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Novel galbonolide derivatives as IPC synthase inhibitors: design, synthesis and in vitro antifungal activities

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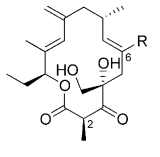
Abstract—A series of novel galbonolide derivatives having a modified methyl enol ether moiety were prepared in total synthetic procedures and evaluated for their in vitro antifungal activities. The antifungal activity was labile to modification of the enol ether functionality and almost all of the modified compounds lacked the activity except for the analogue with an introduction of a methylthio group at the C-6 position, which retained a modest antifungal potency against *Cryptococcus neoformans*.

Galbonolide A (rustmicin, **1**, Fig. 1)[1] is a 14-membered macrolide antibiotic with antifungal activity against clinically important strains such as *Candida albicans* and *Cryptococcus neoformans*. It was originally isolated as an inhibitor of wheat rust fungus by an unknown mode of action, however, researchers at Merck research laboratories recently discovered that fungicidal activities of galbonolide A acted through an inhibitory process against inositol phosphoceramide (IPC) synthase.[2] The inhibition of this essential biosynthetic step is reversible and leads to the accumulation of ceramide in growing cells, leading to cell death. In addition, selective toxicity could be expected because no corresponding enzyme in mammalian sphingolipid biosynthesis pathway exists.[3] Therefore, it seemed an attractive compound possessing a novel antifungal mechanism. Total synthesis of galbonolide B (neorustmicin, **2**)[4] and determination of its absolute stereochemistry have been also reported by Tse.[5]

Several degradation pathways are known, even under physiological conditions, due to the presence of several unstable functional groups.[6] Under basic to neutral conditions, rapid epimerization of the C-2 methyl group to a significantly less potent diastereomer and subsequent translactonization leads to a completely inactive compound. Upon hydrolysis of the enol ether function under acidic condition, an inactive ketone analogue was formed.

At the beginning, our effort focused on constructing more stable functionality as an alternative to the acid-sensitive methylenolether. As a part of the total synthetic approach to galbonolide A analogues, we recently reported the synthesis of 'benzene analogues' that contain benzene ring instead of diene part and more stabilized enolether-equivalent groups.[7] The resulting 'benzene analogues' did not show any appreciable antifungal activities. Therefore, we designed several promising candidates by replacing methoxy group at the C-6 position with hydrogen (**23**), fluoroalkoxy (**25**), methylthio (**26**) and halogen (**24**) and varying the position of methoxy group to C-5 (**27**, **28**). Herein, we describe the synthesis of novel galbonolide A analogues that have the original carbon skeleton (diene part) and their in vitro antifungal activities.[8]



R = OMe : Galbonolide A **1** (Rustmicin)
Me : Galbonolide B **2** (Neorustmicin)

Figure 1. Structure of galbonolide A and B.

Keywords: 14-membered macrolide; IPC synthase; antifungal agent; methylenolether; Macro–Dieckmann cyclization.
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The synthetic route to target compounds **23–28** is shown in Scheme 1. According to the reported synthetic strategy,[5] we prepared the key intermediate **4** from commercially available (*S*)-benzylglycidyl ether (**3**, >99% ee).[9] The construction of α,β-unsaturated esters **6–9** was accomplished by Horner–Wadsworth–Emmons reaction or aldol reaction followed by dehydration using methanesulfonyl chloride (MsCl) in the presence of 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU). After reduction of ester groups with diisobutylaluminum hydride (DIBAL-H), the resultant alcohols were converted to allyl iodides by the action of PPh_3, imidazole and I_2 combination, and subjected to condensation reaction with lithium enolate derived from benzylidene acetal **5** to give adducts **11–14**.

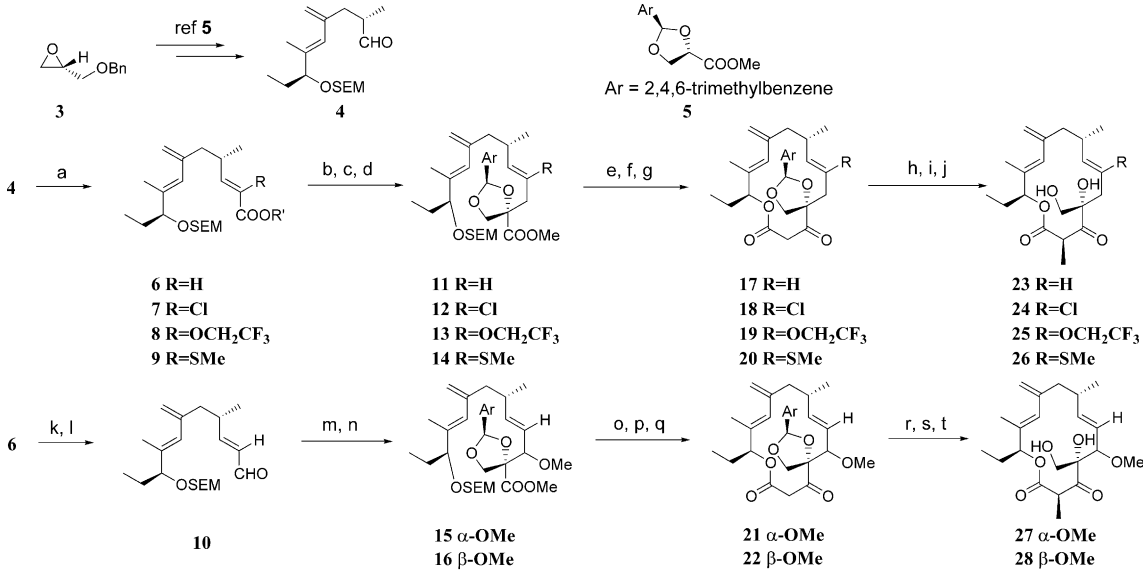
Methoxy analogues **15–16** were prepared starting from α,β-unsaturated ester **6**. Reduction of the ester moiety of **6** with DIBAL-H followed by oxidation by means of tetrapropylammonium perruthenate (TPAP) afforded aldehyde **10**. Aldol reaction with lithium enolate of **5** gave a secondary alcohol as a 1:1 mixture of diastereomers. The resultant alcohols were converted to methyl ethers **15–16** by MeI in the presence of potassium hexamethyldisilazide (KHMDS).

Deprotection of the 2-(trimethylsilyl)ethoxymethyl (SEM) groups of **11–16** with tetraethylammonium fluoride at 90 °C, followed by esterification of the carboxylic acids resulting from hydrolysis with trimethylsilyl (TMS)-diazomethane provided methyl esters. Acetates, precursor of macrocycles, were prepared by acetic anhydride using dimethylaminopyridine (DMAP). Macro–Dieckmann cyclization proceeded smoothly to afford the desired macrocycles **17–22** in good yield (up to 83%). Introduction of a methyl group to the cyclized product followed by epimerization of the methyl group to β-form, if necessary, afforded a protected macrolide. Finally, cleavage of 2,4,6-trimethylbenzylidene acetal under hydrolysis condition (aqueous acetic acid) were conducted to successfully furnish galbonolide A analogues **23–28**.

All the new compounds were evaluated for their in vitro antifungal activity against *C. albicans*, *Cr. neoformans*, *Aspergillus fumigatus* and *Saccharomyces cerevisiae*. Table 1 summarizes the minimum inhibitory concentrations (MICs) and the concentration required for the 80% inhibition of fungal growth (IC_{80}), in which Amphotericin B, fluconazole, galbonolide A, C-2 epimer of galbonolide A and galbonolide B were listed as the reference compounds.

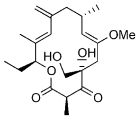
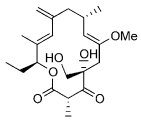
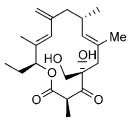
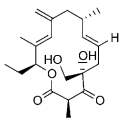
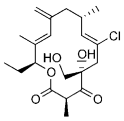
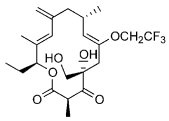
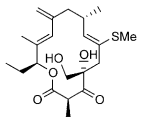
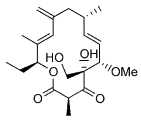
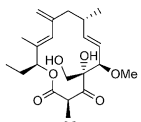
The introduction of functional groups including hydrogen (**23**), chlorine (**24**) and 2,2,2-trifluoroethoxy (**25**) at the C-6 position resulted in a loss of potency. Other analogues (**27**, **28**) with methoxy groups at the C-5 position also lacked activity. Among all derivatives modified at the C-6 position instead of methoxy group, only the methylthio analogue (**26**)[10] showed moderate activity against *Cr. neoformans* ATCC9011 strain with the MIC of 0.5 μg/mL and IC_{80} of 0.063 μg/mL, and weak activity against *C. albicans* ATCC90028 strain and *S. cerevisia* Y9 strain with the MICs of 64 μg/mL, respectively. Although the antifungal activity was reduced as compared with the parent galbonolide A, this is the first antifungal analogue of galbonolide A produced by using total synthetic methodology.

In conclusion, acid-labile methyl enol ether was modified through a total synthetic approach to afford several galbonolide A (rustmicin) analogues, which were evaluated for antifungal activities against a panel of fungi in comparison with galbonolide A. The antifungal



Scheme 1. Reagents: (a) $(EtO)_2P(O)CH_2COOEt$, LiCl, DBU, 80% (*E* only, for **6**), $(EtO)_2P(O)CH_2COOEt$, NaH, NCS, 34% (*E*/*Z* = 5:3, for **7**), $CF_3CH_2OCH_2COOMe$, LHMDS, then MsCl, Et_3N, DBU, 57% (*E*/*Z* = 1:4, for **8**), $MeSCH_2COOEt$, LHMDS, then MsCl, Et_3N, DBU, 75% (*Z* only, for **9**); (b) DIBAL-H, 50–94%; (c) I_2, PPh_3, imidazole; (d) **5**, LHMDS, HMPA, 32–52%; (e) Et_4NF, then $TMSCHN_2$, 40–77%; (f) Ac_2O, DMAP, pyridine 91–97%; (g) LHMDS, HMPA, 66–83%; (h) MeI, *t*-BuOK, 35–86%; (i) *t*-BuOK; (j) AcOH–H_2O, 61–80%; (k) DIBAL-H, 93%; (l) TPAP, NMO, MS4A, 82%; (m) **5**, LHMDS, HMPA; (n) MeI, KHMDS, 60% (*m*+*n*, two steps); (o) Et_4NF, then $TMSCHN_2$, 57%; (p) Ac_2O, DMAP, pyridine, 95–97%; (q) LHMDS, HMPA, 49–65%; (r) MeI, *t*-BuOK, 41–85%; (s) *t*-BuOK, 63–72%; (t) AcOH–H_2O, 74%-quant.

Table 1. In vitro antifungal activities of novel rustmicin analogues

Strain	MIC (IC_{80})[a]					
	AMPH[b]	FLCZ[c]	 Galbonolide A (1)	 C-2 epimer	 Galbonolide B (2)	 23
C. albicans ATCC90028	0.25	(0.5)	4 (1)	>64	>64	>64
Cr. neoformans ATCC9011	0.25	(8)	0.063 (<0.031)	>64	>64 (32)	>64
A. fumigatus TIMM1776	1	(>64)	>64	>64	>64	>64
S. cerevisiae Y9	0.25	(8)	1 (0.5)	>64	>64	NT[d]
		 24	 25	 26	 27	 28
C. albicans ATCC90028		>64 (64)	>64	64 (16)	>64	>64
Cr. neoformans ATCC9011		16 (0.15)	32	0.5 (0.063)	>64	>64
A. fumigatus TIMM1776		>64	>64	>64	>64	>64
S. cerevisiae Y9		NT[d]	NT[d]	64 (32)	NT[d]	NT[d]

[a] MIC (μg/mL) determined by the broth microdilution method and numbers in parentheses indicate IC_{80} values (μg/mL).
[b] Amphotericin B.
[c] Fluconazole.
[d] Not tested.

activity of synthetic analogues proved to be strictly affected by the substituents at the C-6 position and most of the synthetic analogues lost the antifungal activities. However, the substitution of a methylthio group for the methoxy group was tolerated and showed modest antifungal activity against the *Cr. neoformans* strain.

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8. A part of this work was presented by the authors at the 22nd Symposium on Medicinal Chemistry, The Pharmaceutical Society of Japan, Poster No. 1P-17, Nov. 27–29, 2002, Shizuoka, Japan.
9. Purchased from Daiso Co., Ltd, Japan.
10. Selected data for compound **26**: IR (KBr) ν_{max} 2960, 2920, 1709, 1456 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 0.79 (3H, t, $J=7.4$ Hz), 0.94 (3H, d, $J=6.9$ Hz), 1.43 (3H, d, $J=7.0$ Hz), 1.50 (2H, m), 1.69 (3H, s), 1.89 (3H, s), 2.24 (2H, m), 2.66 (2H, AB q, $J=14.8$ Hz), 3.15 (1H, m), 3.66 (2H, AB q, $J=11.9$ Hz), 3.80 (1H, q, $J=7.0$ Hz), 4.85 (1H, s), 4.93 (1H, t, $J=6.1$ Hz), 4.99 (1H, s), 5.71 (1H, d, $J=9.3$ Hz), 5.92 (1H, s); ^{13}C NMR (125 MHz, C_6D_6) δ 9.9, 14.4, 15.2, 15.6, 18.9, 26.2, 34.5, 37.2, 45.6, 50.8, 67.5, 80.9, 82.9, 117.2, 128.0, 135.4, 142.0, 143.6, 168.8, 208.4; FAB-HRMS calcd for $C_{21}H_{32}O_5SNa^+$: 419.1868. Found 419.1873.