Pergamon

Bioorganic & Medicinal Chemistry Letters 12 (2002) 2699–2702

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

Remarkable Influence of the Aromatic Substructure in 9-Methoxystrobilurin Derivatives on their Antifungal Activity

Hiromi Uchiro,[a] Koh Nagasawa,[a] Tomohiro Sawa,[a] Daiju Hasegawa,[a] Tomoya Kotake,[a] Yoshitsugu Sugiura,[a,†] Susumu Kobayashi,[a,*] Kazuhiko Otoguro[b] and Satoshi Ōmura[c]

[a]*Faculty of Pharmaceutical Sciences, Tokyo University of Science, 12 Ichigayafunagawara-machi, Shinjuku-ku, Tokyo 162-0826, Japan*
[b]*Research Center for Tropical Diseases, Research Center for Biological Function, The Kitasato Institute, 5-9-1 Shirokane, Minato-ku, Tokyo 108-8642, Japan*
[c]*Kitasato Institute for Life Sciences, Kitasato University, 5-9-1 Shirokane, Minato-ku, Tokyo 108-8642, Japan*

Received 20 May 2002; accepted 5 July 2002

Abstract—9-Methoxystrobilurin-type β-methoxyacrylate antibiotics (MOSBs) having various aromatic substructures were synthesized. The antifungal activity of the synthesized MOSBs against pathogenic and non-pathogenic fungi was examined, and the obtained results revealed that the antifungal activity of MOSBs was highly dependent on the aromatic substructures. However, no significant correlation was observed between cytotoxicity against human fibroblasts-like cell line and their structural properties. In addition, our results suggested that the strong growth-inhibitory activity of 9-methoxystrobilurin K against human-derived cell lines should be related to its hindered ether-type substructure.

Introduction

Strobilurin A (**1**) was isolated from mycelia of basidomycete *Strobilurus tenacellus* as the first β-methoxyacrylate antibiotic (MOA) by Anke et al. in 1977.[1] The compound exhibits strong antifungal activity against various fungi by binding to cytochrome bc1 (complex III) in a mitochondrial respiration pathway. A certain number of the same class of compounds have been discovered and several artificial analogues are effectively applied for agricultural fungicides.[2] On the other hand, 9-methoxystrobilurin-type β-methoxyacrylates (MOSBs) such as 9-methoxystrobilurin A (**2**) and K (**3**) were isolated in 1995, and **3** indicated strong growth-inhibitory activities toward typical human-derived tumor cell lines, along with the original antifungal activities.[3–5] Recently, total syntheses of several natural-type MOSBs were achieved in our laboratory[6] and further studies are now in progress to clarify the structure–activity relationships of MOSBs. In this paper, we would like to describe an efficient synthesis of several modified MOSBs and the remarkable influence of the aromatic substructures on their antifungal activity (Fig. 1).

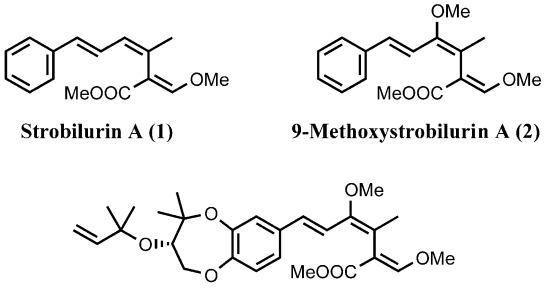


Figure 1. Strobilurin A and 9-methoxystrobilurins.

Molecular Design

According to the original report, 9-methoxystrobilurin A (**2**) and K (**3**) indicated the same level of antifungal activities; however, there is much greater difference in their growth-inhibitory activities toward human-derived tumor cell lines.[3] Surprisingly, the IC_{50} value of **3** for cell growth of HeLaS3 cells reached the order of nM,

*Corresponding author. Tel.: +81-3-3260-8848; fax: +81-3-3260-8848; e-mail: kobayash@ps.kagu.sut.ac.jp
†Present address: Department of Food Chemsitry, Kobe Institute of Health, 4-6 Minatojima-nakamachi, Chuo-ku, Kobe 650–0046, Japan

0960-894X/02/$ - see front matter © 2002 Elsevier Science Ltd. All rights reserved.
PII: S0960-894X(02)00558-9

which is 1000-fold more potent than **2**. This remarkably high growth-inhibitory activity of **3** is probably due to its 'more complicated' aromatic substructure. Therefore, several derivatives **4a–f** modified on the aromatic substructure of **2** were designed to clarify the structure-activity relationships in these two kinds of bioactivities (Fig. 2).

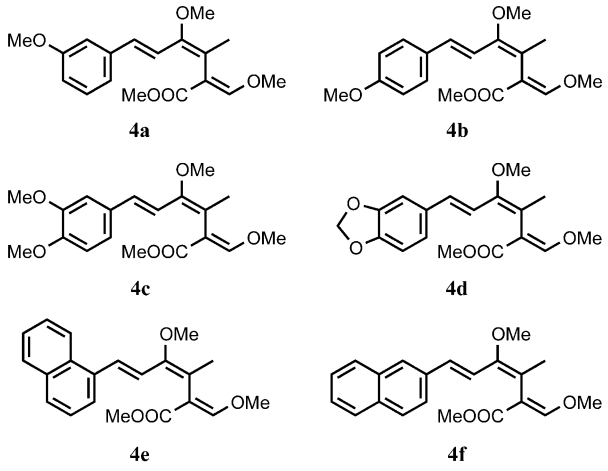


Figure 2. Aromatic modified 9-methoxystrobilurin derivatives.

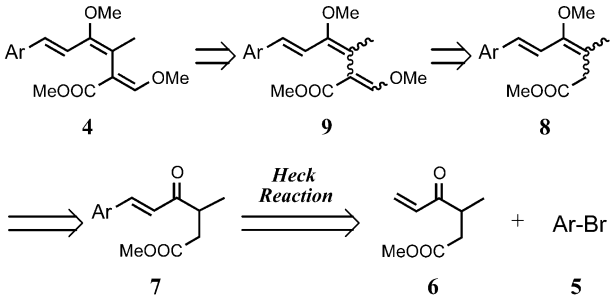
A comparison between their antifungal activities and growth-inhibitory activities (as cytotoxicities) for mammalian normal-type cell would bring about valuable pharmacostructural information for designing a novel 'cell-specific' growth inhibitor. In addition, during the course of this study, it was also expected to clarify the importance of hindered ether-type substituents on the aromatic ring of **3**.

Synthetic Plan

A general synthetic plan for modified MOSBs based on our previous reports[6] is shown in Scheme 1. A key step in the efficient preparation of MOSBs is the Heck reaction of aryl bromides **5** with vinylketone **6**. The vinylketone **6** was already prepared in three steps from commercially available monomethyl itaconate in high yield.[6a] The resulting enone-type intermediates **7** would lead to dienolether **8** by acetalization and successive elimination of methanol under an acidic condition. Further formylation at the α-position of **8** and sequential methylation of the resulting enolic hydroxyl group would afford the corresponding β-methoxyacrylates as a mixture of geometrical isomers **9**. The isomers **9** would be isomerized to the desired MOSBs **4** by ultraviolet lamp irradiation.

Synthesis

The Heck reaction of several aryl bromides **5a–f** with vinylketone **6** proceeded smoothly to give the corresponding enone-type intermediates **7a–f** in good yields (**7a**: 80%; **7b**: 85%; **7c**: 79%; **7d**: 83%; **7e**: 72%; **7f**: 81%). These intermediates **7a–f** were refluxed with



Scheme 1. Syntheic plan for 9-methoxystrobilurin derivatives.

methanol in the presence of trimethyl orthoformate and a catalytic amount of *p*-toluenesulfonic acid for 1 h. Further, the reaction mixtures were evaporated to remove an excess of methanol and once more refluxed in trimethyl orthoformate for 2 h. The desired dienolethers **8a–c**, **8e** and **8f** were obtained in good yields except for **8d** (**8a**: 80%; **8b**: 85%; **8c**: 88%; **8e**: 72%; **8f**: 81%) since the methylenedioxy moiety of **7d** did not survive in the above acidic condition. Therefore, a stepwise enol ether construction via the corresponding carboxylic acid **10d** in a similar manner to the synthesis of **3**[6b] was carried out. The desired intermediate **8d** was thus obtained in 83% yield from **7d**. The prepared dienolethers **8a–f** were formylated by sodium hydride-methyl formate and successively methylated with sodium carbonate-dimethyl sulfate to afford a mixture of three geometrical isomers **9a–f** (the mixture of **4a–f**, **11a–f** and **12a–f**). The mixture was effectively isomerized by ultraviolet lamp irradiation (λ: 365 nm), and the desired MOSBs **4a–f** were isolated along with an unseparable mixture of two other isomers (**11a–f**, **12a–f**). These undesired isomers were again subjected to photoisomerization, and the combined yields of **4a–f** were as follows: (**4a**: 43%, **4b**: 42%, **4c**: 41%, **4d**: 43%, **4e**: 43%, **4f**: 43%).[7] At the same time, the non-isomerized starting mixtures **9a–f** were recovered (**9a**: 43%, **9b**: 42%, **9c**: 41%, **9d**: 43%, **9e**: 43%, **9f**: 43%).

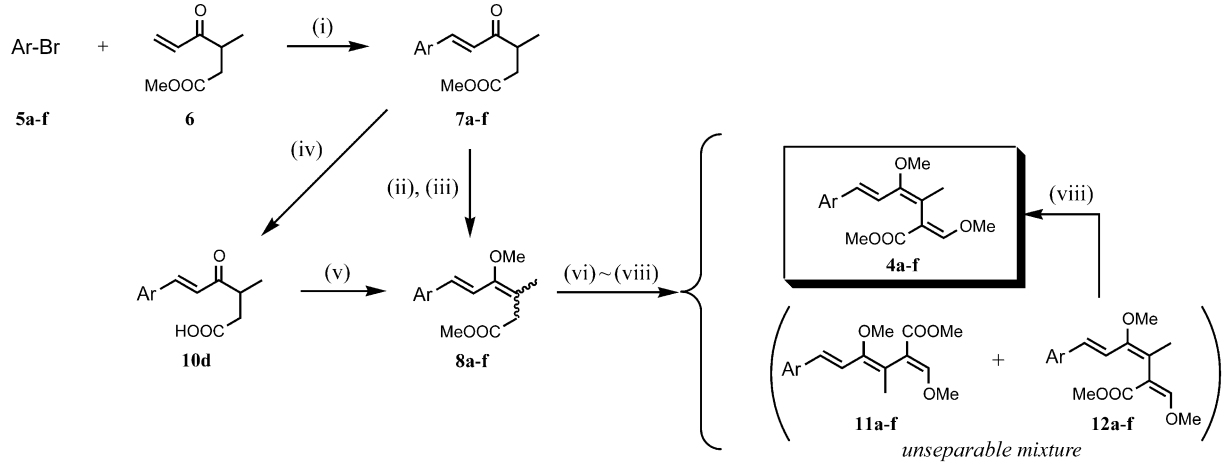
Antifungal Activity

The antifungal activities of synthesized MOSBs against several pathogenic and non-pathogenic fungi, which were maintained by Tokyo University of Science, were examined by a disk diffusion assay, and the obtained data are summarized in Table 1. Interestingly, antifungal activities of synthesized MOSBs were highly dependent on their aromatic substructures. For example, newly synthesized derivatives **4a–c** bearing benzene-type aromatic rings exhibited potent antifungal activities against *Penicillium citrinum*, while naphthalene analogues **4e–f** with larger aromatic substructures gave less sensitivity. In addition, 9-methoxystrobilurin K (**3**) having the largest aromatic moiety was almost inactive against the same fungus. On the other hand, the compounds **4a–d** produced relatively large inhibition zones toward *Saccharomyces cerevisiae* during 12–48 h incubation; however, the zones became gradually obscure and were lost after 96 h. In contrast, compound **4e–f** and 9-methoxystrobilurin K (**3**) evidently showed the

inhibition against *S. cerevisiae* up to 96 h. A similar observation was also found in the case of *Candida albicans*, which is a typical yeast-type pathogenic fungus. Further, it is noted that 9-methoxystrobilurin K (**3**) and compound **4f** were slightly active against *Fusarium solani* which is known as one of the most drug-resistant pathogenic fungi; however, their activity was insufficient similarly to those of the compounds **4a**–**d** against *S. cerevisiae*. These results demonstrated that β-naphthyl-type derivative **4f** has the broadest antifungal spectrum, and the geometrical modification of the aromatic substructure is apparently responsible for their antifungal properties (Scheme 2).

Cytotoxicity

The cytotoxicity of synthesized MOSBs toward a human diploid embryotic cell line (MRC-5) was measured by the previously reported method[8] and their IC_{50}



Scheme 2. Synthesis of 9-methoxystrobilurin derivatives. Reaction conditions: (i) 10 mol% $Pd(OAc)_2$, PPh_3, Et_3N, 100 °C; (ii) 10 mol% *p*-TsOH, $HC(OMe)_2$, MeOH, reflux; (iii) 10 mol% *p*-TsOH, $HC(OMe)_3$, reflux; (iv) NaOHaq–MeOH, rt then HCl; (v) KO′Bu, Me_2SO_4, DMF, −45 to −15 °C; (vi) NaH, HCOOMe, rt; (vii) K_2CO_3, Me_2SO_4, HCOOMe, rt; (viii) hν (λ: 365 nm), acetone-benzene, rt.

Table 1. Biologocal activities of 9-methoxystrobilurins

Compd	Antifungal activity						Cytotoxicity
	Diameter of inhibition zone (mm)[a,b]						IC_{50} (μM)
	Concn (μg/disk)	*Pencillium citrinum* (R-3703)	*Aspergillus fumigatus* (R-1301)	*Fusarium solani* (R-2800)	*Candida albicans* (IFO1594)	*Saccharomyces cerevisiae* (IAM4861)	MRC-5
Nystatin (positive control)	10	12	14	—	16	18	34
2	10	28	31	—	36*i*	49*i*	34
	1	18	15	—	28*i*	40*i*	
	0.1	4	±	—	±	17*i*	
3	10	—	10	25*i*	22	25	0.36
	1	—	8	19*i*	17	20	
	0.1	—	±	—	—	±	
4a	10	25	23	—	32*i*	35*i*	27
	1	19	9	—	27*i*	29*i*	
	0.1	8	—	—	±	16*i*	
4b	10	29	29	—	33*i*	42*i*	35
	1	23	18	—	27*i*	36*i*	
	0.1	9	4	—	±	28*i*	
4c	10	22	20	—	34*i*	43*i*	12
	1	13	±	—	26*i*	38*i*	
	0.1	±	—	—	±	20*i*	
4d	10	18	22	—	32*i*	42*i*	>100
	1	10	±	—	22*i*	36*i*	
	0.1	±	—	—	±	8*i*	
4e	10	17	14	—	15	22	>100
	1	12	10	—	5	18	
	0.1	±	—	—	—	5	
4f	10	19	18	13*i*	26	37	>100
	1	14	13	14*i*	16	30	
	0.1	±	4	—	±	12	

[a]The diameter of each inhibition zone (mean value of two samples) was measured after 48 h incubation.
[b]—, not effective, ±, slightly effective, *i*, incomplete inhibition.

values are listed in Table 1. No significant correlation was observed between cytotoxicity and the structural properties of synthesized MOSBs. It is noteworthy that, among the examined MOSBs, β-naphthyl-type derivative **4f** with the broadest antifungal spectrum showed the lowest cytotoxicity. In contrast, 9-methoxystrobilurin K (**3**) inhibited the growth of MRC-5 cell in 30–500-fold lower concentration than other MOSBs; however, the IC_{50} value was still 40–100-fold higher than those previously reported for several human-derived tumor cell lines. The strong growth-inhibitory activity is probably due to the specific binding affinity of **3** to a target protein in the mammalian cell, which is based on its hindered ether-type sidechain moiety.

Conclusion

Several 9-methoxystrobilurin-type β-methoxyacrylate antibiotics (MOSBs) having variotus aromatic substructures were synthesized. The antifungal activity of synthesized MOSBs is closely related to their aromatic substructures. The β-naphthyl-type derivative **4f** showed the broadest antifungal spectrum and the lowest cytotoxicity. This derivative is expected to be a lead compound as a new and effective disinfectant with a broad antifungal spectrum. Due to lack of significant correlation between cytotoxicity against MRC-5 and the structural properties of MOSBs, the strong growth-inhibitory activity of human-derived tumor cell lines to 9-methoxystrobilurin K should be related to its hindered ether-type substructure. Further extensive studies are now in progress for the design and development of a new 'cell-specific' growth inhibitor.

References and Notes

1. Anke, T.; Oberwinkler, F.; Steglich, W.; Scharmm, G. *J. Antibiotics* **1977**, *30*, 806.
2. Sauter, H.; Steglich, W.; Anke, T. *Angew. Chem., Int. Ed. Engl.* **1999**, *38*, 1328 and references cited therein.
3. Zapf, S.; Werle, A.; Anke, T.; Klostermeyer, D.; Steffan, B.; Steglich, W. *Angew. Chem., Int. Ed. Engl.* **1995**, *34*, 196.
4. Wood, K. A.; Kau, D. A.; Wrigley, S. K.; Beneyto, R.; Renno, D. V.; Ainsworth, A. M.; Penn, J.; Hill, D.; Killacky, J.; Depledge, P. *J. Nat. Prod.* **1996**, *59*, 646.
5. Nicholas, G. M.; Blunt, J. W.; Cole, A. L. J.; Munro, M. H. G. *Tetrahedron Lett.* **1997**, *38*, 7465.
6. (a) Uchiro, H.; Nagasawa, K.; Aiba, Y.; Kobayashi, S. *Tetrahedron Lett.* **2000**, *41*, 4165. (b) Uchiro, H.; Nagasawa, K.; Aiba, Y.; Kotake, T.; Hasegawa, D.; Kobayashi, S. *Tetrahedron Lett.* **2001**, *42*, 4531. (c) Aiba, Y.; Hasegawa, D.; Marunouchi, T.; Nagasawa, K.; Uchiro, H.; Kobayashi, S. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 2783.
7. Physical data of synthesized compounds: **4a**: ^{1}H NMR ($CDCl_3$, 300MHz) δ 1.91 (s, 3H), 3.68 (s, 3H), 3.72 (s, 3H), 3.82 (s, 3H), 3.82 (s, 3H), 6.51 (d, 1H, J=16.0 Hz), 6.68 (d, 1H, J=16.0 Hz, H-7), 6.76 (d, 1H, J=7.9 Hz), 6.90 (s, 1H), 6.98 (d, 1H, J=7.8 Hz), 7.22 (dd, 1H, J=7.8, 7.9 Hz), 7.40 (s, 1H); EI–MS 318 (M^+); **4b**: ^{1}H NMR ($CDCl_3$, 300MHz) δ 1.90 (s, 3H), 3.67 (s, 3H), 3.71 (s, 3H), 3.80 (s, 3H), 3.81 (s, 3H), 6.38 (d, 1H, J=15.8 Hz), 6.66 (d, 1H, J=15.8 Hz), 6.84 (d, 2H, J=8.6 Hz), 7.31 (d, 2H, J=8.6 Hz), 7.39 (s, 1H); EI–MS 318 (M^+); **4c**: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.91 (s, 3H), 3.68 (s, 3H), 3.72 (s, 3H), 3.82 (s, 3H), 3.88 (s, 3H), 3.90 (s, 3H), 6.37 (d, 1H, J=16.0 Hz), 6.66 (d, 1H, J=16.0 Hz), 6.81 (d, 1H, J=8.3 Hz), 6.89 (d, 1H, J=1.7 Hz), 6.96 (dd, 1H, J=1.7, 8.3 Hz), 7.40 (s, 1H); EI–MS 348 (M^+); **4d**: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.90 (s, 3H), 3.66 (s, 3H), 3.71 (s, 3H), 3.81 (s, 3H), 5.94 (s, 2H), 6.52 (d, 1H, J=16.0 Hz), 6.71 (d, 1H, J=16.0 Hz), 6.81 (d, 1H, J=8.3 Hz), 6.89 (d, 1H, J=1.7 Hz), 6.96 (dd, 1H, J=1.7, 8.3 Hz), 7.18–7.40 (m, 3H); 7.40 (s, 1H); EI–MS 332 (M^+); **4e**: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.96 (s, 3H), 3.72 (s, 3H), 3.79 (s, 3H), 3.82 (s, 3H), 6.58 (d, 1H, J=15.6 Hz), 7.40 (s, 1H), 7.50 (d, 1H, J=15.6 Hz), 7.41–7.56 (m, 3H), 7.74 (d, 2H, J=8.1 Hz), 7.83 (dd, 1H, J=2.4, 7.1 Hz), 8.18 (dd, 1H, J=2.0, 7.1 Hz); EI–MS 338 (M^+); **4f**: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.94 (s, 3H), 3.72 (s, 3H), 3.74 (s, 3H), 3.83 (s, 3H), 6.65 (d, 1H, J=15.8 Hz), 6.88 (d, 1H, J=15.8 Hz), 7.38–7.48 (m, 2H), 7.44 (s, 1H), 7.56 (dd, 1H, J=1.8, 8.5 Hz), 7.71–7.82 (m, 4H); EI–MS 338 (M^+).
8. Otoguro, K.; Kohana, A.; Manabe, C.; Ishiyama, A.; Ui, H.; Shiomi, K.; Yamada, H.; Ōmura, S. *J. Antibiotics* **2001**, *54*, 658.