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Diastereoselective enzymatic synthesis of highly substituted 3,4-dihydropyridin-2-ones via domino Knoevenagel condensation–Michael addition–intramolecular cyclization

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ABSTRACT

A direct method to construct 3,4-dihydropyridin-2-ones by enzymatic condensation of aldehyde with cyanoacetamide and 1,3-dicarbonyl compounds was developed. One ring and four new bonds (two C–C, one C–N, one C=C) were formed in one pot. And reaction conditions involving hydrolases, solvents, substrate molar ratios, and hydrolase loading were optimized. A series of new compounds based on the 3,4-dihydropyridin-2-one core were synthesized by the unprecedented three-component domino reaction.

1. Introduction

The synthesis of the 3,4-dihydropyridin-2-ones moiety is an interesting field because its derivatives have exhibited significant biological and pharmacological activities, such as antibacterial,[1] antifungal,[2] and antitumor,[3] also served as HIV-1 specific reverse transcriptase inhibitors.[4] Compounds **1** and **2** (Fig. 1) have been found to show hypolipidemic and 5α-reductase inhibitory activities, respectively. Due to their important medicinal activities, the quick and efficient preparation of this scaffold has drawn much more attention of chemists. So far, some methods have been invented. Pendleton et al.[5] found that the 3,4-dihydropyridin-2-one could be gotten as the byproduct through the reaction of hydrazone with methyl 3-aminocrotonate in hot acetic acid, the yield was only 22%. Yang et al.[6] synthesized the oxirane-containing enamide from the cross-coupling reaction of oxiranecarboxamide with (*E*)-(2-bromovinyl)benzene followed by N-methylation, then in the presence of trifluoroacetic acid and molecular sieve (4 Å), the oxirane-containing enamide underwent 6-*endo*-enamide–epoxide cyclization reaction to produce 3,4-dihydropyridin-2-one derivatives. In recent decades, multicomponent reactions (MCRs) have represented a fascinating tool in organic synthesis owing to the fact that sophisticated products can be synthesized efficiently just by mixing three or more reactants in a single vessel. For example, Paravidino et al.[7]generated the 1-azadiene intermediate in situ via a Horner–Wadsworth–Emmons (HWE) reaction of phosphonates, nitriles, and aldehydes in tetrahydrofuran, and at −78 °C, then the 1-azadiene was trapped by α-aryl isocyanoacetates to form 3,4-dihydropyridin-2-ones. Noguez et al.[8]exposed the mixture of Meldrum's acid, methyl or ethyl acetoacetate, substituted benzaldehyde and ammonium acetate to infrared irradiation for 3 h, a series of 3,4-dihydropyridin-2-ones were gotten in moderated yields (50–75%). Despite several reports have appeared for the synthesis, the importance of this class of molecules means that new method must always be exploited, in particular the mild, efficient, and environmentally friendly entry to this heterocycle.

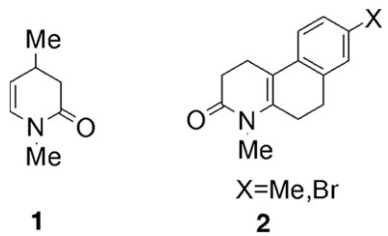

Fig. 1. Compounds with 3,4-dihydropyridin-2-one core.

The pioneered work by Klibanov in the early 1980s initiated nonaqueous enzymology.[9] During the following decades, it was found that they can not only catalyze the 'natural' reactions but also

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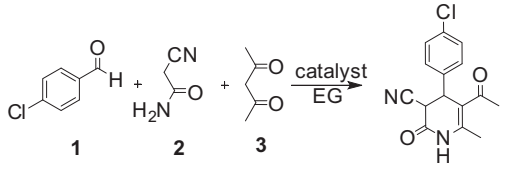
have one or more secondary activities,[10] which defined the catalytic promiscuity of enzymes. Enzymatic promiscuity largely expands the application of enzymes in organic synthesis. For example, Berglund et al. reported the unprecedented carbon–carbon bond formation by an engineered mutant of CAL-B.[11] Seebeck and Hilvert reported that a mutated PLP-dependent racemase could catalyze the aldol reaction.[12] Also, our group reported that D-aminoacylase from *Escherichia coli* catalyzed the Michael addition of 1,3-dicarbonyl compounds to methyl vinyl ketone in organic solvent.[13] MCRs are important for generating high levels of diversity and give rise to complex structures by simultaneous formation of two or more bonds. However, MCRs catalyzed by natural enzymes were very rare, especially the construction of novel heterocycles.[14] Here, we surprisingly discovered a zinc-dependent acylase showed the promiscuous property to form the 3,4-dihydropyridin-2-one ring by the three-component reaction among aldehydes, the cyanoacetamide and 1,3-dicarbonyl compounds in nonaqueous solvent.

2. Results and discussion

Initial endeavor was focused on the search of an efficient enzyme for the one-pot three-component condensation of 4-chlorobenzaldehyde, cyanoacetamide, and acetyl acetone at 50 °C for 24 h in ethylene glycol (EG). The results are summarized in Table 1.

Table 1
Optimization of reaction conditions using different enzymes[a]



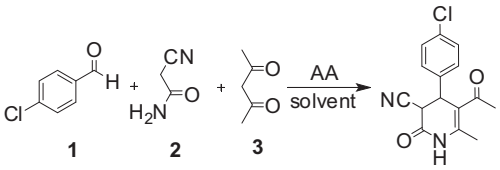
Entry	Catalyst	Yield[b] (%)
1	—	Trace
2	AA[c]	16
3	BSA	17
4	DA	34
5	AA	34
6	MJL	30
7	PGA	5
8	AKL	6
9	CRL	14

[a] Reaction conditions: **1** (0.5 mmol), **2** (0.5 mmol), **3** (0.5 mmol), catalyst (30 mg) in 1 mL EG, 50 °C, 24 h.
[b] Determined by HPLC.
[c] Denatured by urea at 100 °C for 12 h.

As shown in Table 1, the reaction almost could not proceed without enzyme (Table 1, entry 1). Gratifyingly, the yield in the presence of D-Aminoacylase (DA) or Acylase 'Amano' (AA) increased to 34% (Table 1, entries 4 and 5). Amano Lipase M from *Mucor javanicus* (MJL) also showed activity toward the reaction (Table 1, entry 6). While, immobilized penicillin G acylase from *E. coli* (PGA), Lipase AK 'Amano' (AKL) and Lipase from *Candida rugosa* (CRL) had very low catalytic activity (Table 1, entries 7–9). Considering the cost of enzyme, we chose AA as the catalyst. Also, denatured AA or bovine serum albumin (BSA) had a little catalytic effect (Table 1, entries 2 and 3). Finally, AA acted as the catalyst in the following exploration.

With optimal catalyst in hand, different organic solvents were screened to investigate the effect on the reaction. As described in Table 2, the efficiency of solvents was of overwhelming distinction. EG and Di-EG were extremely superior to others (Table 2, entries 3 and 6). Only very poor yield was obtained when EG was substituted by Dimethyl sulfoxide (DMSO), Dimethylformamide (DMF), Glycerol or 1,4-butylene glycol (Table 2, entries 1, 2, 7, and 8).

Table 2
Optimization of reaction conditions using different solvents[a]



Entry	Solvent	Yield[e] (%)
1	DMSO	8
2	DMF	Trace
3	EG	33
4[b]	EG	40
5[c]	EG	55
6[d]	EG	81
7	Di-EG	26
8	Glycerol	3
9	1,4-Butylene glycol	7

[a] Reaction conditions: **1** (0.5 mmol), **2** (0.5 mmol), **3** (0.5 mmol), AA (30 mg) in organic solvent (1 mL), 50 °C, 24 h.
[b] Compound **1** (0.5 mmol), **2** (1.0 mmol), **3** (0.5 mmol), AA (30 mg) in organic solvent (1 mL), 50 °C, 24 h.
[c] Compound **1** (0.5 mmol), **2** (1.0 mmol), **3** (0.75 mmol), AA (30 mg) in organic solvent (1 mL), 50 °C, 24 h.
[d] Compound **1** (0.125 mmol), **2** (1.0 mmol), **3** (0.75 mmol), AA (30 mg) in organic solvent (1 mL), 50 °C, 24 h.
[e] Determined by HPLC.

Meanwhile, week polar solvents, like *n*-hexane, isooctane, and acetonitrile, etc. were also checked, however, no product was detected because the cyanoacetamide failed to dissolve in them. Thus, EG was selected as the reaction solvent to test the ratio of substrates.

The molar ratio of cyanoacetamide and acetyl acetone had an impact on the yield. When the amount of compound **2** increased to 1 mmol, the yield was 40% (Table 2, entries 4 vs 3), then 0.75 mmol compound **3** was applied, the yield increased to 55% (Table 2, entries 5 vs 4 and 3). The dramatic improvement in yield from 55% to 81% was observed when compound **1** was 0.125 mmol. So the best molar ratio of substrates was compound 1 (0.125 mmol), compound **2** (1.0 mmol), and compound **3** (0.75 mmol), after 24 h the yield reached to 81% (Table 2, entry 6).

A survey of catalyst loading was made and the result was shown in Fig. 2. In general, the reaction yield was proportional to the amount of enzyme. When 40 mg enzyme was added to the reaction mixture, the yield was 93%. While the yield scarcely changed along with further increase of enzyme (from 93% to 87%).

Consequently, considering the overall effects of catalyst, solvents, substrate ratio, catalyst loading, and further studies on the exploration of substrate scopes and limitations were carried out. As

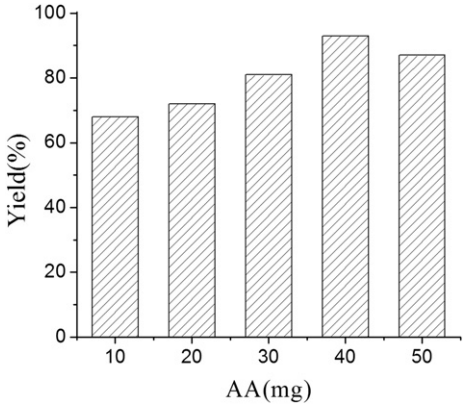
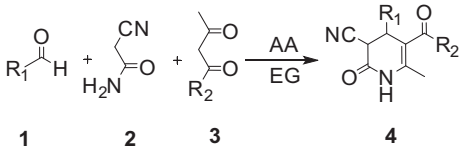


Fig. 2. Influence of catalyst loading on the yield after 24 h.

shown in Table 3, the enzyme-catalyzed one-pot condensation of various aldehydes, 1,3-dicarbonyl compounds and cyanoacetamide proceeded efficiently to desired 3,4-dihydropyridin-2-ones in moderate to excellent yields mostly. It appeared that the aromatic ring could participate in the process irrespective of the electronic nature and the substitution pattern, for example, aromatic systems that possessed substitutions at the *ortho*, *meta* or *para* positions had the yield without substantial loss from 82% to 99% (Table 3, entries 2–4), however, the yield of aromatic aldehydes bearing electron-withdrawing group was higher than that of the aldehydes with electron-donating group (Table 3, entries 3 and 9, 4 and 7, 8). Furthermore, heteroaromatic aldehyde also fit in good yields (Table 3, entries 10 and 19). Aliphatic aldehyde could join in the system successfully with the fact that longer carbon chain got lower yield (Table 3, entries 11 and 12). It was noteworthy that not only acetyl acetone but methyl acetoacetate and acetoacetanilide could also play the role of 1,3-dicarbonyl compounds successfully. Because the product **4** has two adjacent chiral centers, we got the mixture of diastereoisomers whose ratio could be detected in ^{1}H NMR.

Table 3
Substrate scope studies[a]



1 2 3 4

Entry	R_1	R_2	Product	Yield[b] (%)	Ratio of diastereoisomers[c]
1	Ph	Me	**4a**	44	64:36
2	2-$NO_2C_6H_4$	Me	**4b**	99	86:14
3	3-$NO_2C_6H_4$	Me	**4c**	84	86:14
4	4-$NO_2C_6H_4$	Me	**4d**	82	91:9
5	2-ClC_6H_4	Me	**4e**	59	88:12
6	4-ClC_6H_4	Me	**4f**	93	68:22
7	4-MeC_6H_4	Me	**4g**	51	86:14
8	4-$OMeC_6H_4$	Me	**4h**	42	90:10
9	3-OHC_6H_4	Me	**4i**	65	94:6
10	2-Furyl	Me	**4j**	70	95:5
11	CH_3CH_2	Me	**4k**	82	64:36
12	$CH_3CH_2CH_2$	Me	**4l**	50	78:22
13	3-$NO_2C_6H_4$	OMe	**4m**	87	94:6
14	4-ClC_6H_4	OMe	**4n**	64	90:10
15	4-MeC_6H_4	OMe	**4o**	28	83:17
16	CH_3CH_2	OMe	**4p**	35	81:19
17	4-ClC_6H_4	4-NHC_6H_5	**4q**	29	65:35
18	4-MeC_6H_4	4-NHC_6H_5	**4r**	28	74:26
19	2-Furyl	4-NHC_6H_5	**4s**	81	78:14

[a] Reaction conditions: **1** (0.125 mmol), **2** (1 mmol), **3** (0.75 mmol), AA (40 mg) in EG (1 mL), 50 °C, 24 h.
[b] Detected by HPLC.
[c] Detected by ^{1}H NMR.

To gain an insight into the reaction process, we, respectively, prepared two possible intermediates, the Knoevenagel condensation products of p-chlorobenzaldehyde with cyanoacetamide and *p*-chlorobenzaldehyde with acetyl acetone, in order to probe the mechanistic pathway in detail. We found that two condensation products could react with the third component to form the target molecular. We took the mechanism initiated by the condensation of *p*-chlorobenzaldehyde with acetyl acetone, for example (Scheme 1). The zinc ion would coordinate with one carbonyl group of acetyl acetone to increase the nucleophilicity. Then Asp366 deprived one C3–H of acetyl acetone, at the same time, the nucleophile would attack the carbonyl group of aldehyde to form the Knoevenagel condensation product. Next, the interaction of zinc ion with the carbonyl group of cyanoacetamide would enhance the nucleophilic ability of Michael donor, so intermediate **2** was formed. Then, the electron-rich N–H underwent the nucleophilic attack to the carbonyl group of acetyl acetone, intermediate **3** lost one molecular water to get the final product **4**.

3. Conclusion

In summary, we have developed a novel one-pot enzymatic method to construct highly substituted 3,4-dihydropyridin-2-ones derivatives via a three-component condensation of aldehyde, cyanoacetamide, and 1,3-dicarnonyl compound. The simple, convenient and efficient strategy described here is a useful synthetic protocol for the synthesis of potentially bioactive and pharmacological compounds. Further application of this strategy is ongoing in our laboratory.

4. Experimental section

4.1. Materials

D-Aminoacylase from *E. coli* (10,000 U/mg, 1 U is defined as enzyme quantity, which produces 1 mmol of D-Amino acid per 30 min) and Acylase 'Amano' (AA) from *Aspergillus oryzae* (≥30,000 U/g, 1 U is defined as enzyme quantity, which produces 1 mmol of L-Amino acid per 30 min) were purchased from Amano Enzyme Inc (Japan). Amano Lipase M from *M. javanicus* (≥10,000 U/g enzyme activity, pH 7.0, 40 °C). Lipase AK 'Amano' (≥20,000 U/g enzyme activity, pH 7.0, 55 °C). Immobilized penicillin G acylase from *E. coli* (EC 3.5.1.11, immobilized on acrylic beads) was purchased from Hunan Flag Biotech Co. All solvents were analytical grade and were dried by storing over activated 3 Å molecular sieves for 24 h prior to use. All other reagents were used as received.

4.2. Analytical methods

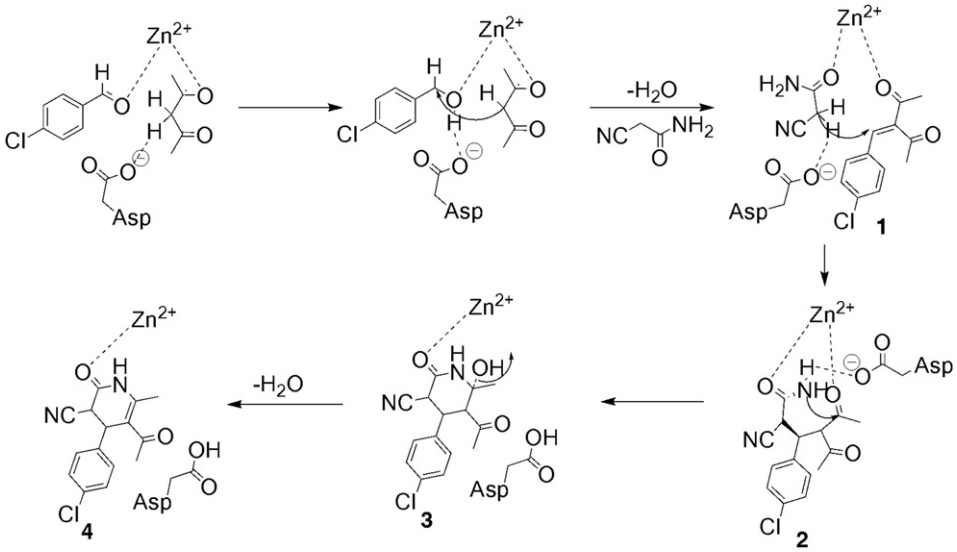
The ^{1}H and ^{13}C NMR spectra were recorded with TMS as internal standard using a Bruker AMX-400 MHz spectrometer. Chemical shifts were expressed in parts per million and coupling constants (*J*) in hertz. Analytical HPLC was performed using an Agilent 1100 series with a reversed-phase Shim-Pack VP-ODS column (150×4.6 mm) and a UV detector (290 nm). IR spectra were measured with a Nicolet Nexus FTIR 670 spectrophotometer. Melting points were determined using XT-4 apparatus and were not corrected. All the known products were characterized by comparing the ^{1}H NMR data with those reported in the literature. The structures of new compounds were confirmed by ^{1}H NMR, ^{13}C NMR, and HRMS.

4.3. General procedure for the three-component condensation of aldehyde, cyanoacetamide, and 1,3-dicarbonyl compound

A mixture of aldehyde (0.125 mmol), cyanoacetamide (1.0 mmol), 1,3-dicarbonyl compound (0.75 mmol), and AA (40 mg) was added to EG (1 mL), and it was stirred at 50 °C for 24 h. After the completion of the reaction, the mixture was separated by silica gel column chromatography using petroleum ether/ethyl acetate mixture (1/1 v/v) as eluent to afford the desired product of 3,4-dihydropyridin-2-ones.

*4.3.1. 5-Acetyl-6-methyl-2-oxo-4-phenyl-1,2,3,4-tetrahydropyridine-3-carbonitrile (**4a**).* Light yellow solid. IR (neat) (ν_{max}/cm^{-1}): 3224, 3139, 2257, 1713, 1668. ^{1}H NMR (400 MHz, $CDCl_3$): δ_H 2.08, 2.12 (s, 3H, –CH_3C=O), 2.37, 2.40 (s, 3H, –CH_3C=C), 3.72–3.73, 4.16–4.18 (d, 1H, *J*=6.4 Hz, –CHC=C), 4.32–4.34, 4.47–4.48 (d, 1H, *J*=6.4 Hz, –CHCN), 7.17–7.40 (m, 4H, Ar–H), 8.51–8.59 (s, 1H, –NH).

*4.3.2. 5-Acetyl-6-methyl-4-(2-nitrophenyl)-2-oxo-1,2,3,4-tetrahydropyridine-3-carbonitrile (**4b**).* Light yellow solid. Mp 106–107 °C. IR (neat) (ν_{max}/cm^{-1}): 3237, 3139, 2254, 1712, 1686. ^{1}H NMR (400 MHz, DMSO-d_6): δ_H 1.63, 1.66 (s, 3H, –CH_3C=O), 1.85,



Scheme 1. Proposed mechanism of lipase-catalyzed reaction of aldehyde with cyanoacetamide and 1,3-dicarbonyl compounds.

1.89 (s, 3H, $-CH_3C{=}C$), 3.77–3.78, 4.47–4.49 (d, 1H, J=7.6 Hz, $-CHC{=}C$), 4.54–4.55, 4.86–4.88(d, 1H, J=7.2 Hz, $-CHCN$), 6.82–7.61 (m, 4H, Ar–H), 10.11, 10.28 (s, 1H, –NH). ^{13}C NMR (100 MHz, DMSO-d_6): δ_C 19.4, 30.8, 35.4, 115.1, 115.6, 125.8, 128.9, 130.0, 133.0, 134.8, 148.2, 149.1, 162.8, 196.6. HRMS (EI) calcd for $C_{15}H_{13}N_3O_4$ [M^+]: 299.0906, found: 299.0910.

*4.3.3. 5-Acetyl-6-methyl-4-(3-nitrophenyl)-2-oxo-1,2,3,4-tetrahydropyridine-3-carbonitrile (**4c**).* Light yellow solid. IR (neat) (ν_{max}/cm^{-1}): 3261, 3167, 2257, 1717, 1679. ^{1}H NMR (400 MHz, DMSO-d_6): δ_H 2.03, 2.04 (s, 3H, $-CH_3C{=}O$), 2.28, 2.29 (s, 3H, $-CH_3C{=}C$), 4.21–4.22, 4.63–4.65 (d, 1H, J=6.4 Hz, $-CHC{=}C$), 4.75–4.76, 4.83–4.85(d, 1H, J=6.8 Hz, $-CHCN$), 7.60–8.15 (m, 4H, Ar–H), 10.56, 10.64 (s, 1H, –NH).

*4.3.4. 5-Acetyl-6-methyl-4-(4-nitrophenyl)-2-oxo-1,2,3,4-tetrahydropyridine-3-carbonitrile (**4d**).* Light yellow solid. Mp 105–106 °C. IR (neat) (ν_{max}/cm^{-1}): 3266, 3165, 2257, 1719, 1677. ^{1}H NMR (400 MHz, DMSO-d_6): δ_H 1.99, 2.01 (s, 3H, $-CH_3C{=}O$), 2.24, 2.25 (s, 3H, $-CH_3C{=}C$), 4.55–4.57 (d, 1H, J=6.4 Hz, $-CHC{=}C$), 4.68–4.69, 4.80–4.82 (d, 1H, J=6.8 Hz, $-CHCN$), 7.42–8.13 (m, 4H, Ar–H), 10.47, 10.58 (s, 1H, –NH). ^{13}C NMR (100 MHz, DMSO-d_6): δ_C 19.1, 30.3, 40.0, 41.0, 114.4, 115.7, 124.3, 124.4, 129.3, 129.9, 145.1, 147.7, 148.0, 162.8, 196.8. HRMS (EI) calcd for $C_{15}H_{13}N_3O_4$ [M^+]: 299.0906, found: 299.0909.

*4.3.5. 5-Acetyl-4-(2-chlorophenyl)-6-methyl-2-oxo-1,2,3,4-tetrahydropyridine-3-carbonitrile (**4e**).* White solid. Mp 220–221 °C. IR (neat) (ν_{max}/cm^{-1}): 3221, 3134, 2251, 1716, 1675. ^{1}H NMR (400 MHz, DMSO-d_6): δ_H 2.02, 2.03 (s, 3H, $-CH_3C{=}O$), 2.28, 2.31 (s, 3H, $-CH_3C{=}C$), 4.82–4.84 (d, 1H, J=6.8 Hz, $-CHC{=}C$), 4.98–5.00 (d, 1H, J=6.8 Hz, $-CHCN$), 7.11–7.52 (m, 4H, Ar–H), 10.50, 10.65 (s, 1H, –NH). ^{13}C NMR (100 MHz, DMSO-d_6): δ_C 19.1, 30.0, 37.4, 40.3, 114.8, 115.5, 128.5, 128.6, 130.4, 130.4, 133.8, 135.5, 147.8, 162.7, 196.9. HRMS (EI) calcd for $C_{15}H_{13}N_2O_2Cl$ [M^+]: 288.0666, found: 288.0664.

*4.3.6. 5-Acetyl-4-(4-chlorophenyl)-6-methyl-2-oxo-1,2,3,4-tetrahydropyridine-3-carbonitrile (**4f**).* Light yellow solid. Mp 173–174 °C. IR (neat) (ν_{max}/cm^{-1}): 3255, 3167, 2257, 1712, 1675. ^{1}H NMR (400 MHz, DMSO-d_6): δ_H 2.11, 2.14 (s, 3H, $-CH_3C{=}O$), 2.37, 2.40 (s, 3H, $-CH_3C{=}C$), 3.69–3.70, 4.17–4.18 (d, 1H, J=6.8 Hz, $-CHC{=}C$), 4.34–4.35, 4.48–4.49 (d, 1H, J=6.8 Hz, $-CHCN$), 7.12–7.36 (m, 4H, Ar–H), 8.75, 8.77 (s, 1H, –NH). ^{13}C NMR (100 MHz, DMSO-d_6): δ_C 19.0, 30.1, 40.7, 41.4, 114.7, 116.0, 129.2, 130.2, 133.3, 136.2, 147.3, 162.9, 197.1. HRMS (EI) calcd for $C_{15}H_{13}N_2O_2Cl$ [M^+]: 288.0661, found: 288.0660.

*4.3.7. 5-Acetyl-6-methyl-2-oxo-4-p-tolyl-1,2,3,4-tetrahydropyridine-3-carbonitrile (**4g**).* Light yellow solid. IR (neat) (ν_{max}/cm^{-1}): 3234, 3139, 2260, 1702, 1678. ^{1}H NMR (400 MHz, DMSO-d_6): δ_H 2.00 (s, 3H, Ar-CH_3), 2.22, 2.23 (s, 3H, $-CH_3C{=}O$), 2.27 (s, 3H, $-CH_3C{=}C$), 4.0.3–4.04, 4.37–4.38 (d, 1H, J=4.0 Hz, $-CHC{=}C$), 4.52–4.53, 4.71–4.73(d, 1H, J=4.0 Hz, $-CHCN$), 7.07–7.15 (m, 4H, Ar–H), 10.39, 10.51 (s, 1H, –NH).

*4.3.8. 5-Acetyl-4-(4-methoxyphenyl)-6-methyl-2-oxo-1,2,3,4-tetrahydropyridine-3-carbonitrile (**4h**).* Light yellow solid. IR (neat) (ν_{max}/cm^{-1}): 3257, 3172, 2210, 1713, 1608. ^{1}H NMR (400 MHz, DMSO-d_6): δ_H 2.00 (s, 3H, $-CH_3C{=}O$), 2.26, 2.27 (s, 3H, $-CH_3C{=}C$), 3.68, 3.69 (s, 3H, $-OCH_3$), 4.36–4.37 (d, 1H, J=6.4 Hz, $-CHC{=}C$), 4.50–4.51, 4.69–4.70 (d, 1H, J=6.8 Hz, $-CHCN$), 6.87–7.14 (m, Ar–H, 4H), 10.38, 10.51 (s, –NH, 1H).

*4.3.9. 5-Acetyl-4-(3-hydroxyphenyl)-6-methyl-2-oxo-1,2,3,4-tetrahydropyridine-3-carbonitrile (**4i**).* Yellow solid. Mp 249–250 °C. IR (neat) (ν_{max}/cm^{-1}): 3355, 3262, 2255, 1712, 1664. ^{1}H NMR (400 MHz, DMSO-d_6): δ_H 1.99 (s, 3H, $-CH_3C{=}O$), 2.23, 2.24 (s, 3H, $-CH_3C{=}C$), 4.29–4.30 (d, 1H, J=6.4 Hz, $-CHC{=}C$), 4.45–4.46, 4.69–4.70 (d, 1H, J=6.8 Hz, $-CHCN$), 6.57–7.13 (m, 4H, Ar–H), 9.67, 9.70 (s, 1H, –OH), 10.35, 10.48 (s, 1H, –NH). ^{13}C NMR (100 MHz, DMSO-d_6): δ_C 18.9, 30.1, 41.5, 115.0, 115.1, 115.5, 116.2, 119.1, 130.4, 138.8, 146.9, 157.8, 163.2, 197.4. HRMS (EI) calcd for $C_{15}H_{14}N_2O_3$ [M^+]: 270.1011, found: 270.1004.

*4.3.10. 5-Acetyl-4-(furan-2-yl)-6-methyl-2-oxo-1,2,3,4-tetrahydropyridine-3-carbonitrile (**4j**).* Yellow solid. IR (neat) (ν_{max}/cm^{-1}): 3286, 3179, 2260, 1707, 1663. ^{1}H NMR (400 MHz, DMSO-d_6): δ_H 1.71, 1.73 (s, 3H, $-CH_3C{=}O$), 1.76, 1.79 (s, 3H, $-CH_3C{=}C$), 3.73–3.75, 4.14–4.15 (d, 1H, J=6.0 Hz, $-CHC{=}C$), 4.22–4.23 (d, 1H, J=6.4 Hz, $-CHCN$), 5.89–5.90, 5.90–5.91 (d, 1H, J=1.2 Hz, -C=CH),5.92–5.93, 5.93–5.94 (d, 1H, J=2.0 Hz, O–C=HC=CH), 7.07 (d, 1H, J=1.2 Hz, O–CH=), 9.95, 10.13 (s, 1H, –NH).

*4.3.11. 5-Acetyl-4-ethyl-6-methyl-2-oxo-1,2,3,4-tetrahydropyridine-3-carbonitrile (**4k**).* Yellow liquid. IR (neat) (ν_{max}/cm^{-1}): 3258, 3152, 2253, 1717, 1617. ^{1}H NMR (400 MHz, DMSO-d_6): δ_H 0.94–0.98 (t, 3H,

J=7.6 Hz, $-CH_2CH_3$), 1.39–1.87 (m, 2H, $-CH_2CH_3$), 2.32, 2.33 (s, 3H, $-CH_3C=O$), 2.38, 2.39 (s, 3H, $-CH_3C=C$), 3.18–3.21, 3.32–3.37 (m, 1H, −CHC=C), 3.54–3.55, 3.90–3.92 (d, 1H, J=5.6 Hz, −CHCN), 8.66, 8.72 (s, 1H, −NH). ^{13}C NMR (100 MHz, DMSO-d_6): δ_C 11.0, 11.1, 19.5, 19.6, 23.5, 24.6, 30.4, 30.5, 35.9, 36.7, 39.5, 39.6, 114.8, 115.4, 116.5, 117.0, 143.0, 143.7, 162.3, 163.9, 196.7, 196.8. HRMS (EI) calcd for $C_{11}H_{14}N_2O_2$ [M^+]: 206.1055, found: 206.1053.

*4.3.12. 5-Acetyl-6-methyl-2-oxo-4-propyl-1,2,3,4-tetrahydropyridine-3-carbonitrile (**4l**).* Yellow solid. Mp 138–139 °C IR (neat) (ν_{max}/cm^{-1}): 3221, 3134, 2251, 1716, 1675. ^{1}H NMR (400 MHz, $CDCl_3$): δ_H 0.91–0.94 (t, 3H, J=6.8 Hz, $-CH_2CH_3$), 1.26–1.77 (m, 4H, $-CH_2CH_2-$), 2.29–2.33 (s, 3H, $-CH_3C=O$), 2.36–2.39 (s, 3H, $-CH_3C=C$), 3.26–3.29, 3.37–3.41 (m, 1H, −CHC=C), 3.52, 3.88–3.89 (d, 1H, J=5.2 Hz, −CHCN), 8.66, 8.72 (s, 1H, −NH). ^{13}C NMR (100 MHz, $CDCl_3$): δ_C 13.7, 14.2, 19.5, 19.6, 19.8, 20.0, 30.4, 30.6, 32.7, 33.6, 35.5, 36.2, 37.7, 39.8, 114.8, 115.3, 116.8, 117.7, 142.8, 143.3, 162.4, 163.9, 196.6, 196.7. HRMS (EI) calcd for $C_{12}H_{16}N_2O_2$ [M^+]: 220.1212, found: 220.1213.

*4.3.13. Methyl 5-cyano-2-methyl-4-(3-nitrophenyl)-6-oxo-1,4,5,6-tetrahydropyridine-3-carboxylate (**4m**).* White solid. IR (neat) (ν_{max}/cm^{-1}): 3272, 3182, 2268, 1709, 1639. ^{1}H NMR (400 MHz, DMSO-d_6): δ_H 2.33, 2.36 (s, 3H, $-CH_3C=C$), 3.50, 3.51 (s, 3H, $-OCH_3$), 4.21–4.22, 4.56–4.58 (d, 1H, J=6.8 Hz, −CHC=C), 4.67–4.68, 4.90–4.92 (d, 1H, J=7.6 Hz, −CHCN), 7.60–8.16 (m, 4H, Ar−H), 10.64, 10.75 (s, 1H, −NH).

*4.3.14. Methyl 4-(4-chlorophenyl)-5-cyano-2-methyl-6-oxo-1,4,5,6-tetrahydropyridine-3-carboxylate (**4n**).* White solid. IR (neat) (ν_{max}/cm^{-1}): 3275, 3170, 2254, 1711, 1637. ^{1}H NMR (400 MHz, DMSO-d_6): δ_H 2.30, 2.35 (s, 3H, $-CH_3C=C$), 3.51 (s, 3H, $-OCH_3$), 4.35–4.36 (d, 1H, J=6.8 Hz, −CHC=C), 4.48–4.49, 4.81–4.82 (d, 1H, J=7.2 Hz, −CHCN), 7.17–7.37 (m, 4H, Ar−H), 10.49, 10.66 (s, 1H, −NH).

*4.3.15. Methyl 5-cyano-2-methyl-6-oxo-4-p-tolyl-1,4,5,6-tetrahydropyridine-3-carboxylate (**4o**).* White solid. IR (neat) (ν_{max}/cm^{-1}): 3283, 3181, 2265, 1713, 1639. ^{1}H NMR (400 MHz, DMSO-d_6): δ_H 2.25, 2.27 (s, 3H, $-CH_3Ph$), 2.32, 2.38 (s, 3H, $-CH_3C=C$), 3.54, 3.55 (s, 3H, $-OCH_3$), 4.07–4.08, 4.30–4.31 (d, 1H, J=6.8 Hz, −CHC=C), 4.44–4.45, 4.87–4.89 (d, 1H, J=6.8 Hz, −CHCN), 7.05–7.15 (m, 4H, Ar−H), 10.50, 10.62 (s, 1H, −NH).

*4.3.16. Methyl 5-cyano-4-ethyl-2-methyl-6-oxo-1,4,5,6-tetrahydropyridine-3-carboxylate (**4p**).* White solid. Mp 133–134 °C. IR (neat) (ν_{max}/cm^{-1}): 3229, 3137, 2248, 1701, 1648. ^{1}H NMR (400 MHz, DMSO-d_6): δ_H 0.79–0.83 (t, 3H, J=8.0 Hz, $-CH_2CH_3$), 1.29–1.68 (m, 2H, $-CH_2CH_3$), 2.20, 2.25 (s, 3H, $-CH_3C=C$), 3.06–3.09, 3.15–3.19 (m, 1H, −CHC=C), 3.65, 3.68 (s, 3H, $-OCH_3$), 3.89–3.90, 4.58–4.59 (d, 1H, J=6.0 Hz, −CHCN), 10.30, 10.50 (s, 1H, −NH). ^{13}C NMR (100 MHz, DMSO-d_6): δ_C 10.6, 18.4, 23.7, 35.7, 36.0, 51.6, 105.3, 116.7, 147.4, 164.2, 166.8. HRMS (EI) calcd for $C_{11}H_{14}N_2O_3$ [M^+]: 222.1004, found: 222.1001.

*4.3.17. 4-(4-Chlorophenyl)-5-cyano-2-methyl-6-oxo-N-phenyl-1,4,5,6-tetrahydropyridine-3-carboxamide (**4q**).* Yellow solid. Mp 157–158 °C. IR (neat) (ν_{max}/cm^{-1}): 3281, 3161, 2257, 1712, 1652. ^{1}H NMR (400 MHz, DMSO-d_6): δ_H 1.98, 2.06, 2.07 (s, 3H, $-CH_3C=C$), 4.33–4.35 (d, 1H, J=7.2 Hz, −CHC=C), 4.77–4.79 (d, 1H, J=6.8 Hz, −CHCN), 6.99–7.49 (m, 9H, Ar−H), 9.85, 9.93 (s, 1H, −NH), 10.15 (s, 1H, −NHPh). ^{13}C NMR (100 MHz, DMSO-d_6): δ_C 17.7, 41.1, 41.3, 111.6, 116.2, 120.2, 124.4, 128.9, 129.1, 129.2, 130.2, 130.3, 133.3, 136.2, 136.9, 138.5, 138.5, 138.8, 162.5, 165.8. HRMS (EI) calcd for $C_{20}H_{16}N_3O_2Cl$ [M^+]: 365.0935, found: 365.0931.

*4.3.18. 5-Cyano-2-methyl-6-oxo-N-phenyl-4-p-tolyl-1,4,5,6-tetrahydropyridine-3-carboxamide (**4r**).* White solid. Mp 108–109 °C. IR (neat) (ν_{max}/cm^{-1}): 3278, 3140, 2257, 1709, 1654. ^{1}H NMR (400 MHz, DMSO-d_6): δ_H 2.06, 2.07,2.12 (s, 3H, $-CH_3Ph$), 2.22, 2.27 (s, 3H, $-CH_3C=C$), 4.36–4.38, 4.84–4.86 (d, 1H, J=7.2 Hz, −CHC=C), 4.31–4.33 (d, 1H, J=7.2 Hz, −CHCN), 6.97–7.58 (m, 9H, Ar−H), 9.68, 9.71 (s, 1H, −NH), 10.20, 10.21 (s, 1H, −NHPh). ^{13}C NMR (100 MHz, DMSO-d_6): δ_C 17.8, 21.0, 41.1, 41.4, 112.2, 116.5, 120.0, 120.0, 123.9, 128.2, 128.9, 129.0, 129.5, 129.7, 134.4, 137.7, 138.4, 139.3, 162.7, 165.5. HRMS (EI) calcd for $C_{21}H_{19}N_3O_2$ [M^+]: 345.1477, found: 345.1476.

*4.3.19. 5-Cyano-4-(furan-2-yl)-2-methyl-6-oxo-N-phenyl-1,4,5,6-tetrahydropyridine-3-carboxamide (**4s**).* Yellow solid. Mp 98–99 °C. IR (neat) (ν_{max}/cm^{-1}): 3284, 3134, 2259, 1712, 1654. ^{1}H NMR (400 MHz, DMSO-d_6): δ_H 2.07 (s, 3H, $-CH_3C=C$), 4.42–4.44, 4.82–4.84 (d, 1H, J=6.4 Hz, −CHC=C), 4.61–4.63 (d, 1H, J=6.8 Hz, −CHCN), 6.29–6.30 (d, 1H, J=2.8 Hz, −CH=C), 6.40 (s, −OCH, 1H), 7.03–7.07 (t, 1H, J=7.2 Hz, O−CH=CH), 7.27–7.62 (m, 5H, Ar−H), 9.75, 9.80 (s, 1H, −NH), 10.21, 10.29 (s, 1H, −NHPh). ^{13}C NMR (100 MHz, DMSO-d_6): δ_C 17.9, 30.0, 36.1, 108.1, 109.5, 110.9, 116.3, 120.1, 123.9, 129.0, 129.0, 129.2, 139.4, 139.6, 143.7, 151.2, 162.7, 165.1. HRMS (EI) calcd for $C_{18}H_{15}N_3O_3$ [M^+]: 321.1113, found: 321.1112.

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References and notes

1. Li, Q.; Mitscher, L. A.; Shen, L. L. *Med. Res. Rev.* **2000**, *20*, 231–293.
2. Cox, R. J.; Ohagan, D. *J. Chem. Soc., Perkin Trans. 1* **1991**, 2537–2540.
3. (a) Nagarajan, M.; Xiao, X. S.; Antony, S.; Kohlhagen, G.; Pommier, Y.; Cushman, M. *J. Med. Chem.* **2003**, *46*, 5712–5724; (b) Hasvold, L. A.; Wang, W. B.; Gwaltney, S. L.; Rockway, T. W.; Nelson, L. T. J.; Mantei, R. A.; Fakhoury, S. A.; Sullivan, G. M.; Li, Q.; Lin, N. H.; Wang, L.; Zhang, H. Y.; Cohen, J.; Gu, W. Z.; Marsh, K.; Bauch, J.; Rosenberg, S.; Sham, F. L. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 4001–4005.
4. (a) Dolle, V.; Fan, E.; Nguyen, C. H.; Aubertin, A. M.; Kirn, A.; Andreola, M. L.; Jamieson, G.; Tarragolitvak, L.; Bisagni, E. *J. Med. Chem.* **1995**, *38*, 4679–4686; (b) Wai, J. S.; Williams, T. M.; Bamberger, D. L.; Fisher, T. E.; Hoffman, J. M.; Hudcosky, R. J.; Mactough, S. C.; Rooney, C. S.; Saari, W. S.; Thomas, C. M.; Goldman, M. E.; Obrien, J. A.; Emini, E. A.; Nunberg, J. H.; Quintero, J. C.; Schleif, W. A.; Anderson, P. S. *J. Med. Chem.* **1993**, *36*, 249–255.
5. Pendleton, M. D.; Beddoes, R. L.; Andrew, N. J.; Butters, M.; Joule, J. A. *J. Chem. Res., Synop.* **1997**, 86–87.
6. Yang, L.; Zheng, Q. Y.; Wang, D. X.; Huang, Z. T.; Wang, M. X. *Org. Lett.* **2008**, *10*, 2461–2464.
7. Paravidino, M.; Scheffelaar, R.; Schmitz, R. F.; De Kanter, F. J. J.; Groen, M. B.; Ruijter, E.; Orru, R. V. A. *J. Org. Chem.* **2007**, *72*, 10239–10242.
8. Noguez, M. O.; Marcelino, V.; Rodríguez, H.; Martí, O.; Martínez, J. O.; Pérez, F. J.; Suárez, M.; Miranda, R. *Int. J. Mol. Sci.* **2011**, *12*, 2641–2649.
9. Kilbanov, A. M. *CHEMTECH* **1986**, *16*, 354–359.
10. (a) Bornscheuer, U. T.; Kazlauskas, R. J. *Angew. Chem., Int. Ed.* **2004**, *43*, 6032–6040; (b) Copley, S. D. *Curr. Opin. Chem. Biol.* **2003**, *7*, 265–272; (c) O'Brien, P. J.; Herschlag, D. *Chem. Biol.* **1999**, *6*, R91–R105.
11. Svedendahl, M.; Hult, K.; Berglund, P. *J. Am. Chem. Soc.* **2005**, *127*, 17988–17989.
12. Seebeck, F. P.; Hilvert, D. *J. Am. Chem. Soc.* **2003**, *125*, 10158–10159.
13. Xu, J. M.; Zhang, F.; Liu, B. K.; Wu, Q.; Lin, X. F. *Chem. Commun.* **2007**, 2078–2080.
14. (a) Li, K.; He, T.; Li, C.; Feng, X. W.; Wang, N.; Yu, X. Q. *Green. Chem.* **2009**, *11*, 777–779; (b) Lai, Y. F.; Zheng, H.; Chai, S. J.; Zhang, P. F.; Chen, X. Z. *Green. Chem.* **2010**, *12*, 1917–1918; (c) Zhang, P. F.; Chai, S. J.; Lai, Y. F.; Xu, J. C.; Zheng, H.; Zhu, Q. *Adv. Synth. Catal.* **2011**, *353*, 371–375.