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A novel series of potent cytotoxic agents targeting G2/M phase of the cell cycle and demonstrating cell killing by apoptosis in human breast cancer cells

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Abstract—Breast cancer, a leading cause of mortality in women, warrants the development and biological evaluation of new anti-cancer agents. A novel series of thiopyridine triazine derivatives was synthesized and investigated in the human breast cancer cell line, MDA-MB-468. SM40, the most potent derivative, induced a G2/M arrest and apoptosis with a possible involvement of p53. The cytotoxicity of SM40 was also examined against the NCI 60 cell line panel and its potency was rationalized using molecular modeling. Results suggest that SM40 is a promising cytotoxic agent.

Breast cancer carries a high mortality rate, globally, regardless of therapeutic advances. Among the treatment choices available, chemotherapy is one of them despite its many disadvantages including toxic side effects and the development of resistance. Ideal drugs would be small molecules, soluble and relatively stable in aqueous media, more potent, less toxic, tissue specific and have increased bioavailability. Thus successful treatment of cancer demands the synthesis and characterization of new drugs with novel mechanism of action.

We have proposed our rationale in designing simple molecules to improve specificity and potency.[1–4] Here, we report the synthesis of new thiopyridine derivatives of 1,3,5-triazine (Scheme 1) and their biological response in an estrogen receptor α (ERα) negative human breast cancer cell line, MDA-MB-468. We have also examined the cytotoxic potency of these derivatives in 60 human cancer cell lines (NCI). The NCI has selected compound **C** (Chart 1), SM40, for further studies.

Compounds were synthesized in a straightforward one or two-step high yielding procedure as outlined in Scheme 1. All compounds were characterized by NMR, mass, and elemental analysis, respectively. SM40 (compound **C**, Chart 1), 2,4,6-tris(pyridin-2-ylthio)-1,3,5-triazine, proved to be the most potent in this group of compounds (Chart 1).

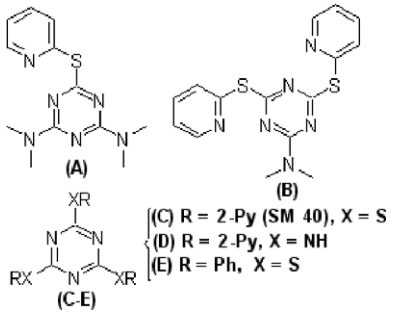

Chart 1.

Keywords: Cytotoxic agent; MDA-MB-468; Cell cycle; TUNEL assay; Electron micrographs; Apoptosis; Immunohistochemistry; Subcellular localization; p53273.His; Molecular modeling; Triazine; Thiopyridine triazine.
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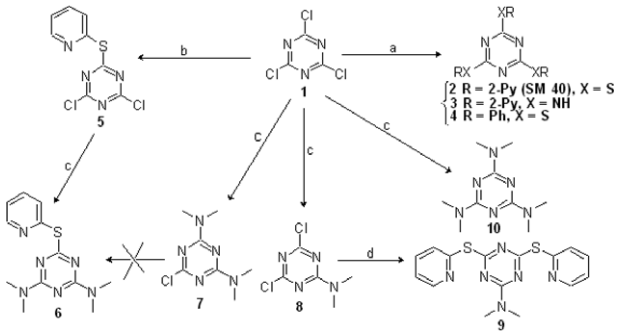

Scheme 1. Synthetic strategy for compound **2** (SM40) and other derivatives. Reagents and conditions: For **2**, (a) **1** in dry acetone (25 °C) and 2-thiopyridine [9 equivalents (equiv) in acetone], stirred for 2–3 h, 70%. For **3**, (a) **1** and excess 2-aminopyridine in methanol, stirred for 6–7 h, 50%. For **4**, (a) **1** and excess benzenethiol in refluxing benzene (5–6 h), 60%. For **5**, (b) **1** in dry acetone, cooled to −15 °C and 2-thiopyridine in dry acetone added drop-wise, separated as solid product, hydrochloride salt. For **6**, (c) **5** and dimethylamine (6 equiv) in methanol/water, stirred 7–8 h at 25 °C, 70%. For **8**, (c) **1** in dry acetone, cooled to −15 °C and dimethylamine (1 equiv) in dry acetone added dropwise; For **9**, (c) **8** (1 equiv) and excess 2-thiopyridine in acetone stirred for 2–3 h at 25 °C, 60%. For SM40: ^{1}H NMR (400 MHz, CDCl3) δ 8.50 (m, 3H, 3× 6-pyridyl), 7.50 (m, 6H, 3× 3,4-pyridyl), 7.19 (m, 3H, 3 × 5-pyridyl); *m/z* 408; Anal. Calcd for $C_{18}H_{12}N_6S_3$: C 52.92%, H 2.96%, N 20.57%; found C 52.69%, H 3.00% and N 20.49%.

It is well documented that compounds containing the 1,3,5-triazine moiety have many interesting properties and this moiety has been used to develop molecules in a number of research fields[1,2,5–14] including cancer drug design;[1,2,5,7–10] for example, hexamethylmelamine[5] **10** (Scheme 1) is a potent cytotoxic agent against a number of cancers including breast cancer but its use is limited due to its undesirable side effects such as vomiting and nausea after administration.

Additionally, as a selective inhibitor, 1,3,5-triazine based molecules have been found to act in many different targets which include: HIV-1 reverse transcriptase;[9] estrogen receptor beta;[10] glutathione *S*-transferase;[11] *M. tuberculosis* dihydrofolate reductase;[12] photosynthetic reaction center,[13] and urate oxidase.[14]

As exemplified in the above targets, proper modification of 1,3,5-triazine ring system could lead to the development of novel target-specific drugs. Careful evaluation using molecular modeling and docking tools revealed that none of these targets are suitable for SM40. With the exception of the 1,3,5-triazine ring, nothing is structurally common to any of the other inhibitors that are bound to the above targets and SM40 is no exception. Therefore, the target for SM40 is unknown and remains to be identified. To the best of our knowledge, triazine derivatives are numerous but thiopyridine derivatives of triazine possessing novel biological properties are rare.[1,2]

The preparation of some of the derivatives has been described elsewhere.[1] S-2-pyridyl, NH-2-pyridyl, and S-phenyl derivatives (2, 3, and 4; Scheme 1) were prepared to establish the requirement of nitrogen atom in second position of the thioheterocyclic ring. In a relatively small number of derivatives, it has been observed that both the nitrogen atom in a specific position of the heterocyclic ring (second position with respect to sulfur atom) and the sulfur atoms are essential for activity. For this series of compounds, the concentration required for 50% growth inhibition (GI_{50}) against 60 cell lines ranged from 0.146 to 100 μM as evaluated by the NCI. Preliminary results of cytotoxic potency show an interesting structure–activity relationship in these derivatives given by the mean GI_{50} of 60 human cancer cell lines (NCI, USA); **A** (GI_{50} = 96.99 μM, **B** (GI_{50} = 99.19 μM), **C** (GI_{50} = 3.17 μM), **D** (GI_{50} > 100 μM), and **E**

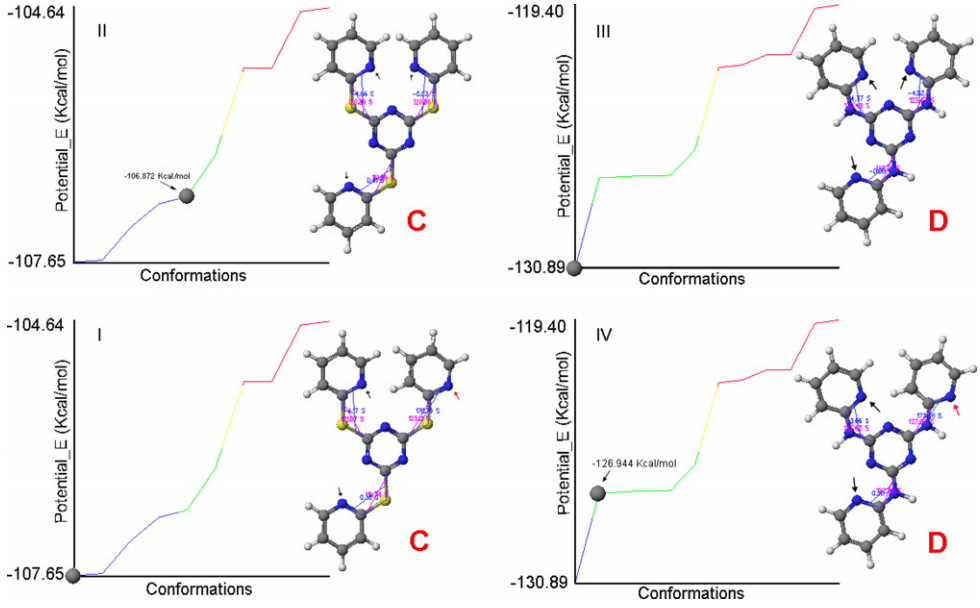

Figure 1. Conformational analysis for **C** and **D**; **I** is the lowest energy (−107.65 kcal/mol) structure for **C** that has similar conformation, with respect to heterocyclic ring nitrogen atoms (as shown using arrows), with **D** shown on the right, **IV**, but **IV** (−126.94 kcal/mol) is not the lowest energy structure for **D**; **III** is the lowest energy (−130.89 kcal/mol) structure for **D** that has similar conformation with **C** as shown on the left, **II**. Conversion of **III** to **IV** requires 3.95 kcal/mol of energy.