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Design and synthesis of 4-morpholino-6-(1,2,3,6-tetrahydropyridin-4-yl)-*N*-(3,4,5-trimethoxyphenyl)-1,3,5-triazin-2-amine analogues as tubulin polymerization inhibitors

Suresh Narva [a], Surendar Chitti [a], Suresh Amaroju [a], Debanjan Bhattacharjee [b], Bala Bhaskara Rao [b], Nishant Jain [b], Mallika Alvala [c], Kondapalli Venkata Gowri Chandra Sekhar [a,*]

[a] Department of Chemistry, Birla Institute of Technology and Science, Pilani, Hyderabad Campus, Jawahar Nagar, Hyderabad 500 078, Telangana, India
[b] Centre for Chemical Biology, CSIR-Indian Institute of Chemical Technology, Tarnaka, Hyderabad 500007, Telangana, India
[c] National Institute of Pharmaceutical Education and Research-Hyderabad, Hyderabad 500037, Telangana, India

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ABSTRACT

A series of thirty-seven 1,3,5-triazine analogues have been synthesized, characterized and evaluated for their antiproliferative activity against a panel of four different human cancer cell lines such as HeLa, HepG2, A549 and MCF-7. Most of the 1,3,5-triazine analogues exhibited promising antiproliferative activity against tested cancer cell lines. Among all the synthesized compounds, **8j** showed potent activity against the cancer cell lines such as HeLa, HepG2, A549 and MCF-7 with IC_{50} 12.3 ± 0.8, 9.6 ± 0.4, 10.5 ± 1.0 and 11.7 ± 0.5 μM respectively. **8j** was taken up for elaborate biological studies and the cells in the cell cycle were arrested in G2/M phase. In addition, **8j** was examined for its effect on the microtubule system with a tubulin polymerization assay, immunofluorescence. **8j** showed remarkable inhibition of tubulin polymerization. Molecular docking studies were also carried out to understand the binding pattern. The studies suggested that **8j** has a good binding affinity of −7.949 towards nocodazole binding site of tubulin while nocodazole has −7.462.

Antimitotic agents are very important class of drugs used in the treatment of cancer. These antimitotic agents generally target tubulin and inhibit the formation of the mitotic spindle.[1,2] Microtubules are formed by the polymerization of the α,β-tubulin heterodimers. The microtubules are the key components of eukaryotic cells and are very crucial in several cellular functions like cell division, giving proper shape to the cell, transportation and in cell movement.[3,4] Inhibiting the polymerization of tubulin or interference with microtubule, interrupts the cell motility and mitosis of cell functions[5–7] Hence microtubule is a vital target for developing anticancer drugs.[8] The innovation and development of analogues that affect tubulin polymerization is of huge importance.[9–11] Colchicine and combretastatin A-4 bind to tubulin and inhibit its polymerization into microtubules.[12–14] The synthesis of tubulin polymerization inhibitors has enthused significant attention in the anticancer drug discovery.

The 1,3,5-triazine scaffold occupies an outstanding position in organic chemistry and medicinal chemistry. It has been broadly used in organic reactions,[15–20] due to its specific structure and electronic properties. 1,3,5-Triazines have a wide array of biological activities like antiprotozoal,[21] anticancer,[22–25] antimalarial,[26] antiviral,[27] and antimicrobial.[28,29] Nitrogen containing triazine inhibits the action of an inducible membrane protein which is useful to increase the efflux of the cytotoxic agents and acts at dissimilar targets to varied pharmacological properties.[30] In 1,3,5-triazine 2-, 4- and 6 positions are occupied by different reactivity of chlorine atoms; each chlorine is controlled by different temperatures. This phenomenon has amplified interest in this moiety and allows researchers to introduce various substitutions by replacing the chlorine atoms at various temperatures for the preparation of mono-, di- and tri-substituted 1,3,5-triazines.[31,32] Some herbicides like atrazine, cyanazine, simazine, trietazine and resin modifiers like melamine and benzoguanamine have 1,3,5-triazine as the basic structure[33,34] and drugs like Altretamine (antineoplastic agent), Triethylenemelamine (chemotherapy drug) also contain 1,3,5-triazine nucleus.[37]

On the other side 3,4,5-trimethoxy substitution enhances the antiproliferative activity. Ursolic acid (UA) inhibits tumor initiation and promotion and activates routes leading to apoptosis and also suppresses the cell proliferation. Several modifications have been

* Corresponding author.
E-mail addresses: kvgcs.bits@gmail.com, kvgc@hyderabad.bits-pilani.ac.in (K.V.G.C. Sekhar).

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introduced in UA and screened for potential antitumor agents. Novel UA derivatives modified at the C-3 and the C-28 positions were reported as potential antitumor agents. Among all UA derivatives trimethoxy substituted analogue (**A**) exhibited excellent *in vitro* cytotoxicity. It induced apoptosis through G1 cell cycle arrest and also through both intrinsic and extrinsic apoptotic pathways.[35–37]

Resveratrol, commonly found in grape skins, is a natural polyphenolic phytoalexin.[38] Its analogues displayed various cancer chemo-preventive properties.[39] A series of trimethoxy derivatives of resveratrol were reported as anticancer agents against various human cancer cell lines.[40,41] Among these, (*E*)-3,4,5,4-tetramethoxystilbene (**B**) exhibited potent anticancer activity and was active by 30–100 folds in comparison to resveratrol.[42] Trimethoxy substituted hetero aromatic analogue (**C**) of the resveratrol showed potent growth inhibition in 85% of the cancer cell lines.[43]

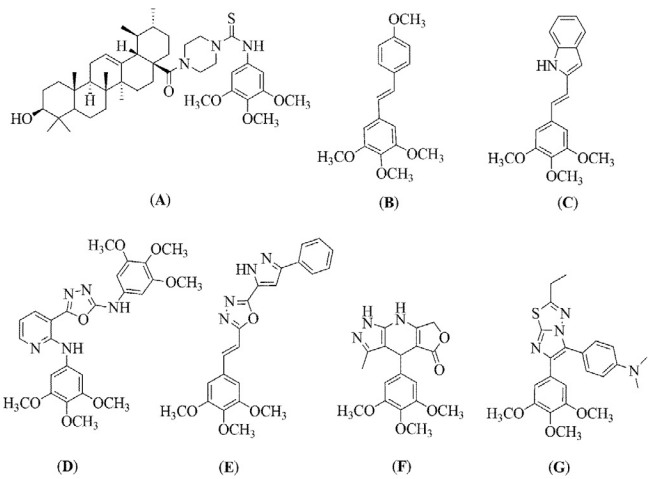


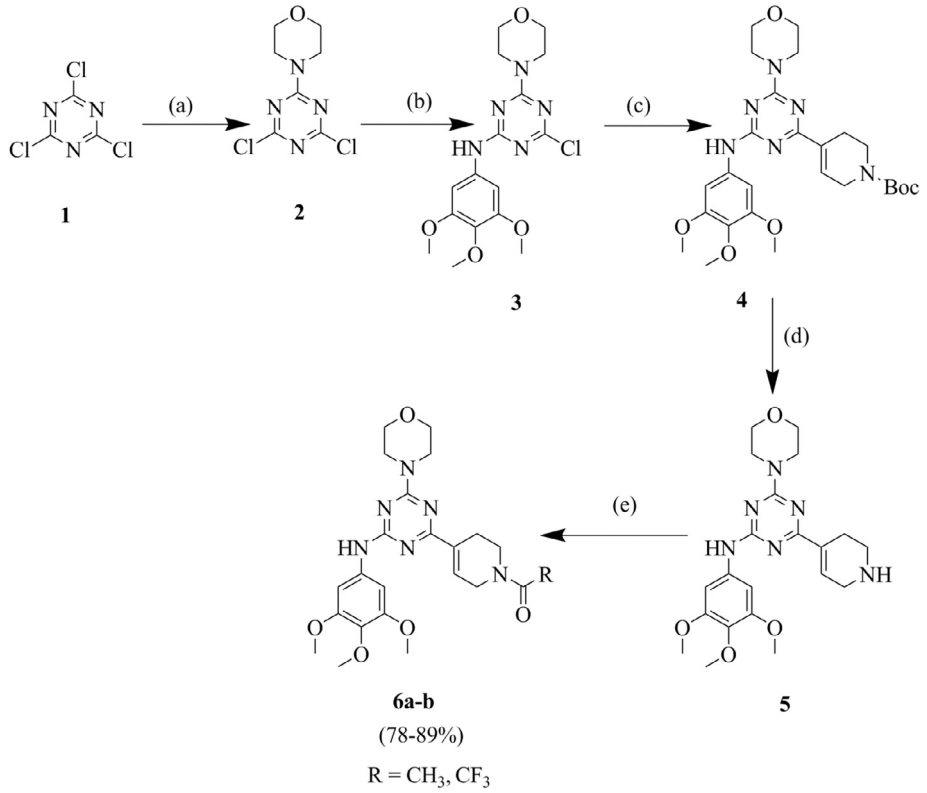
Fig. 1. Trimethoxy containing anticancer agents.

3,4,5-Trimethoxyphenyl ring is present in most of the synthesized cytotoxic agents, tubulin inhibitors and naturally occurring biologically active compounds, including colchicine, combretastatin A-4, ursolic acid derivative (**A**), resveratrol derivatives (**B**, **C**).

Incorporation of an oxadiazole ring to 2-anilinonicotinyl linked sulfonyl hydrazide scaffold (**D**) showed potential antitumor activity that considerably inhibited the tubulin polymerization.[44] Pyrazole-oxadiazole conjugate containing trimethoxy derivative (**E**) exhibited potent cytotoxicity and inhibited the tubulin polymerization.[44] Trimethoxy containing dihydropyridopyrazole derivative (**F**) inhibited tubulin polymerization and disrupted mitotic spindles.[44] 2-Ethyl-6-(3,4,5-trimethoxyphenyl)-5-aryl–imidazothiadiazole analogue (**G**) showed significant cytotoxicity activity, arrested cell cycle at G2/M phase and disrupted the microtubule.[44] Trimethoxy containing anticancer agents are depicted in Fig. 1.

As part of our research program aimed to develop new tubulin polymerization inhibitors, a series of novel 1,3,5-triazine derivatives were synthesized by substituting chlorine atoms of cyanuric chloride (2,4,6-trichloro-1,3,5-triazine) with nucleophilic groups to make a huge diversity of substitutions, according to Scheme 1.

The synthesized compounds were evaluated for their antiproliferative activity. The synthesis of new 4-morpholino-6-(1,2,3,6-tetrahydropyridin-4-yl)-*N*-(3,4,5-trimethoxyphenyl)-1,3,5-triazin-2-amine analogues is illustrated in Scheme 1. The synthesis of the substituted 1,3,5-triazines was carried out based on previously reported procedures.[45–47]. 2,4,6-Trichloro-1,3,5-triazine (Cyanuric chloride) **1** was reacted with morpholine at 0 °C to give the 4-(4,6-dichloro-1,3,5-triazin-2-yl)morpholine **2**, which was reacted



Scheme 1. Synthetic protocol to achieve the compounds **6a–b.** Reagents and Conditions: (a) morpholine, Et_3N, Acetone, −20 °C, 20 min (b) 3,4,5-trimethoxy aniline, DIPEA, 1,4-dioxane, RT, 6 h (c) *N*-Boc-1,2,5,6-tetrahydropyridine-4-boronic acid pinacol ester, K_2CO_3, $Pd(dppf)Cl_2$, 1,4-dioxane:H_2O, reflux, 6 h (d) CH_2Cl_2, trifluoroacetic acid, 0 °C-RT, 1 h (e) acetic anhydrides, Et_3N, CH_2Cl_2, 0 °C-RT, 2 h.