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Divergent synthesis of Thapsigargin analogs

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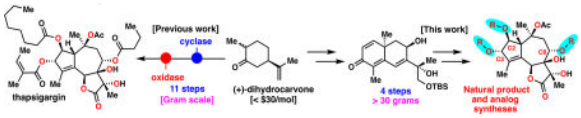
Abstract

Thapsigargin (**3**) is a potent inhibitor of the SERCA-pump protein, with potential for application in a variety of medicinal areas. The efficient and scalable syntheses of thapsigargin (**3**) and nortrilobolide (**2**) have been disclosed previously. To demonstrate the modularity of the previous routes, three natural products (compounds **6**, **13, 15**) and four analogs (compounds **17–20**) have been divergently prepared from a common building block featuring varied acyl chains at the C2, C3, and C8 positions. Biological tests revealed that all of the compounds prepared displayed promising activity profiles.

Graphical Abstract



Keywords

Natural products; Total Synthesis; SERCA inhibition; Divergent synthesis

The strategy of divergent synthesis was first codified by Boger in 1984 with his landmark syntheses of azafluoranthene alkaloids.[1] As defined, divergence *"requires that an identical intermediate (preferably an advanced intermediate) be converted, separately to at least two members of the class of compounds. Divergent total syntheses are distinct from partial total synthesis in which one member is interconverted to a second member of the class of compounds."* Since then numerous new terms have been developed to describe exactly the same idea. The essence of a divergent synthesis strategy requires the design of a key intermediate that can be further diversified to a set of molecules related to the target scaffold. This maneuver is widely used by all medicinal chemists to rapidly interrogate SAR. In the

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arena of natural product synthesis, such an approach can facilitate access to multiple members and analogs within a class.

In 2009 our lab described a divergent approach to terpenes that mimics the two-phase biosynthetic pathway of this large class of natural products.[2] The practical advantage of the two-phase terpene synthesis strategy resides in its capability to deliver analogs with diverse oxygenation patterns for biological evaluation, without the need to alter the parent route. The utility of this strategy was demonstrated multiple times in various total syntheses[3] and also in the elucidation of the ingenol oxidase phase "barcode".[4] Similarly, the previously disclosed two-phase routes to access of nortrilobolide (**2**) and thapsigargin (**3**) from (+)-dihydrocarvone (**1**)[5] (Figure 1) can be repurposed for the synthesis of medicinally relevant analogs.[6] The therapeutic potential of thapsigargin analogs is demonstrated in mipsargargin,[7a] a prodrug of the parent natural product that is currently in PhII clinical trials in several cancer diseases.[7b] The employed route to access the parent guaianolide natural products featured modular installation of individual oxygen atoms along with their attached acyl groups, which in turn effectively generated a suitable template for facile analog synthesis. In the case of thapsigargin (**3**), for instance, the angelate and the butyrate group found at the C2 and C8 position, respectively, can easily be swapped via simple acylation transforms. Additionally, the C2 acyl group was installed via an alpha oxidation reaction.[8] Changing the acid and anhydride components during this oxidation would enable access to a variety of C2 acyl analogs.

The syntheses of compounds **6**, **13**, **15**, and **17–20** were accomplished by a two-phase strategy similar to that previously reported.[5] Since intermediate **4** (prepared from (+)-dihydrocarvone (**1**) in 4 steps) has been scalably prepared in greater than 30-gram batches by an outside vendor, it was used as the starting point for analog synthesis. From this intermediate, nortrilobolide (**2**) was accessed in 6 steps.[5] Heating **2** in methanol in the presence of triethylamine[9] selectively cleaved the butyrate at the C8 position leading to an intermediate secondary alcohol. This compound was subsequently acylated with senecioic anhydride to afford thapsivillosin F (**6**) in 52% yield over 2 steps. Next, the C2 oxidized natural products were targeted with variations at the C2 and C8 positions. In 3 steps,[10] compound **4** was converted to the [5.7] enone **7** by way of a Barton photochemical rearrangement[11]. This intermediate would serve as the divergence point to access two natural products of the thapsigargin family with varying acyl chains at the C2 and C8 positions. The identity of the photo-rearranged product was confirmed via x-ray crystallography of the acylated compound **7a**. Acylation with butyric anhydride in the presence of 4-dimethylamino pyridine (DMAP) led to butyrate **8** in near quantitative yield. Subsequent permanganate mediated alpha oxidation[8] in the presence of hexanoic acid and hexanoic anhydride furnished the corresponding hexanoate **9** in 60% yield. Acidic deprotection furnished diol **10**, which underwent a smooth diastereoselective dihydroxylation[12] to afford tetra-ol **11** in 51% over 2 steps. Parikh-Doering oxidation[13] led to lactone **12**. In two further steps, Thapsigarcin (**13**) could be accessed in 50% yield by way of a stereoselective reduction[14] ($Zn(BH_4)_2$) and an acylation. Thapsivillosin C (**15**), the C8 variant in this family of natural products, was accessed in an analogous manner. Thus,

starting from **7**, acylation with S-(+)-2-methylbutyric anhydride provided the C8 acylated congener **14** that was processed in 6 steps to thapsivillosin C (**15**).

A significant amount of SAR information has been gathered on the role of the C8 acyl group on the bioactivity of thapsigargin (**3**).[15] In contrast, the C3 allylic alcohol has received less attention. It is known in literature as well as from in-house experience that the C3 alcohol is notoriously resistant to various angeloylation conditions.[16] In addition, esterification to generate angelate esters without isomerization to the thermodynamically more stable tiglate ester can be a challenge for production of active pharmaceutical ingredients.[17] As a result, an equipotent analog without the C3-angelate ester moiety, but otherwise similar properties as the natural product, would serve as a stronger starting point for a discovery program. In this vein, 4 additional analogs (**17–20**) were prepared using simple acylation conditions from allylic alcohol **16**. Indeed, cellular characterization of analogs **17–20** for their ability to increase cytosolic calcium concentration via SERCA inhibition[18] revealed three of them (**17–19**) to have potency comparable to thapsigargin (**3**) and thapsigarcin **(13)**. In contrast, a significant, approx. 10-fold loss of potency was observed for analogue **20**.

In summary, the concise divergent approach to thapsigargin and related natural products has been leveraged to access a series of potent non-competitive SERCA inhibitors. The two-phase approach utilized herein strategically enabled various acyl chains to be directly embedded in the parent route without unnecessary redox or protecting group manipulations. Since the most relevant sites of SAR studies reside in the acyl chains of the thapsigargins, an efficient route to easily install these groups is of high value. Finally, compound **18** and **19** are examples of highly potent analogs of thapsigargin without the epimerization-prone C3-angelate moiety, thus rendering them attractive starting points for further manipulations.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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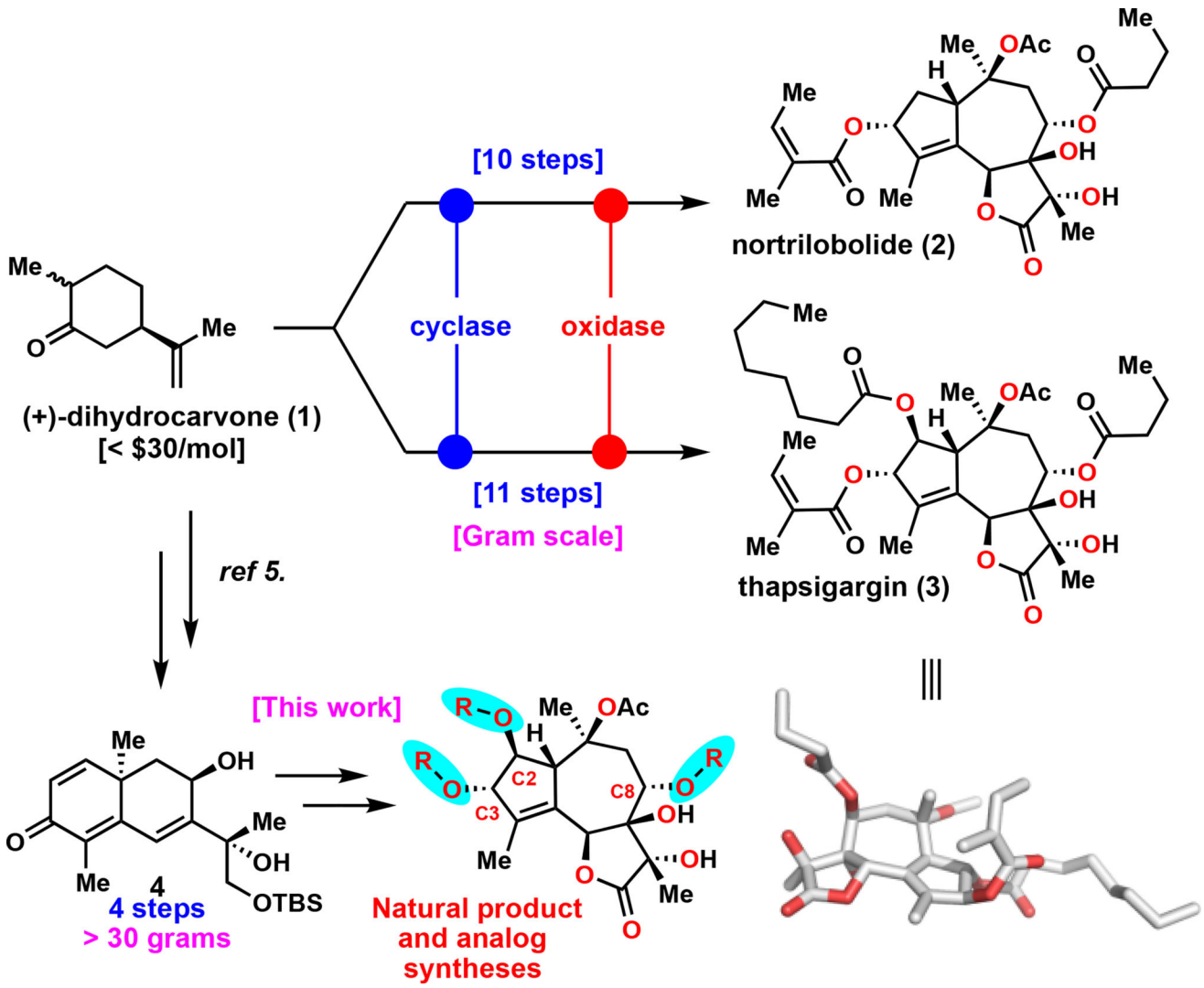

Figure 1.

Two-phase terpene synthesis strategy enables divergent, scalable, and modular access of complex guaianolide natural products and analogs.

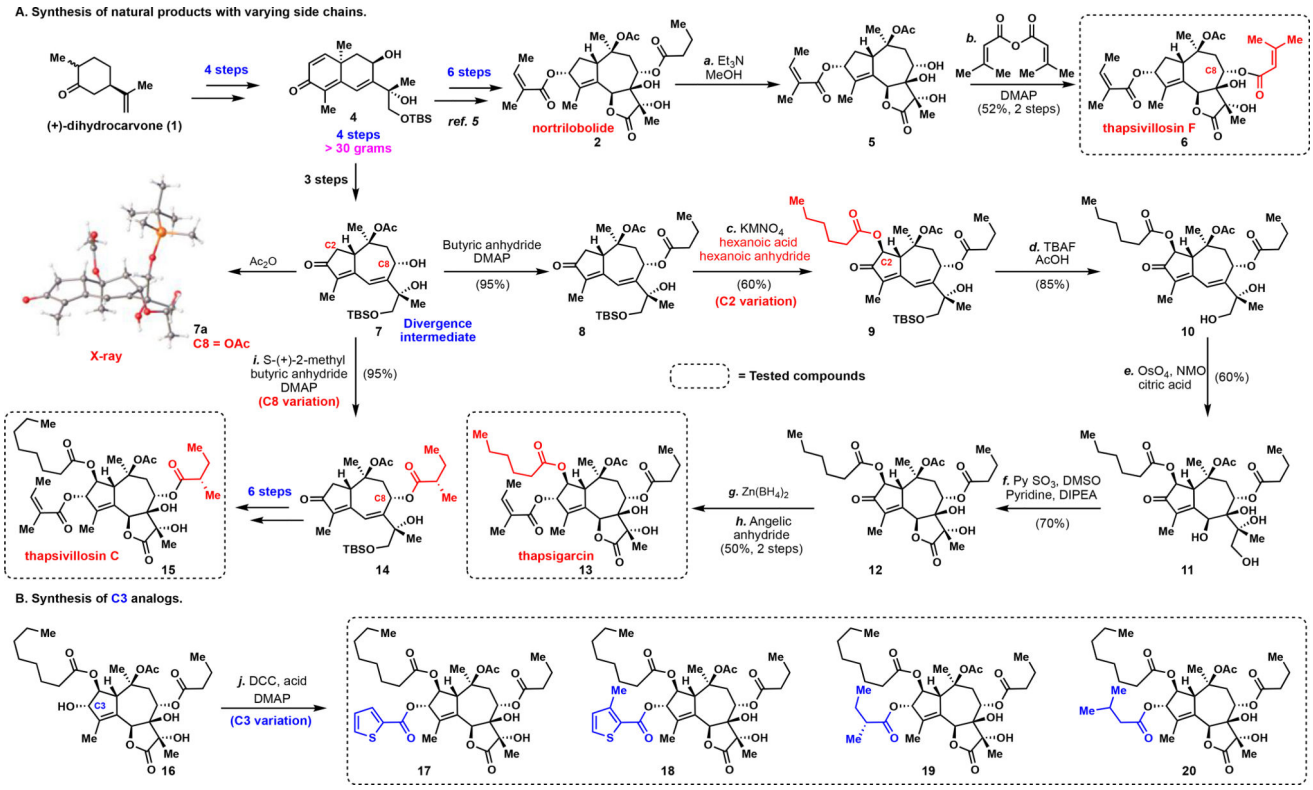

Figure 2.

Divergent access of the thapsigargin family of natural products and analogs

Reagents and conditions: (a) Et_3N, MeOH, 60 °C, 30 min; (b) Senecioic anhydride, DMAP, dichloromethane (52% yield, 2 steps); (c) $KMnO_4$ (2.1 equiv), hexanoic acid (35 equiv), hexanoic anhydride (9 equiv), PhH (1.95 mL), 20 h, 85 °C (60%); (d) TBAF/AcOH (10 equiv), THF (0.1 M), 0 °C (85%); (e) Citric acid (2 equiv), NMO (2 equiv), OsO_4 (0.1 equiv), *t*BuOH/H_2O/Acetone (0.1 M), 50 °C, 4.5h (60%); (f) DIPEA (20 equiv), pyridine (30 equiv), $PySO_3$ (20 equiv), DMSO, dichloromethane, 0 °C, 0.5 h (70%); (g) $Zn(BH_4)_2$, Et_2O, 4 h, - 20 °C (84%); (h) angelic anhydride, $NaHCO_3$, 80 °C, 16 h (60%); (i) (S)-(+)-2-methylbutyric anhydride (1.3 equiv), DMAP (0.8 equiv), dichloromethane (0.1 M), 25 °C, 12 h (95%); (j) DCC (4 equiv), DMAP (2 equiv), 25 °C (80–85%).

	IC_{50} SERCA Inhibition (nM)				
Compound	**Run #1**	**Run #2**	**Run #3**	**Mean**	***SD***
Thapsigargin (**3**)	31	29	51	**37**	12
Thapsivillosin F (**6**)	246	204	268	**239**	33
Thapsigarcin (**13**)	81	73	90	**81**	9
Thapsivillosin C (**15**)	220	220	298	**246**	45
Analog **17**	73	72	117	**87**	26
Analog **18**	43	30	119	**64**	48
Analog **19**	65	58	104	**76**	25
Analog **20**	436	311	576	**441**	133

Figure 3.
IC_{50} SERCA inhibition values (nM) of analogs. SD = standard deviation.