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A Novel Microtubule Destabilizing Entity from Orthogonal Synthesis of Triazine Library and Zebrafish Embryo Screening

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To overcome the nonspecific toxicities of current chemotherapeutic and radiation therapies, influencing microtubule functionality has become a widespread strategy in the design of anticancer drugs.[1] The recently characterized myoseverin, a tubulin binder with a novel purine structure, has demonstrated promising ability in surmounting the major problems of currently known tubulin binding drugs.[2] The moderate activity of myoseverin (low μM IC_{50}) remains to be improved through a thorough structure−activity relationships (SAR) study.

To accelerate the development of an optimized myoseverin-like anti-tubulin drug, we designed a novel triazine library utilizing computer aided modeling and known SAR data from myoseverin derivatives (Figure 1). The triazine scaffold has three-fold symmetry, and therefore positional modification is much more flexible than in purine.

Triazine has elicited considerable interest as an ideal combinatorial library scaffold due to its ease of manipulation and the low price of the starting material, resulting in the publication of several triazine libraries in the literature.[3] All of the reported library synthesis procedures utilize the reactivity differences of the three reaction sites. This stepwise amination approach, however, was difficult to generalize for a variety of nucleophiles with various reactivities; thus, each reaction step may accumulate byproducts yielding impure library compounds at the last stage. Herein, we report a new straightforward orthogonal synthetic pathway, which can be used in the general preparation of a trisubstituted triazine library (Scheme 1).

In this new route, three types of building blocks were prepared separately and assembled by orthogonal reactions. The building blocks are (i) PAL-resin bound primary amine, (ii) amine, alcohol, or thiol attached (at 2 position) 4,6-dichloro-[1,3,5]triazine, and (iii) a series of primary and secondary amines. For Building block I, primary amines including a variety of benzylamine were attached to PAL aldehyde resins using the reductive amination reaction. Building block II especially accommodated alkyl/aryl alcohols, bulky amines, and anilines with low nucleophilic reactivity, utilizing the high reactivity of the first substitution reaction on triazine trichloride. Building block III is composed of many different primary and secondary amines, but not anilines due to their low reactivity.

Using this straightforward synthetic procedure with readily available building blocks, we synthesized more than 100 triazine

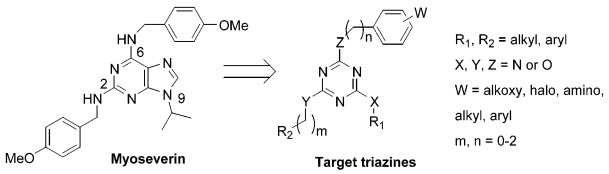
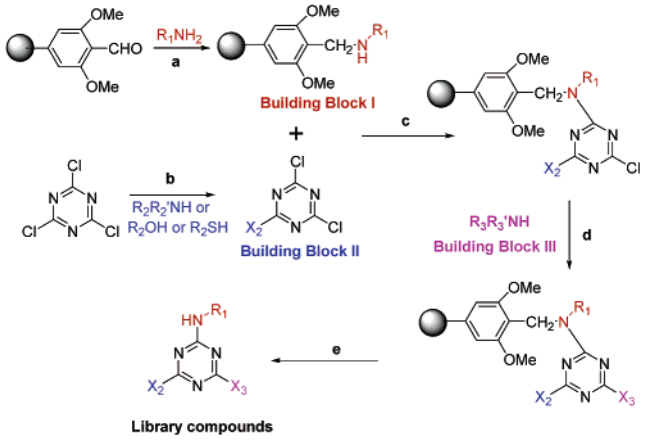


Figure 1. Structure of myoseverin and triazine library structures.

Scheme 1[a]



[a] (a) R_1NH_2 (5 equiv), 2% acetic acid in THF, room temperature, 1 h, followed by $NaB(OAc)_3H$ (7 equiv), room temperature, 12 h. (b) $R_2R_2'NH$, R_2OH, or R_2SH (1 equiv) in THF, 0 °C, 1 h. (c) Building block II (4 equiv), DIEA (4 equiv) in THF, 60 °C, 3 h. (d) $R_3R_3'NH$ (4 equiv), DIEA (4 equiv) in NMP:*n*-BuOH = 1:1, 120 °C, 3 h. (e) 5% TFA in DCM, 10 min.

compounds that share a structural motif similar to that of myoseverin. The purity and identity of all of the products were monitored by LC-MS, and more than 90% of the compounds demonstrated >98% purity.

The synthesized triazine library compounds were first screened using zebrafish embryos.[4] A series of compounds induced very similar morphological changes as the known microtubule disrupters myoseverin and nocodazole at 10 μM concentration. 1K cell stage embryos (3 h post fertilization: 3hpf), over the course of 5 h of compound treatment, were characterized by delays in epiboly, as well as larger blastoderm cells. Arrows mark the extent of migration of the blastoderm over the yolk (Figure 2, upper row). Moreover, treatment of somite stage embryos induced a characteristic developmental arrest (Figure 2, lower row).

To confirm that the selected compounds acted on tubulin directly, the compounds were subjected to an in vitro polymerization assay using purified bovine brain tubulin.[5] The representative active

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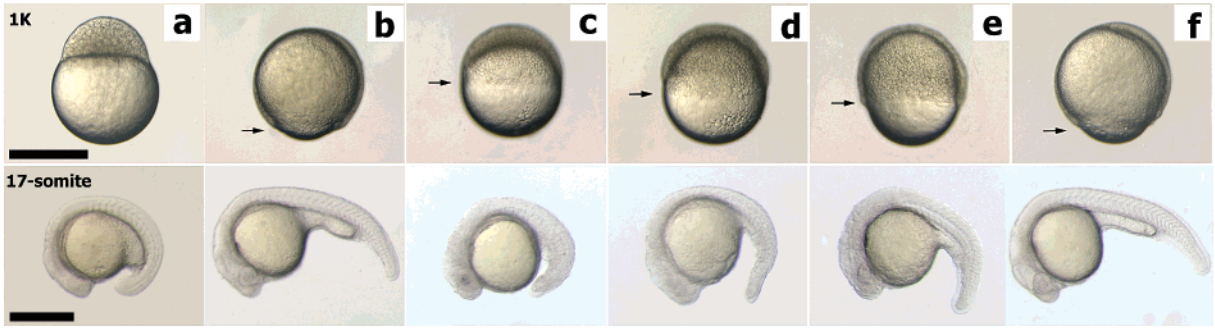

Figure 2. Morphological changes of zebrafish embryos upon treatment with different compounds. (a) upper: 1K cell stage embryo (3 hpf); lower: 17-somite stage embryo (17 hpf). (b)−(f) After 5 h treatment of the compounds: (b) 1% DMSO, (c) nocodazole (10 μM), (d) myoseverin (10 μM), (e) **1** (active triazine, 10 μM), (f) **S79** (inactive triazine, 10 μM) in 1% DMSO/Hanks-derived buffer (scale bar, 100 μm).

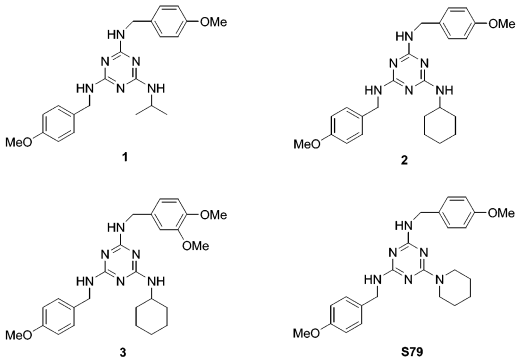

Figure 3. Structures of the representative active compounds (**1**−**3**) and inactive compound (**S79**).

Table 1. In Vitro Microtubule Disassembly and Growth Inhibition of U937 Cells by New Triazine Compounds and Known Inhibitors

	MIC of tubulin polymerization (μM)[a]	GI_{50} in U937 (μM)
nocodazole	10	0.05
myoseverin	20	10
1	10	9
2	20	5
3	5	1

[a] MIC: minimum inhibitory concentration.

compounds (shown in Figure 3) were also tested in U937 human leukemia cells to measure their cell growth inhibition (Table 1).[6] While compound **1** and **2** showed activities comparable to that of myoseverin, the best compound, **3**, demonstrated at least a 4−10-fold improvement in tubulin assays and U937 cell growth inhibition.

In conclusion, we have developed a straightforward solid phase synthetic pathway for a highly pure trisubstituted triazine library, and through zebrafish embryo screening we have identified members of this library that have novel anti-tubulin activities in vitro and in vivo. This high-quality triazine library will ultimately provide a rich source of biologically active synthetic molecules, just as its relative purine libraries have. Diversity extension of the triazine library and its application to the development of better derivatives and other biological screening are currently being studied.

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Supporting Information Available: Full experimental procedures and characterization data (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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