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Design and synthesis of β-methoxyacrylate analogues via click chemistry and biological evaluations

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Abstract—A library of potential antifungal triazole-modified β-methoxyacrylate analogues was designed and synthesized via a Cu(I)-catalyzed 1,3-dipolar alkyne-azide coupling reaction or 'click chemistry'. Subsequent biological screening revealed that some compounds displayed low to moderate antifungal activity toward pathogenic fungi and low phytotoxicity.

The Strobilurins are an important class of natural products produced by higher fungi that possess potent and effective antifungal activity.[1] These naturally occurring antibiotics such as strobilurin A (**1**) share a β-methoxyacrylate moiety as a common and critical structural element. This family of compounds binds specifically at Q_0 site of the mitochondrial cytochrome bc_1 complex and consequently inhibits the mitochondrial respiratory chain.[2] There has been extensive industrial development of these compounds and their analogues for use as agricultural chemicals,[3] including azoxystrobin (**2**) and kresoxim-methyl (**3**). These two compounds are the most significant examples of these structural analogues as they are able to control a wide range of economically important fungal pathogens[3] (Fig. 1).

Structurally, the synthetic β-methoxyacrylates are composed of three portions (Fig. 2):[1] (1) the pharmacophore, which is essential for antifungal activity; (2) the side chain, which is necessary for an optimal lipophilicity and can be modified in a broad range; and (3) the aromatic bridge, which connects the pharmacophore with the side chain, providing the required geometry for the biological activity and photo-stability.

During the past five years, the Huisgen 1,3-dipolar cycloaddition reaction (Scheme 1), or click chemistry, a concept introduced by Sharpless, has been experiencing a growing popularity in biomedical and drug research.[4] Generally, this reaction has the following advantages: (1) high product yield without the need

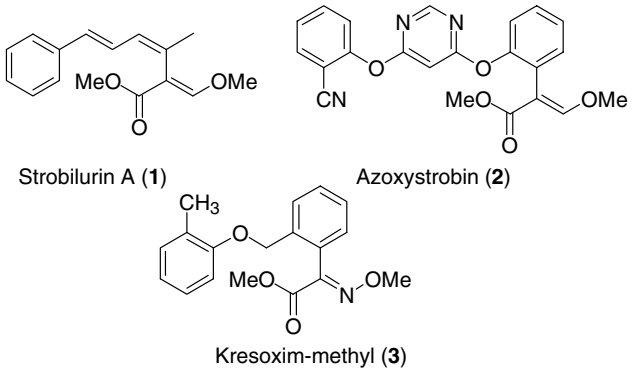

Figure 1. Structures of naturally occurring Strobilurins and synthetic analogues.

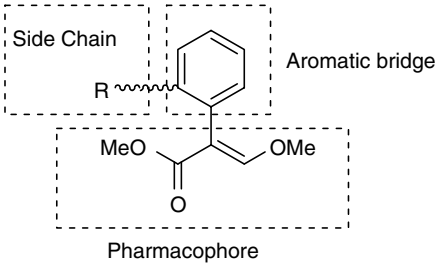

Figure 2. General structure for β-methoxyacrylates.

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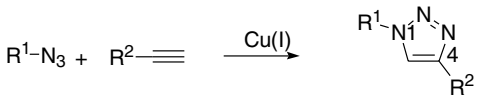


Scheme 1. Cu(I)-catalyzed Huisgen [2 + 3] cycloaddition.

for chromatographic purification; (2) inoffensive by-products; (3) mild reaction conditions, operating in a water–alcohol system; and (4) the azido group and alkyne are tolerant to most chemical manipulations. Therefore, this transformation is especially useful for drug discovery since it is a reliable linking reaction. Moreover, the triazole moiety usually has favorable physicochemical properties. It often interacts with the biological target through hydrogen bonding and dipole interactions. Approaches based on 'click chemistry' were shown to be a highly versatile and effective strategy for the synthesis and identification of biologically active leads with a number of biological activities,[5] such as antitumor,[5a] antibacterial,[5b] and antiviral activities;[5c] metalloprotease,[5d] HIV protease,[5e,f] sulfotransferase,[5g] fucosyltransferase,[5h] protein tyrosine phosphatase,[5i] and acetylcholinesterase inhibitors.[5j] However, the application of this approach to the modification of antifungal β-methoxyacrylates has not been explored.

Herein, we describe, for the first time, a 'click chemistry' protocol for the rapid synthesis of a small library of β-methoxyacrylate antifungal antibiotic analogues. Our library design was based on the general structure of β-methoxyacrylates (Fig. 3). The pharmacophores and aromatic bridges were preserved and the side chain was attached to the aromatic bridges via a triazole ring. Presumably, our strategy has the following advantages: (1) the linkage by a triazole ring allows a parallel synthesis strategy for the analogues, thus facilitating the library synthesis; (2) the [1,2,3]-triazoles are stable to acidic and basic hydrolysis as well as oxidation and reduction, thus providing ideal stability for the analogues; (3) the triazole moiety is capable of participation in hydrogen bonding, dipole–dipole, and π-stacking interactions, thus improving both the potency and specificity of the resulting analogues.

In order to test the hypothesis that click chemistry could be feasibly used for the rapid parallel synthesis of β-methoxyacrylate analogues, a library of 110 triazole-modified analogues was designed. The library's diversity was insured through the use of five azides, with varied

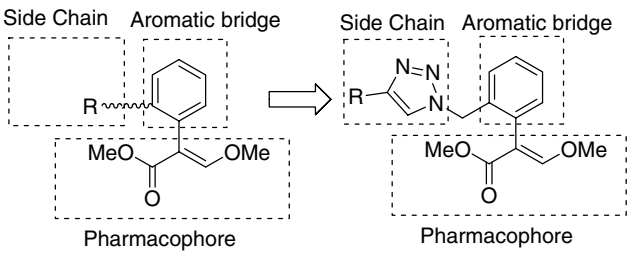


Figure 3. Design of triazole-modified β-methoxyacrylates.

pharmacophores and aromatic bridges (Fig. 4), and 22 commercially available alkynes as the side chain (Fig. 4). When devising a suitable azide building block, two key criteria were considered: (1) that the pharmacophore and aromatic bridge were retained without major variations, and that (2) the attachment of the azido group to the aromatic bridge was facile. Both criteria were fulfilled by the method shown in Scheme 2. The bromides **4**[6] and **6**[7] were synthesized based on published methods. The desired azides **5** and **7** were prepared in high yield by nucleophilic substitution of bromides **4** and **6** with sodium azide in DMSO at room temperature[8] (Scheme 2). Therefore, a total of five different azides were synthesized. Additionally, 22 alkynes with a variety of substituted aromatic rings and cycloalkenes were obtained commercially.

Next, the 110-member β-methoxyacrylate library was assembled using 'click chemistry' (Fig. 5). Each of the five azides was mixed with each of the 22 alkynes (in slight excess; see Supporting information for details) in a *t*-BuOH/H_2O solution, followed by the addition of catalytic sodium ascorbate and $CuSO_4 \cdot 5H_2O$. The 'click reaction' proceeded very well at room temperature. The resulting products were extracted with ethyl acetate. All the products were submitted to MS analysis to verify their authenticity. Indeed, all the mass spectra were consistent with the anticipated product structure (see Supporting information for details). The purity of each member was then assessed by HPLC. The results showed that all the products were over 70% pure (see Supporting information for details).

The 110-member library was screened for antifungal activity in a bioassay consisting of 3-mm diameter mycelial plugs of *Verticillium lamellicola* incubated in 50 μg/mL crude products in Tinline minimal medium agar. In this screening, fifteen products displayed growth inhibition of the fungus comparable to that seen in 50 μg/mL azoxystrobin (a commercial fungicide) (see Supporting information for details). All the 15 crude products were then purified by flash column chromatography. The purified compounds and, as controls, the 10 alkyne building blocks of these compounds were then retested for their effect on the mycelia growth of *V. lamellicola* (see Table 1 for details).

The resulting bioassay revealed that four compounds from the library with a chlorine atom on the aromatic bridge displayed the greatest activity (see Fig. 6 for structures and Table 1 for bioassay results). Of the alkyne starting material, only one, B18, ethynyl *p*-tolyl sulfone (B18) showed very strong fungicidal activity at the concentration of 50 μg/mL (see Table 1), resulting in complete inhibition.

The minimum inhibitory concentration (MIC) of the four compounds (A3B1, A3B13, A3B18, and A3B20) and ethynyl *p*-tolyl sulfone (B18) was analyzed in 96-well plates with 1-mm diameter mycelia pieces incubated in varying concentrations of compounds in Czapek Dox minimal medium. The MIC was defined as the lowest concentration at which no mycelial growth was evident.

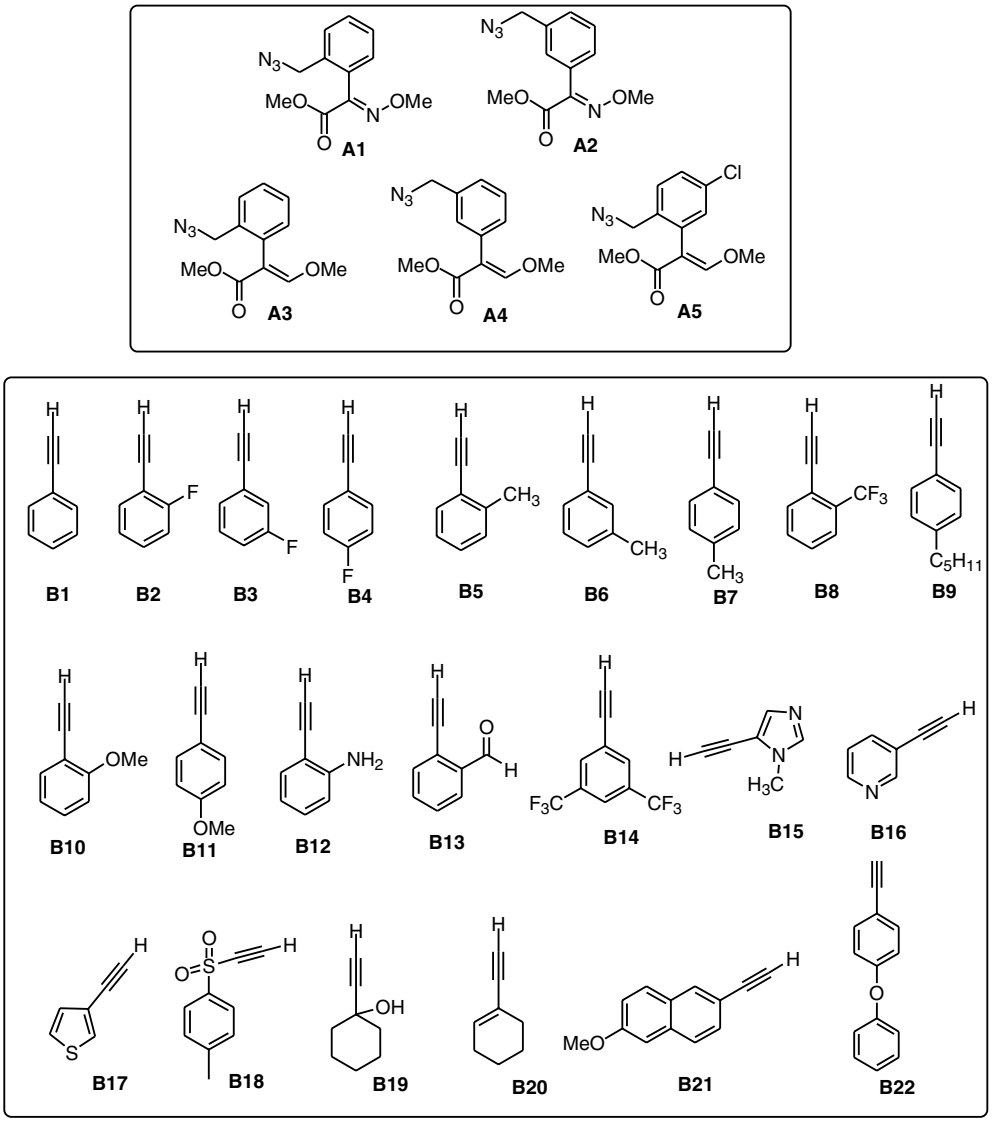
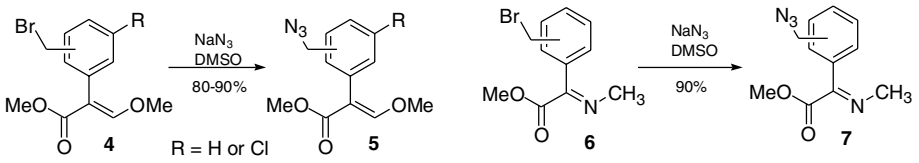


Figure 4. Designed azide and alkyne building blocks.



Scheme 2. Synthesis of azide building blocks.

The fungi tested were *Alternaria brassiceae*, *A. brassicola*, *Aspergillus flavus*, *Fusarium oxysporum*, and *V. lamellicola*. The bioassay results showed that all four triazole-modified compounds displayed low to moderate antifungal activities (see Table 2). Surprisingly, the alkyne B18 (ethynyl *p*-tolyl sulfone) showed excellent antifungal activity (MIC = 4–5 μg/mL) toward the five tested fungi. Although, ethynyl *p*-tolyl sulfone was previously reported to be fungicidal, the activity was very weak toward the tested fungus *Aspergillus niger* (MIC = 3400–10,000 μg/mL).[9] In terms of its structural simplicity and excellent activity toward plant pathogenic fungi, ethynyl *p*-tolyl sulfone should be considered as a good lead for further study. Experiments are now underway to elucidate the mode of action.

Finally, the phytotoxicity of the four identified triazole-modified methoxyacrylates and ethynyl *p*-tolyl sulfone was evaluated and compared to that of the commercial fungicide azoxystrobin by a seed germination assay on *Raphanus sativus*. As shown in Table 3, all four triazole-modified methoxyacrylates had little or no inhibitory effect on the seed germination and plant growth. Further, ethynyl *p*-tolyl sulfone (B18) treatment resulted

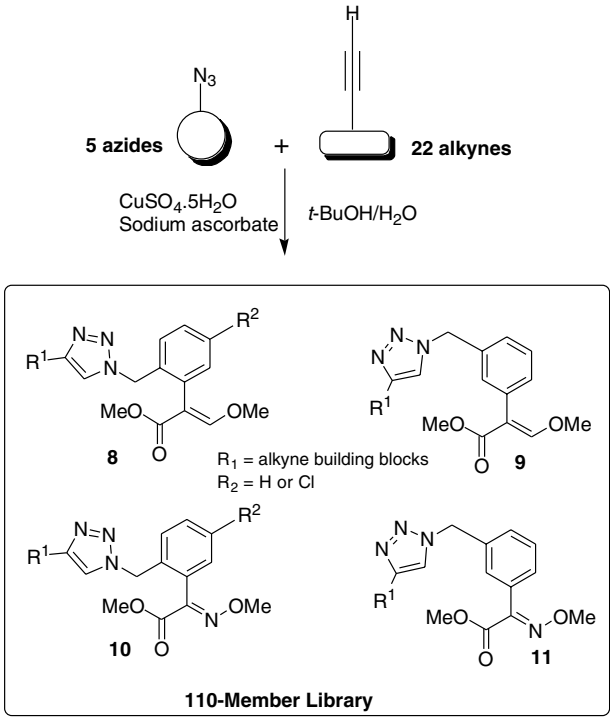


Figure 5. 110-Member library synthesis.

Table 1. Effect on growth of *Verticillium lamellicola* by 50 μg/mL of triazole-modified methoxyacrylates and alkyne building blocks

Sample[a]	Diameter[b]	Sample[a]	Diameter[b]	Sample[a]	Diameter[b]
B1	9.0	A1B8	9.0	A3B20[e]	6.5
B4	9.5	A1B11	9.0	A4B8	8.5
B8	8.5	A1B18	9.5	A4B18	9.0
B11	10	A2B4	9.0	A5B12	8.5
B12	9.0	A2B16	8.5	A5B18	9.0
B13	8.5	A2B18	9.0	Azox[c]	6.5
B16	8.5	A2B19	9.5	DMSO[d]	9.5
B18[e]	0.0	A3B1[e]	7.5		
B19	10.0	A3B13[e]	7.5		
B20	9.0	A3B18[e]	8.0		

[a] See Figures 4 and 6 for compound composition.
[b] Total diameter (mm) including 3 mm plug inoculum.
[c] Azoxystrobin (a commercial fungicide) was used for comparison.
[d] DMSO was used as control.
[e] The activity was comparable with azoxystrobin.

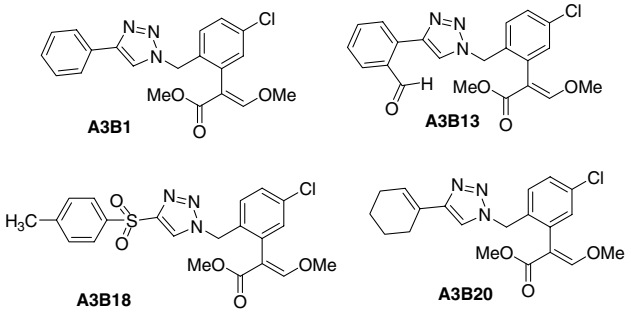


Figure 6. Antifungal compounds identified from screening.

Table 2. Minimum inhibitory concentrations of selected compounds for a representative group of pathogenic fungi

Fungus	A3B1[a]	A3B13[a]	A3B18[a]	A3B20[a]	B18[a]
Alternaria brassiceae	1	1	0.4	1	0.004
Alternaria brassicola	1	1	0.5	1	0.004
Aspergillus flavus	>1.5	1.5	0.5	>1.5	0.004
Fusarium oxysporum	1	1	0.5	1.5	0.004
Verticillium lamellicola	1.5	1	0.5	1.5	0.004

[a] Concentrations are given in mg/mL.

Table 3. Effect on growth of *Raphanus sativus* of four identified triazole-modified methoxyacrylates and ethynyl *p*-tolyl sulfone at 50 μg/mL

Compound	Length of hypocotyl[a] (mm)	Std. dev.
Control[b]	31.0	±10.2
A3B1	18.8	±8.3
A3B13	25.0	±9.1
A3B18	30.7	±12.9
A3B20	14.0	±10.2
B18	56.2	±2.2
Azoxystrobin	2.0	±2.9

[a] Values are means of six seedlings for each compound.
[b] 0.25% DMSO.

in growth promotion. In contrast, the commercial fungicide azoxystrobin showed extensive growth inhibition.

The structure–activity relationship (SAR) of β-methoxyacrylate analogues has been well studied through variation of the pharmacophore, side chain, and aromatic bridge.[1,10–12] In our study, although general SAR of the compounds is not evident, the bioassay results still provide some information for further structural modification. Briefly, the presence of the side chain in the ortho-position of the bridge ring, as in **8** and **9** (Fig. 5), is more favorable than that of the connection to the meta-position, as in **10** and **11** (Fig. 5). This result is consistent with the previous observation.[10] After introducing the [1,2,3]-triazole moiety to the side chain, only weak to moderate potency was observed, which is 5- to 10-fold lower than that of azoxystrobin. This may be due to the removal of the ether linkage in the molecule, resulting in the loss of side chain flexibility. Interestingly, introduction of a chlorine atom to the aromatic bridge exhibited enhanced efficacy.

In summary, for the first time a small library of β-methoxyacrylate analogues was designed and synthesized using the 'click chemistry' approach. Compounds synthesized by this method are of high quality, allowing for simple purification and screening in a high-throughput manner. Our study identified four candidate hits which had low to moderate inhibitory activity against five pathogenic fungi. Those compounds did not harm seed germination or plant growth. Our strategy therefore lays the cornerstone for the future exploration of more potent and selective β-methoxyacrylate fungicides. Moreover, a potent fungicide for plant pathogens was identified. This compound should be a good lead for further research and development.

Acknowledgments

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Supplementary data

Experimental procedures and characterization data (^{1}H, ^{13}C NMR and MS); HPLC, MS, and library screening bioassay data. Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2007.01.021.

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