ACS Combinatorial Science

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Combinatorial Solid-Phase Synthesis of 4,6-Diaryl and 4-Aryl, 6-Alkyl-1,3,5-triazines and Their Application to Efficient Biofuel Production

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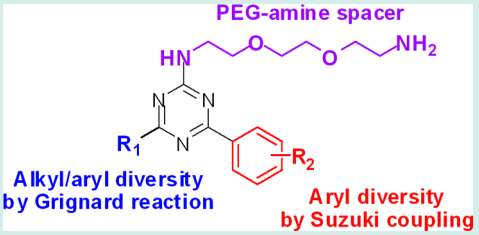
Supporting Information

ABSTRACT: Herein we report the solid-phase synthesis of a combinatorial aryl, alkyl-triazine library and its application to biofuel production. The combination of Grignard reactions and solid supported Suzuki coupling reactions afforded unique 120 triazine compounds with high purities and minimum purification steps. Through an unbiased phenotypic screening for improved biofuel generation in oleaginous yeast, we found one diaryl triazine derivative (**E4**) which increased the biolipid production up to 86%.



KEYWORDS: *triazines, Grignard reaction, Suzuki coupling, biolipids, high-throughput screening, chemical genetics*

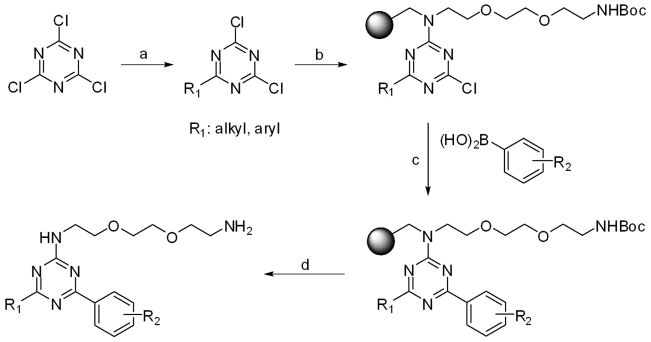
The ease of manipulation and wide range of biological properties of the triazine scaffold have attracted the interest of combinatorial and medicinal chemists to explore its derivatization.[1−5] As a result, a number of synthetic approaches reported by our group and others have converted the triazine structure into a key scaffold in drug discovery programs.[6−11] 1,3,5-Triazine derivatives can be readily prepared from cyanuric chloride using amine, thiol, and alcohol building blocks that involve the formation of *C*-heteroatom bonds.[12−16] These syntheses have been adapted to solid-phase methodologies to facilitate the construction of large collections.[17−21] In contrast, the derivatization of the triazine scaffold forming C−C bonds has been much lesser explored. For instance, although aryl−aryl motifs are well-known pharmacophores and widely employed in the derivatization of privileged scaffolds,[22,23] the synthesis of diaryl or aryl and alkyl-substituted triazines are limited to a few examples.[24] Our group recently reported efficient synthetic schemes for triazine libraries with single C−C bonds using either Grignard reactions[25] or Suzuki couplings.[26] We envisioned that a triazine library with double C−C bond derivatization may open a new chemical space for the discovery of novel bioactivities.

Lipids are one of the most important topics of biological research because numerous diseases, including diabetes, obesity, and cancer, are caused by their abnormal uptake, synthesis, metabolism, storage, or mobilization.[27,28] In addition, the global energy crisis has made lipid research even more important, since biolipids produced by microorganisms and plants hold a promising potential to provide sustainable fuel for the human society. Chemical genetics approaches have been used to find chemicals that alter the neutral lipid accumulation in mammalian cells and simple animal models such as *Caenorhabditis elegans* and zebrafish. These experiments led to identification of new factors that regulate lipid metabolism[28−31] and opened new ways to treat lipid-related diseases. We envisioned that similar approaches might be useful to discover new chemicals that can increase biofuel production in microorganisms and plants, since neutral lipids can be readily converted to biofuel. We screened our new library of 4,6-diaryl and 4-aryl,6-alkyl triazines in a forward chemical genetics approach to identify compounds that increased neutral lipid accumulation in *Yarrowia lipolytica,* an oleaginous yeast well-known for its versatile lipid-related metabolic pathways.[32]

Herein we report the solid-phase synthesis of 4,6-diaryl and 4-aryl, 6-alkyl 1,3,5-triazines by combining Grignard reactions and Suzuki couplings and their evaluation for efficient biofuel generation, which yielded to the discovery of **E4** as a unique chemical regulator of the biolipid production. We designed the orthogonal synthesis of a two-dimensional library by combining a solution phase Grignard reaction followed by a solid-phase Suzuki coupling (Scheme 1). Cyanuric chloride was initially derivatized by 12 different Grignard reagents under reported conditions[25] including alkyl and aryl groups on the 6-position of the triazine scaffold (e.g., linear and β-branched alkyl groups,

Received: January 20, 2012
Revised: May 14, 2012
Published: June 11, 2012

Scheme 1. Synthesis of 4,6-Diaryl and 4-Aryl, 6-Alkyl-1,3,5-triazine Compounds[a]



[a]Reagents and conditions: (a) R_1-MgX, THF, 0 °C to r.t., 8 h; (b) PAL-PS amine-derivatized resin, THF, DIEA, 60 °C, 3 h; (c) $Pd(PPh_3)_4$, dioxane, R_2-$B(OH)_2$, Cs_2CO_3, 90 °C, 15 h; (d) TFA-DCM (1:9), r.t., 0.5 h.

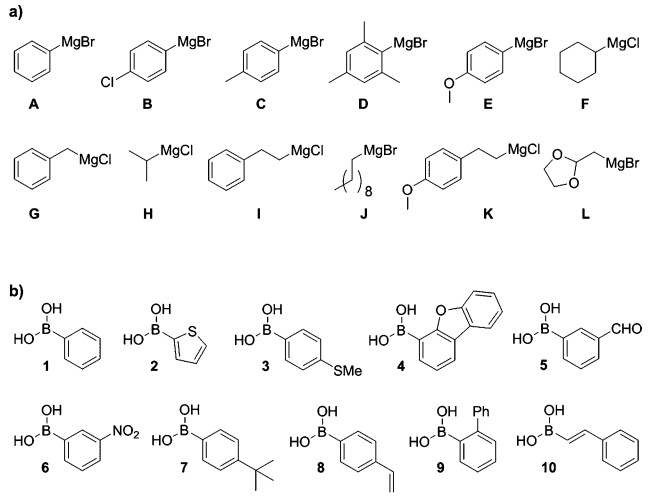


Figure 1. Building blocks for library synthesis: (a) Grignard reagents, (b) boronic acids.

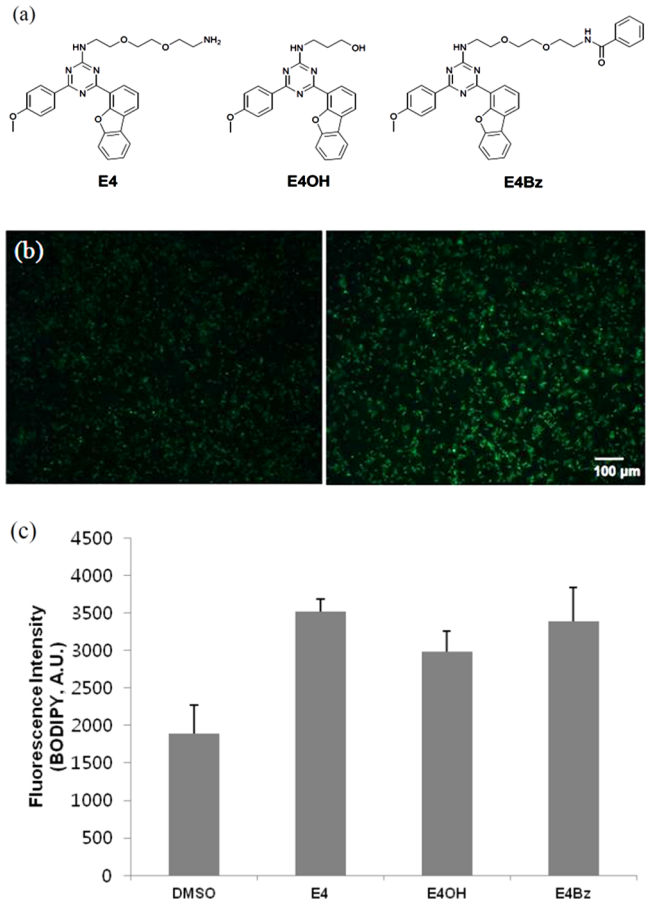


Figure 2. (a) Chemical structures of E4 and the analogues E4OH and E4Bz; (b) Fluorescent microscope images of BODIPY-stained *Y. lipolytica* cells treated with DMSO (*left*) and 10 μM of E4 (*right*). Cells were grown for 12 h at 28 °C in an air-shaking incubator (180 rpm) after being treated with DMSO or E4; (c) BODIPY fluorescence average intensity of cells treated with E4, E4OH, E4Bz and DMSO (10 μM) (n = 3, bars as SD). The same number of cells (OD_{600} = 0.5) was used for all the experiments.

differently substituted aromatic rings, Figure 1a). The resulting 2,4-dichloro-6-alkyl-1,3,5-triazines were loaded onto an amine-derivatized PAL-polystyrene (PAL-PS) resin to accelerate their further combinatorial derivatization.[33] PAL-PS resins are compatible with palladium-catalyzed cross-coupling conditions,[34−36] and have been used for the preparation of tagged-triazine libraries.[6−8]

Polymer-supported triazines were then derivatized using optimized Suzuki coupling conditions[26] with 10 different boronic acids that included both electron-withdrawing and electron-donating groups (Figure 1b). A final acidic cleavage with a trifluoroacetic acid-dichloromethane solution (1:9) rendered a collection of 4,6-diaryl and 4-aryl, 6-alkyl 1,3,5-triazines (120 compounds) in average purities around 90% without running any purification step (Supporting Information, Table S1).

Next we examined the library in a chemical genetics approach for efficient biofuel generation. To identify triazine compounds that promoted lipid accumulation in *Y. lipolyica*, we cultured the cells to midlog phase, treated them with the individual compounds of the library, and analyzed their neutral lipid content by staining the cells with a lipid sensing fluorescent BODIPY dye (D3922, Invitrogen).[37] In this screening we found that only one triazine compound (**E4**, Figure 2a) induced a significant increase of the lipid production, with a fluorescence intensity enhancement up to 86% at 10 μM (Figure 2b). Furthermore, we observed that that none of the single C−C bond triazine derivatives from previously synthesized libraries (588 Grignard[25] and 160 Suzuki derivatives[26]) showed any significant activity, asserting the unique biochemical properties of the newly synthesized triazines. To examine the role that the ethyleneglycol group played in the activity of **E4**, we synthesized and tested two analogues with shorter and aromatic versions of the linker (i.e., respectively **E4OH** and **E4Bz**, Figure 2a). All three compounds showed a similar dose-dependent increase of the lipid accumulation (Supporting Information, Figure S4), which indicated the substituted diaryl triazine core as the main one responsible for the biological activity. Moreover, none of these compounds did show substantial toxicity or inhibition of the cell growth, even at high concentration. Finally, we determined the amount of triacylglycerol (TAG) extracted from the same number of cells treated with **E4**, **E4OH**, and **E4Bz** and compared it to DMSO-treated control cells. This experiment confirmed the capacity of **E4** and its analogues to increase the content of TAG (Supporting Information, Figure S4a). The

dx.doi.org/10.1021/co300007a | *ACS Comb. Sci.* 2012, 14, 395−398

amount of biochemically extracted TAG was quantified by densitometry on iodine-stained TLC plates, and corroborated the results obtained by fluorescence staining. These results proved the application of 4,6-diaryl 1,3,5-triazines as candidate molecules to increase lipid accumulation in *Y. lipolytica*.

In conclusion, we prepared in solid-phase a collection of 4,6-diaryl and 4-aryl, 6-alkyl 1,3,5-triazines using Grignard reactions and Suzuki couplings and performed an unbiased phenotypic screening to discover **E4** as a unique small molecule with efficient biolipid generation activity. Further biochemical studies toward target identification and mechanism elucidation will be reported in due course.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Synthetic procedures for the library synthesis, characterization data for the hit compounds and procedures for the bioassays. This material is available free of charge via the Internet at http://pubs.acs.org.

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Y.S.L. and Y.T.C. conceived and designed the research and wrote the manuscript. J.Y.H.K. performed the biological experiments and wrote the manuscript. J.W.L., W. S.L., H.-H.H., and M.V. performed organic synthesis and wrote the manuscript. J.T.B. carried out organic synthesis. All authors have given approval to the final version of the manuscript.

Funding

The authors are grateful to A*STAR SSCC Grant (SSCC10/024) and the Global Frontier Program (2010-0029723) of the Ministry of Education, Science and Technology of Korea for the financial support.

Notes

The authors declare no competing financial interest.

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