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Synthesis and evaluation of trehalose-based compounds as anti-invasive agents

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ABSTRACT

Brartemicin is a trehalose-based inhibitor of tumor cell invasion produced by the actinomycete of the genus *Nonomuraea*. In order to explore the preliminary structure–activity relationship and obtain more potent inhibitors, a series of brartemicin analogs were synthesized through the Mitsunobu coupling of the secondary hydroxyls benzyl protected α,α-D-trehalose with benzoic acid derivatives, followed by modification of functional groups and deprotection. These compounds were evaluated for their inhibitory activity against invasion of murine colon 26-L5 carcinoma cells in vitro. Among the synthetic analogs tested, 6,6′-bis(2,3-dimethoxybenzoyl)-α,α-D-trehalose (**5e**) was found to be the most potent anti-invasive agent, exhibited a 2.6-fold improvement with regard to the parent natural product brartemicin, and it is considered to be a promising lead molecule for the anti-metastasis.

A characteristic feature of cancer is dissemination into distant, often specific tissues. Cancer cells invade beyond the constraints of the normal tissue from which they originate; this invasion permits them to enter into the circulation from where they can reach distant organs and eventually form secondary tumors, called metastasis. Metastasizing is a complex, multi-step biochemical process, which includes detachment of cancer cells from primary tumor, migration, adhesion, and invasion of cancer cells into the blood or lymphatic vessels, extravasation out of the vessel, and finally, interactions with the target tissue and grows out to micrometastasis and macrometastasis/secondary tumors.[1] Metastasis is responsible for 90% of cancer patient deaths, and it remains a major hurdle in cancer treatment.[2] Anticancer drug development strategies are generally aimed at direct inhibition of cancer cell growth. Most current chemotherapy treatments are antiproliferatives, which seek to retard growth of the primary tumor or even reduce the existing tumor burden. However, many tumor cells are unaffected by this type of chemotherapy. Indeed, some cells are able to stay dormant for months, or even years, and to grow later into new tumors.[3] Furthermore, migrating cells are known to show a decreased proliferation rate and are thus less sensitive to standard chemotherapy.[3] As invasion is a measure of tumor cell activity and crucial at all the different steps of the metastasis process, closely related to the final outcome of cancer, treatments directly targeting metastatic disease by specifically inhibiting the invasive behavior of tumor cells are extremely attractive. Such approaches might extend the patient's lifespan, even though they might not directly inhibit late-stage tumor proliferation. The development of anti-invasive and anti-metastasis drugs is a major challenge in current cancer research.[4]

Natural products are important sources in drug discoveries and developments. In the area of cancer, over the time frame from around the 1940s to 2007, of the 155 small molecules, 47% are either natural products or directly derived therefrom.[5] In recent years, a number of natural products are identified to have good activities against tumor invasion and metastasis.[6,7] Trehalose-6,6′-dimycolate (TDM, **1**, Fig. 1), the major trehalose-based natural glycolipid component on the surface of the mycobacterial cell wall, shows significant antitumor and anti-metastasis activities.[8,9] However, due to its significant toxicity, TDM is not yet applicable for practical use. Brartemicin (**2**), another trehalose-containing metabolite, recently isolated from the culture broth of the actinomycete of the genus *Nonomuraea*, inhibits the invasion of murine colon carcinoma 26-L5 cells with an IC_{50} value of 0.25 μg/mL (0.39 μM), and has no cytotoxicity against the same cell line even at the concentration of 10 μg/mL.[10] Brartemicin is composed of a trehalose scaffold and two identical 2,4-dihydroxy-6-methylbenzoate units, which are connected by two ester bonds. This combinatorial structural feature prompted us to synthesize more analogs and assess the preliminary structure–activity relationship of this class of compounds as anti-invasive agents. We herein report the synthesis of brartemicin derivatives, preliminary structure–activity relationship investigations and in vitro anti-invasive activities.

The trehalose-based compounds were synthesized by the coupling of the secondary hydroxyls benzyl protected trehalose with benzoic acid derivatives and the following modification of

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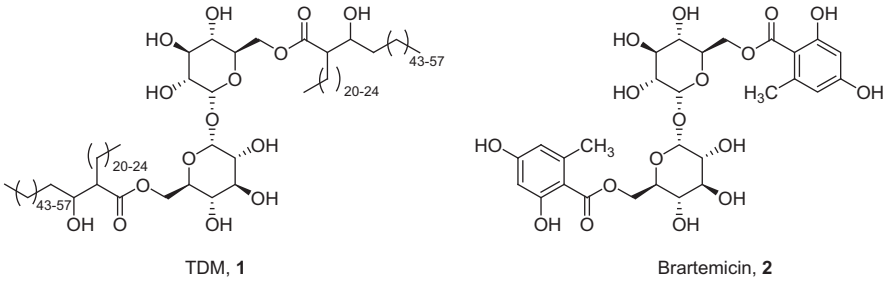
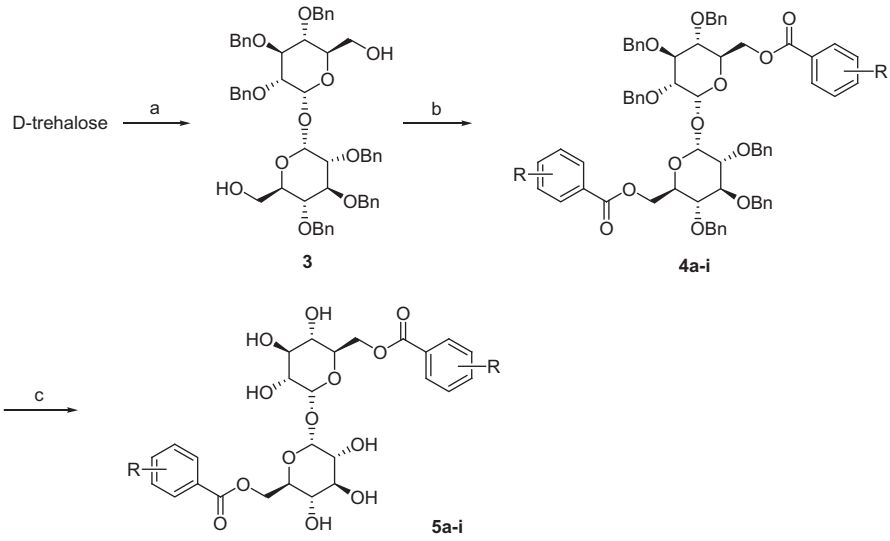


Figure 1. Natural trehalose-based anti-invasive and anti-metastatic agents.



Scheme 1. Reagents and conditions: (a) (i) TrCl, Py, (ii) BnBr, NaH, (iii) TsOH·H_2O; (b) ArCOOH, DIAD, Ph_3P; (c) H_2, 10% Pd-C, EtOH/AcOEt.

functional groups and deprotection. Nine analogs possessing various types of functionality at the different positions of the benzene ring were prepared as shown in Scheme 1.

The secondary hydroxyls benzyl protected trehalose **3** was prepared by a slight modification of Gilbertson's procedures.[11] Reaction of trityl chloride with the commercially available α,α-D-trehalose dihydrate in pyridine protected the two primary hydroxyls. In the following benzylation step, Gilbertson's group used a catalytic amount of tetrabutylammonium iodide, excess NaH and BnBr in DMF solution.[11] We found the benzylation could be carried out without the use of tetrabutylammonium iodide. The crude bistritylated trehalose was dissolved in anhydrous DMF, treated portion wise with excess NaH and stirred at 0 °C for 1 h, followed by dropwise addition of benzyl bromide. After stirring at room temperature for 48 h, instead of pouring the reaction mixture into 10% $NaHCO_3$ and ether solution,[11] the solvent DMF was distilled in vacuo and the residue was extracted with EtOAc. Finally, the removal of the trityl group by treatment the crude benzylated product with *p*-toluenesulfonic acid furnished the trehalose with the secondary hydroxyls benzyl protected and the primary hydroxyls free (**3**). These modified procedures led to the synthesis of **3** with an improved yield from 25% to 35.1%. Acylation of the 6- and 6′-primary hydroxyls had been tried using acyl chloride, Steglich esterification,[12] and Mitsunobu reaction.[13,14] The later proved to be the choice and suitable for various substrates. Reaction of **3** with benzoic acid derivatives in anhydrous THF in the presence of triphenylphosphine and DIAD (diisopropyl azodicarboxylate) led to the esters **4a–i** in generally good yields (64.3–87.5%, Table 1).[15] Finally, removal of the benzyl protective groups and functionality alteration by hydrogenolysis generated the brartemicin analogs **5a–i**.[16] To our surprise, the reduction of nitro group in ethanol solution led to the ethylamino compound **5i** as the major product in a low yield (35.8%, Table 1). Its structure was confirmed by ^{1}H NMR, ^{13}C NMR, and ESI-MS.[17]

The synthetic trehalose-based compounds were tested for in vitro inhibitory activity against invasion of murine colon 26-L5 cells using Transwell chamber with 8.0 μm diameter polyvinylpyrrolidone-free carbonate filters coated with matrigel.[18,19] Cytotoxicity was also examined in the same cell line. All compounds tested exhibited no cytotoxicity even at the concentration of 10 μg/mL. The anti-invasion assay results are summarized in Table 1.

Among the compounds with different functionality at the 2-position of the benzoic acid ring, 2-methyl substituted analog **5b** was not active, while 2-hydroxyl and 2-methoxy substituted derivatives **6** and **5a** maintained the anti-invasive activity, though they were slight less potency than natural brartemicin (**2**). In addition, the 2-hydroxyl analog **6** at 1.0 μg/mL exhibited 35% inhibition,[10] while the methylated compound **5a** showed an increased potency (entries 1 and 10). On the other hand, the 4-hydroxybenzoic acid derivative **5d** (entry 4) had an IC_{50} of 1.0 μg/mL, its methylated analog **5c** (entry 3) lost anti-invasive activity. The 3,4,5-trimethoxy-substituted and 3-methoxy-4-fluorobenzoic esters **5g** and **5f** (entries 6 and 7) also exhibited no anti-invasive abilities. These results demonstrated the subtle requirements for the active substituents at these positions. For the 2,6-disubstituted analogs, both 2,6-difluoro- and 2-ethylamino-6-methylbenzoic acid esters **5h** and **5i** (entries 8 and 9) maintained the anti-invasive activities. Therefore, the 2-hydroxyl group in brartemicin could be replaced

Table 1
Synthesis of brartemicin analogs and their anti-invasive activity in vitro

Entry	R	Yield (%)		Murine colon 26-L5 cells	
		4a–j	**5a–j**	Compounds	IC_{50} (μg/mL)
1	2-OCH_3	**4a**, 82.8	**5a**, 70.2	**5a**	1.0
2	2-CH_3	**4b**, 87.5	**5b**, 63.3	**5b**	NA[a]
3	4-OCH_3	**4c**, 81.8	**5c**, 69.5	**5c**	NA
4	4-OBn (**4d**); 4-OH (**5d**)	**4d**, 68.0	**5d**, 64.7	**5d**	1.0
5	2,3-$(OCH_3)_2$	**4e**, 74.2	**5e**, 60.2	**5e**	0.10
6	3,4,5-$(OCH_3)_3$	**4f**, 68.8	**5f**, 58.7	**5f**	NA
7	3-OCH_3-4-F	**4g**, 64.3	**5g**, 82.2	**5g**	NA
8	2,6-F_2	**4h**, 83.0	**5h**, 66.3	**5h**	1.0
9	2-CH_3-6-NO_2 (4i); 2-EtNH-6-CH_3 (5i)	**4i**, 76.7	**5i**, 35.8	**5i**	1.0
10	2-OH			**6**[b]	<1.0[c]
11	2,3-$(OH)_2$			**7**[b]	<1.0[d]
12	2,4-$(OH)_2$-6-CH_3			Brartemicin	0.25

[a] No activity.
[b] Ref. 10.
[c] 35% inhibition at 1.0 μg/mL.
[d] 40% inhibition at 1.0 μg/mL.

by methoxy-, ethylamino-, and fluoro- groups. It is noteworthy that the 2,3-dimethoxy-substituted analog **5e** was not only much more potent than its 2,3-dihydroxyl counterpart **7**, but also superior to the natural brartemicin. Compound **5e** exhibited an IC_{50} of 0.10 μg/mL (0.15 μM), was 2.6-fold more potent than brartemicin (0.39 μM).

Unlike the natural anti-invasive inhibitor myxochelins,[20] brartemicin analogs did not inhibit the MMP-2 and MMP-9 protease activities that are related to degradation of extracellular matrix, an important step for the tumor cell invasion. In addition, brartemicin analogs did not affect the tumor cell migration, differing from the natural anti-invasive inhibitor lupinacidins.[21] Further investigation is needed to elucidate the mode of action of the brartemicin analogs.

In summary, a series of nine novel trehalose-based brartemicin analogs are synthesized and evaluated for their inhibitory activity against invasion of murine colon 26-L5 carcinoma cells. Among the synthetic analogs tested, 6,6′-bis(2,3-dimethoxybenzoyl)-α,α-D-trehalose (**5e**) was found to be the most potent anti-invasive agent, exhibited a 2.6-fold improvement with regard to the parent natural product brartemicin and showed no cytotoxicity even at the 100 times of its effective anti-invasive concentration, and is considered to be a promising lead molecule for the antimetastasis. Based on our preliminary SAR analysis, more trehalose-based brartemicin derivatives will be prepared and studied for anti-invasive activity both in vitro and in vivo in the near future.

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15. *Typical synthetic procedures*: Triphenylphosphine (300 mg, 1.14 mmol), 2,3-dimethoxybenzoic acid (186 mg, 1.02 mmol), and diisopropyl azodicarboxylate (226 μl, 1.14 mmol) were added to a stirred solution of 2,2′,3,3′,4,4′-hexabenzyl-α,α-D-trehalose (**3**, 300 mg, 0.34 mmol) in dry THF (10 mL) at 0 °C. After stirring for 2 h at the same temperature, the reaction mixture was diluted with ice water and extracted with EtOAc. The organic layer was dried over anhydrous $MgSO_4$, filtered, and concentrated. The residue was purified by silica gel column chromatography (hexane/EtOAc, 15–3:1) to give **4e** (305.6 mg, 74.2%) as a colorless oil. ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.24–7.37 (m, 16H), 7.02–7.05 (m, 2H), 5.20 (d, *J* = 3.6 Hz, 1H), 5.01 (d, *J* = 10.2 Hz, 1H), 4.88 (dd, *J* = 7.8, 10.8 Hz, 2H), 4.68 (q, *J* = 12.0 Hz, 2H), 4.58 (d, *J* = 10.2 Hz, 1H), 4.38 (dd, *J* = 3.6, 12.0 Hz, 1H), 4.28–4.32 (m, 2H), 4.08 (t, *J* = 9.6 Hz, 1H), 3.86 (s, 3H), 3.84 (s, 3H), 3.68 (t, *J* = 9.6 Hz, 1H), 3.57 (dd, *J* = 3.6, 9.6 Hz, 1H). MS (ESI) *m/z* 1211.5 [MH^+].
16. *Typical procedures*: To a solution of **4e** (255 mg, 0.21 mmol) in a EtOAc/EtOH (1:1) solution (5 mL) was added 10% Pd/C (100 mg), and the mixture was stirred at room temperature under an atmosphere of H_2 for 18 h. The reaction mixture was filtered through a pad of Celite and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (CH_2Cl_2/MeOH, 15:1–6:1) to give **5e** (85 mg) in 60.2% yield as a white solid, mp 135–137 °C. ^{1}H NMR (600 MHz, DMSO-d_6): δ 7.25 (d, *J* = 7.8 Hz, 1H), 7.20 (d, *J* = 7.8 Hz, 1H), 7.15 (t, *J* = 7.8 Hz, 1H), 5.18 (d, *J* = 5.4 Hz, 1H), 4.94 (d, *J* = 4.8 Hz, 1H), 4.87–4.89 (m, 2H), 4.40 (d, *J* = 10.8 Hz, 1H), 4.30 (dd, *J* = 4.8, 11.4 Hz, 1H), 4.00–4.08 (m, 1H), 3.83 (s, 3H), 3.74 (s, 3H), 3.60 (td, *J* = 9.0, 4.8 Hz, 1H), 3.23–3.30 (m, 2H). ESI-MS *m/z* 671.4 [MH^+], 693.4 [MNa^+].
17. *Compound* **5i**: White solid, mp101–103 °C. ^{1}H NMR (600 MHz, DMSO-d_6): δ 7.16 (t, *J* = 8.4 Hz, 1H), 6.54 (d, *J* = 8.4 Hz, 1H), 6.45 (d, *J* = 8.4 Hz, 1H), 6.19 (s, 1H), 5.20 (d, *J* = 5.4 Hz, 1H), 4.91–4.93 (m, 3H), 4.45(d, *J* = 10.8 Hz, 1H), 4.26 (dd, *J* = 4.8, 11.4 Hz, 1H), 4.04–4.07 (m, 1H), 3.57–3.60 (m, 1H), 3.26–3.32 (m, 1H), 3.19–3.28 (m, 1H), 3.06–3.13 (m, 2H), 2.32 (s, 3H), 1.15 (t, *J* = 7.2 Hz, 3H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 170.9, 150.9, 141.3, 133.5, 120.1, 115.4, 110.5, 95.6, 74.7, 73.3, 72.4, 71.5, 65.1, 39.1, 23.4, 15.1. ESI-MS *m/z* 665.3 [MH^+].
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