have investigated against B16F10 (mouse melanoma), A549 (human lung cancer) and PANC-1 (human pancreatic cancer) cell lines by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay (MTT assay)[29a–c] with doxorubicin as positive control. The data from this assay enables to predict the cell viability profiles. The inhibitory activity of these compounds in B16F10 cells was summarized in Figure 2. Treatment of these compounds (**7a**–**7t**) in cultured cancer cells in dose dependent manner for 48 h inhibited cell proliferation and particularly the effect is more pronounced in B16F10 cells (Fig. 2). Interestingly, some of the compounds exhibited the significant cytotoxic activity against the B16F10 cells compare to A549 cells and PANC-1. The cytotoxicity values indicated that the compounds were more potent on melanoma (B16F10) and lung cancer (A549) than pancreatic (PANC-1) cancer cells. Among the series, compounds **7k**, **7l**, **7m** and **7n** displayed IC_{50} values of 7, 11.6, 3.4, and 5.4 μM, respectively against the B16F10 cancer cells and compounds **7k**, **7l**, **7p**, **7q** and **7r** showed IC_{50} value of 12.5, 12.6, 12.6, 10 and 10.8 μM respectively, against A549 lung cancer cells. Even though the other compounds also showing the cytotoxicity in B16F10 and A549 cells, we have considered the only compounds with less IC_{50} values and represented in Table.1. The cytotoxicity



7a. X = O, R_2 = dimethylamino
7b. X = O, R_2 = diethylamino
7c. X = O, R_2 = pyrrolidino
7d. X = O, R_2 = piperidino
7e. X = O, R_2 = morpholino
7f. X = O, R_2 = thiomorpholino
7g. X = O, R_2 = 2-(piperazin-1- yl)benzonitrilo
7h. X = O, R_2 = 1-(2-methoxyphenyl)piperazino
7i. X = S, R_2 = 1-methylpiperazino
7j. X = S, R_2 = dimethylamino
7k. X = S, R_2 = diethylamino
7l. X = S, R_2 = ethanamino
7m. X = S, R_2 = propan-1-amino
7n. X = S, R_2 = pyrrolidino
7o. X = S, R_2 = piperidino
7p. X = S, R_2 = morpholino
7q. X = S, R_2 = thiomorpholino
7r. X = S, R_2 = phenyl(piperazin-1-yl)methanono
7s. X = S, R_2 = 1-(pyridin-2-yl)piperazino
7t. X = S, R_2 = 1-(4-fluorophenyl)piperazine

Scheme 1. Reagents and conditions: (a) DMF, rt, 2 h; (b) $SnCl_2{\cdot}2H_2O$, ethanol, reflux, 2 h; (c) chloroacetylchloride, DMF, 0 °C–rt, 2 h; (d) 2° amines, DMF, 70 °C, 3 h.