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Promiscuous acylase-catalyzed aza-Michael additions of aromatic N-heterocycles in organic solvent

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Abstract—A novel and efficient enzymatic promiscuous protocol for aza-Michael addition of aromatic N-heterocycles to α,β-unsaturated compounds has been described. The reactions were catalyzed by promiscuous zinc-active-site acylase in organic solvent at 50 °C. The strategy works with a broad range of N-heterocycles to afford the corresponding Michael adduct with good yields in several hours (0.5–6 h). This catalytic promiscuity is the first example of metal-active-site enzyme-catalyzed aza-Michael addition for aromatic N-heterocycles.

The synthesis of biologically interesting compounds is an area of growing interest in organic and in some of bio-organic chemistry. N-Substituted aromatic N-heterocyclic compounds, such as imidazoles, triazoles, pyrazoles, pyrimidines and their derivatives obtained through aza-Michael addition are pharmacologically active and may be applied as potential therapeutic alternatives.[1]

In general, the aza-Michael addition requires basic conditions or acidic catalysts that may lead to environmentally hazardous residues or undesirable side-products.[2] To avoid typical disadvantages resulting from the presence of such catalysts, a great number of alternatives have been studied in the past few years, such as $CeCl_3$,[3a] $InCl_3$,[3b] $Yb(OTf)_3$,[3c] $Bi(OTf)_3$,[3d] $Cu(OTf)_2$,[3e] $LiClO_4$,[3f] β-cyclodextrin,[3g] polyethylene glycol,[3h] heterogeneous solid acids,[3i] ionic liquids,[3j–l] and water.[3m] Recently, several serine-active-site hydrolases were found to be able to catalyze aza-Michael additions. Wild-type[4a] and the Ser105Ala mutant of CAL-B[4b] could catalyze the Michael-type addition of thiol and amine nucleophiles to a range of α,β-unsaturated carbonyl compounds. Unfortunately, these works were focused on the enzymatic Michael addition of aliphatic amines and the related reports about aromatic N-heterocycles were rare.

In our previous work, we examined the aza-Michael addition of aromatic N-heterocycles catalyzed by serine-active-site hydrolases[5] and found the reaction usually requires one or more days. In this Letter, we surveyed the aza-Michael additions of zinc-active-site acylases and surprisingly found that two zinc-active-site acylase, D-aminoacylase 'Amano' from *Escherichia coli* (DA) and acylase 'Amano' from *Aspergillus oryzae* (AA), could catalyze the reaction of aromatic N-heterocycles in several hours. This catalytic promiscuity is the first example about metal-active-site enzyme-catalyzed aza-Michael addition for aromatic N-heterocycles. Under the optimal conditions, Michael derivatives of diversified pentacyclic N-heterocycles and pyrimidines were successfully obtained under mild conditions.

Acylase-catalyzed Michael addition of imidazole and α,β-unsaturated carbonyl compounds in organic medium has been achieved and it is shown in Scheme 1. Eighteen derivatives of aromatic N-heterocycles have been obtained in moderate to high yields. The results are summarized in Tables 3 and 4. The Michael addition of 4-nitro-imidazole (**1a**) and methyl acrylate (**2a**) was first examined. When 0.2 mmol **1a** and 2 equiv of **2a** were added to 2 ml DMSO containing 10 mg DA, a single product was prepared in 95% isolated yield at 50 °C after 24 h. A similar result was observed for AA. The structure of the additional compounds was confirmed by IR, ^1H NMR, ^{13}C NMR, and ESI-MS. We also monitored the formation of the product by HPLC and it is worthwhile to mention that no byproduct was detected

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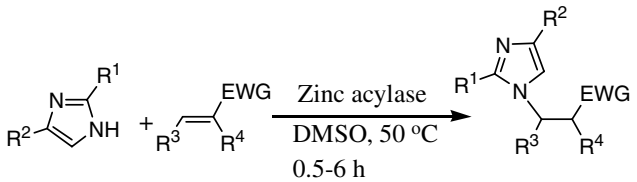


Scheme 1. Acylase-catalyzed Michael addition of imidazole and α,β-unsaturated carbonyl compounds in organic solvent.

resulting from acylation reaction, hydrolytic reaction or other reactions.

We designed some control experiments to demonstrate the catalytic specificity of DA and AA. The reaction yields and the initial reaction rates were compared, which are summarized in Table 1. From this study, the addition without any catalyst led to a very low yield (<0.5%) even after 24 h. In contrast, more than 95% yield was obtained in the presence of 10 mg DA or AA. The initial reaction rates of these cases were up to 333-fold and 638-fold faster than the process in absence of a biocatalyst, respectively. The initial rate is practically proportional to the enzyme amount.

When the enzyme (AA) is pre-treated with urea at 100 °C in order to completely denaturalize the protein, the rate is practically equal to the bovine serum albumin (BSA), suggesting that the tertiary structure of the biocatalyst is necessary to catalyze the reaction. Then control reactions were run with inhibited DA and AA by adding 50 mM non-competitive inhibitor $ZnCl_2$.[6] The inhibited enzyme did not show any acylase activity to catalyze the hydrolysis of *N*-acetyl-D-methionine, and the specific activity for the Michael addition was as that of BSA protein. All these results suggest that the tertiary structure and the specific active sites of zinc-acylase are responsible for the Michael addition reaction. Comparing with serine-active-site hydrolyses such as *Candida antarctica* lipase B (CAL-B), alkaline protease from *Bacillus subtilis* (PA) and Amano Lipase M from *Mucor javanicus* (MJL), zinc-active-site acylase could catalyze the reaction more efficiently. Among the two acylase, AA could catalyze the enzymatic Michael addition more efficiently. Thus AA was selected for the next steps.

Table 1. Michael addition between 4-nitro-imidazole (**1a**) and methyl acrylate (**2a**) in the presence of different catalysts

Catalyst	Time (h)	Yield[a] (%)	V_0 (M m min^{-1})	V_r[b]
No catalyst	24	0.4	4.2×10^{-5}	1.0
AA	6	98.2	0.027	638
DA	6	97.5	0.014	333
DA[c]	6	92.7	0.007	165
DA denatured[d]	24	8.7	5.1×10^{-4}	13
BSA	24	6.7	4.6×10^{-4}	10.9
DA inhibited[e]	24	6.0	5.0×10^{-5}	1.2
CAL-B	24	66.8	0.004	95
MJL	24	73.6	4.6×10^{-3}	110
PA	24	53.4	2.6×10^{-3}	63

[a] Experimental conditions: 0.3 M imidazole (4-nitro-imidazole), 0.6 M methyl acrylate, 10 mg enzyme, 2 ml DMSO, 50 °C, 6 h. All yields were detected by HPLC.
[b] Relative initial reaction rate to the reaction in the absence of enzyme.
[c] DA 2.5 mg ml^{-1}.
[d] Enzyme predenatured with urea at 100 °C for 24 h.
[e] Enzyme inhibited by 50 mM $ZnCl_2$.

Table 2. Solvents screening for enzymatic Michael addition[a]

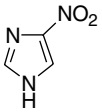
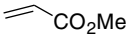
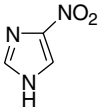
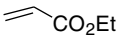
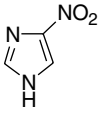
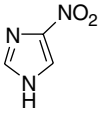
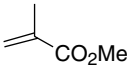
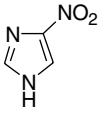
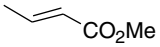
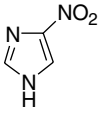
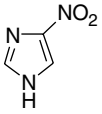
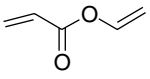
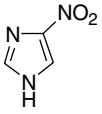
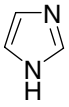
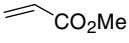
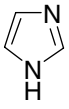
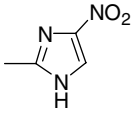
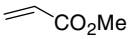
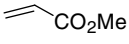
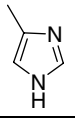
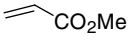
Solvents	log *P*	Yield (%) of imidazole[b]	Yield (%) of 4-nitro-imidazole[b]
DMSO	−1.3	94	97
DMF	−1.0	92	97
Dioxane	−0.5	55	n.d.
THF	0.46	70	n.d.
Pyridine	0.65	60	n.d.
Isopropyl ether	1.9	69	n.d.
Toluene	2.6	77	n.d.
n-Hexane	3.9	93	n.d.

[a] Experimental conditions: 0.3 M imidazole (4-nitro-imidazole), 0.6 M methyl acrylate, 15 mg AA, 1 ml solvents, 50 °C, 6 h.
[b] All yields were determined by HPLC. n.d. means no reaction was found.

Reaction media is an important factor in the enzymatic reaction. We examined the enzymatic Michael addition in different organic solvents with log *P* value ranging from −1.3 to 4.9 and the results are shown in Table 2. The log *P* value of the solvents is the widely used parameter to describe solvent polarity and their possible effects on the enzyme activity, where *P* is the partition coefficient of a given solvent between water and *n*-octanol in a two-phase system.[7] It was found that the reaction only took place in DMSO and DMF. No obvious reaction was detected in other solvents. This may be attributed to the low solubility of 4-nitro-imidazole. In order to exclude the influence, we examined the Michael addition of imidazoles (**1b**) and **2a**. The results are also shown in Table 2. Similar results were observed in DMF and DMSO. In other solvents, the Michael adducts could also be obtained in moderate to high yield. To our surprise, those solvents with a higher log *P* value exhibited higher conversion. The reaction proceeded smoothly and good yield was obtained in non-polar solvents such as *n*-hexane. Based on the good yield and substrate solubility, DMSO was selected in the following reactions.

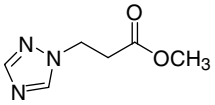
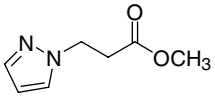
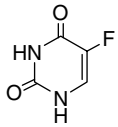
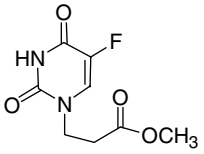
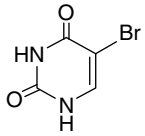
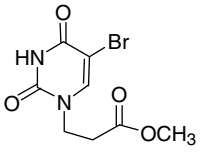
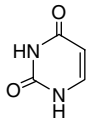
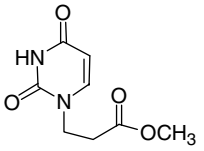
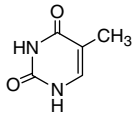
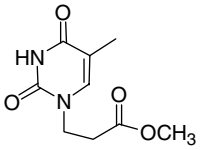
The scope of this method was investigated with a variety of structurally diverse imidazoles and α,β-carbonyl compound under Michael additions to generate the corresponding derivatives in moderate to good yields. The results are summarized in Table 3. The reactivity decreased with increasing chain length of the acceptor, the longer chain the acrylates had, the lower the yield was obtained. Apart from acrylate ester, acrylonitrile could also react with imidazoles in good yields. Vinyl acrylate and butenone showed higher reactivity than methyl acrylate and ethyl acrylate. In order to extend the scope of this method, α,β-substituted Michael acceptors like methyl α-methylacrylate and methyl crotonate were tested under the same conditions. Both of the

Table 3. Michael addition of imidazoles to α,β-carbonyl compound promoted by AA[a]

Entry	Imidazole	α,β-Carbonyl compound	Time (h)	Yield[b] (%)
1			3	(**3a**) 96
2			3	(**3b**) 90
3			24	(**3c**) 19
4			24	(**3d**) 16
5			24	(**3e**) 37
6			3	(**3f**) 90
7			0.5	(**3g**) 85
8			0.5	(**3h**) 82
9			3	(**3i**) 83
10			3	(**3j**) 75
11			6	(**3k**) 68
12			6	(**3l**) 82
13			6	(**3m**) 83

[a] Reactions were carried out on 0.3 mmol scale of substrate with 2 equiv acceptor in 1 ml DMSO at 50 °C, 15 mg AA was added.
[b] Isolated yields.

Table 4. Michael addition of other aromatic N-heterocycles with methyl acrylate catalyzed by AA[a]

Entry	Substrate	Product	Time (h)	Yield[b] (%)
1			3	(**5a**) 95
2			3	(**5b**) 56
3			3	(**5c**) 97
4			3	(**5d**) 91
5			3	(**5e**) 93
6			3	(**5f**) 90

[a] Reactions were carried out on 0.3 mmol scale of substrate with 2 equiv acceptors in 1 ml DMSO at 50 °C, 15 mg AA was added.
[b] Yields was determined by HPLC.

acceptors showed rather lower activity because of the strong steric hindrance. To our disappointment, no enantioselectivity could be observed for the above tested reactions with the catalysis of DA or AA. The reason for this will be further investigated.

Comparable behavior was observed using imidazole as substrate. The four substituted imidazoles examined underwent Michael addition with methyl acrylate favorably and all substituted imidazoles could be obtained from moderate to good yields. The reactivity decreased by the following order: 4-nitro-imidazole, imidazole, and 4-methyl-imidazole. The reactivity was in accordance with the nucleophilicity of imidazole derivatives. Substituted imidazoles with strong steric hindrance, such as 2-methyl-4-nitro-imidazole, underwent Michael addition much more slowly.

Having obtained favorable results with imidazoles, we then examined the addition of other N-heterocycles to methyl acrylate. Other five membered N-heterocycles such as pyrazole and triazole also exhibited good Michael addition activity. Triazole reacted faster due to its stronger nucleophilicity than pyrazole. More complicated N-heterocycles, such as pyrimidines, can also be used as a substrate to obtain the corresponding Michael adducts. All the substituted pyrimidines derivatives were obtained in high yield. Among the examined pyrimidines, fluorouracil, and bromouracil reacted faster than uracil and thymine. It is worthwhile to mention that only *N*-1 adducts were observed (monitored by ^{1}H NMR and HPLC). This result reveals that AA exhibited higher regioselectivity compared to the chemical transformation.

In this study, a facile and an efficient biotransformation method to perform aza-Michael addition of aromatic N-heterocycles has been developed by utilizing two promiscuous zinc-active-site acylases as biocatalysts. The structural influence of the Michael donor and acceptor was systematically studied. This strategy has expanded the application of catalytic promiscuities in organic synthesis. The obtained N-heterocyclic derivatives may be potentially bioactive and could be used as pharmacological alternatives.

Acknowledgments

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References and notes

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