Journal of the Iranian Chemical Society (2019) 16:1891–1902
https://doi.org/10.1007/s13738-019-01662-x

ORIGINAL PAPER

Glycoside- and acyclic nucleoside-based 6-cyclohexyl-4-aryl-2-oxonicotinonitrile: synthesis and antimicrobial evaluation

Abdalla E. Hassan[1,2] · Reham A. I. Abou-elkhair[1,2] · Samar M. Mohamed[2] · Ahmed H. Moustafa[2] · Reda M. Fikry[2] · Fathy A. Yassen[2]

Received: 6 October 2018 / Accepted: 2 April 2019 / Published online: 9 April 2019

Abstract
Antimicrobial resistance is a global public health threat that signals the identification of new antimicrobial agents with different modes of action. The synthesis and the antimicrobial evaluations of a series of 2-oxonicotinonitriles (2-ONNs) derivatives are described. The parent 2-ONN derivative **3** was synthesized by one-pot four-component reaction: acetyl cyclohexane, *p*-anisaldehyde, ethyl cyanoacetate, and NH_4OAc, in high yield. Density functional theory geometrical equilibrium of the tautomeric forms of **3** concluded the predominance of the keto tautomer. Base-catalyzed coupling of **3** with several organohalides/alkylating agents provided branching and solubilizing groups to the 2-ONN derivative **3**. Alkylated ONNs and free acyclic nucleoside mimics/glycosides derived from the 2-ONN derivative **3** were tested for their antibacterial and antifungal activities. The acyclic nucleoside mimic **13** and the N^1-propargyl derivative **16** and N^1-allyl derivative **18** showed significant activities against *S. aureus*, and *M. luteus*; meanwhile, significant anti-*E. coli* activity was observed with compounds **3** and **18**. On the other hand, a significant antifungal activity was observed with **3** and with its N^1-glucoside derivatives **4** and **5** against *Candida albicans* and *Aspergillus niger*.

Keywords 2-Oxonicotinonitriles · Acyclic nucleosides · 2-Oxonicotinonitriles glycosides · Antibacterial activity · Antifungal activity

Introduction

Emergence of antimicrobial resistance, especially multidrug bacterial resistance has posed worldwide problem in medicine [1, 2]. The Center for Disease Control and Prevention (CDC) estimates that more than 2 million individuals become infected with drug-resistant bacteria annually, and greater than 23,000 individual dies as a result of those infections [3]. Gram-positive bacterium Staphylococcus aureus (Staph) is the causative agent of multitude of diseases including bacteremia or sepsis, pneumonia, endocarditis, and osteomyelitis. Staph became resistant to several antibiotics such as methicillin-resistant Staphylococcus aureus (MRSA) [4–10], vancomycin-intermediate Staphylococcus aureus (VISA) [11, 12], and vancomycin-resistant Staphylococcus aureus (VRSA) [13–16]. *Micrococci luteus* are another Gram-positive bacterium that have been reported to cause pneumonia, meningitis associated with ventricular shunts, septic arthritis, bacteremia, peritonitis, endophthalmitis, and endocarditis. *Micrococci* have shown resistance to several antibiotics including streptomycin [17]. *Pseudomonas aeruginosa* is a Gram-negative bacterium that tends to infect people with immunodeficiency and those with indwelling catheters or on respirators. Infection with *pseudomonas* can lead to urinary tract infections (UTI), sepsis, pneumonia, and pharyngitis. *Pseudomonas* colonizes the lungs of patients with cystic fibrosis (CF) and contributes to the chronic progressive pulmonary disease and death rate in CF. *P. aeruginosa* is a Gram-negative bacterium that is intrinsically resistant to β-lactam-based antibiotics such as cephalothin and ampicillin [18–22]. Escherichia coli is the most common

✉ Abdalla E. Hassan
habdallaa@aol.com

[1] Applied Nucleic Acids Research Center, Zagazig University, Zagazig, Egypt

[2] Chemistry Department, Faculty of Science, Zagazig University, Zagazig, Egypt

Gram-negative pathogen in human, and multiple antibiotic resistance *E. coli* strains have been reported [23–26].

2-Oxonicotinonitriles (2-ONN) ring is a core moiety in several biologically important compounds [27, 28]. This motif has been found in HIV-1-non-nucleoside reverse transcriptase inhibitors [27, 29, 30], non-nucleosides HBV inhibitors, and HCV NS5B polymerase inhibitors [31–35], anti-HSV-1 agents [36] (Fig. 1). In addition to their antiviral activity, 2-pyridinone derivatives are known to possess activity against wild type [37] and resistant bacterial strains [38]. Pyridone derivatives have also shown antifungal [39] and antiprotozoal activity [40, 41]. We have identified 2-ONN derivatives with significant anti-SARS and anti-*Bacillus subtitis* activity (**1**, **2**; Fig. 1) [42]. The structural features of the 2-ONNs derivatives **1**, **2** have two aryl substituents and a glycosyl/polar alkyl moieties at the 4 and 6, and N-1 positions, respectively. Though the molecular target for these 2-ONNs is still unknown, it is legitimate, and the structure activity relationship studies of these entities could provide useful antimicrobial agents. It is of interest to probe the effect of substituent at the C-6 and *N*-1 positions on the antimicrobial activities. Herein, we report on the synthesis of the 4-(*p*-methoxyphenyl)-6-cyclohexyl-2-ONNs derivatives **3–20** (Table 2; general structures I-II, Fig. 1) and their antimicrobial evaluations.

Results and discussions

Chemistry

Methods available in the literature for the synthesis of 4,6-disubstituted 2-ONN derivatives involve condensation of 2-substituted acrylamide with enolates [43], one-pot four-component condensation reaction of aromatic aldehyde, ketone, ethyl cyanoacetate in the presence of ammonium acetate [44–48].The later procedure was adapted with minor modification for the synthesis of the 2-ONN derivative **3**. Treatment of cyclohexyl methyl ketone with equimolar amounts of *p*-anisaldehyde, ethyl cyanoacetate, and NH_4OAc in reflux EtOH gave the 2-ONN derivative **3** in good yield (Scheme 1).

A schematic representation of the reaction mechanism for the one-pot four-component reaction is illustrated in Fig. 2. The reaction may commence with aldol condensation providing the chalcone intermediate [I], which then reacts with the ethyl cyanoacetate to give the intermediate [II] which cyclizes with ammonia generated from ammonium acetate to give the corresponding dihydropyridine ring [III]. Oxidation of the intermediate [III] would give the pyridone derivative **3**. 2-Pyridones are known to exist in two tautomeric forms: amide form and 2-hydroxypyridine form [49, 50], where the distribution ratio of these tautomers, among other factors, could affect the *N*-/*O*-alkylation selectivity at the 2-ONN ring. Keto–enol tautomerism in 2-pyridone/2-hydroxypyridine is considered as an intramolecular hydrogen transfer reaction, where the hydrogen atom is transferred between the *N* and the *O* sites of the molecule through a transition state (A3) (Fig. 3). Spectroscopic data show the predominance of the keto form over the enol form. IR spectrum of **3** showed $\nu_{C=O}$ 1647 cm^{-1}, and its ^{13}C-NMR spectrum showed the signal for (C2) at 161.8 ppm. Energies of the tautomers of the 2-ONN derivative **3** as well as that of the transition state were calculated via density functional theory (DFT) at ωB97X-D/6-31G* and level of theory [51]. Energies listed in Table 1 coincide with the experimental results supporting the predominance of the keto form (A1) for **3** over the enol form (A3) tautomer (Fig. 4). The calculation results show that the energy of the transition state closer to the enol form compared with the keto form (Table 1). The local ionization potential and electrostatic potential maps are used to identify the nucleophilic or the electrophilic centers in a molecule.

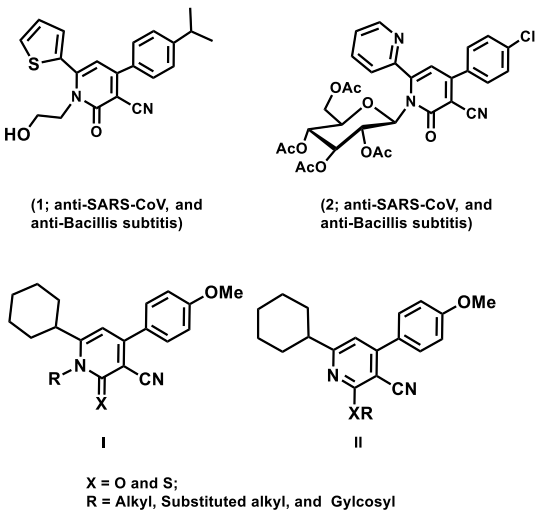


Fig. 1 Structures of biologically active pyridone derivatives (1–2) and the newly synthesized 2-ONN (I, II) derivatives

Scheme 1[a]

[a]Reagents and conditions. a) EtOH, 24 h, reflux, 75%.

Scheme 1 Reagents and conditions. **a** EtOH, 24 h, reflux, 75%

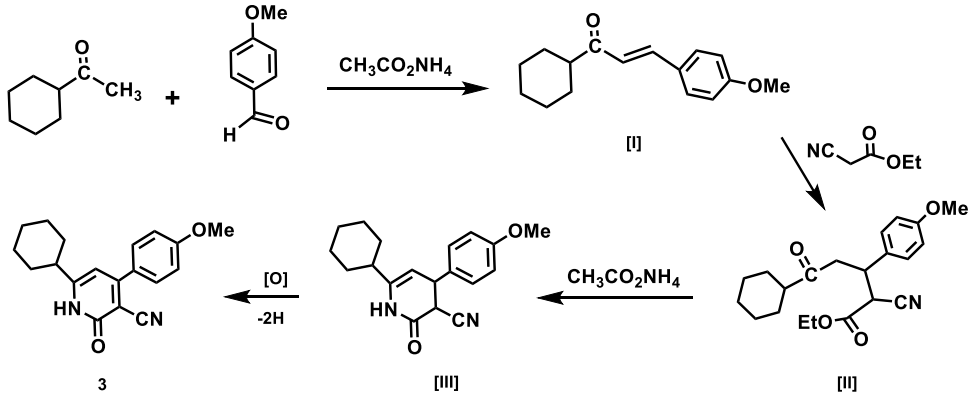


Fig. 2 A plausible mechanism for the one-pot four-component synthesis of **3**

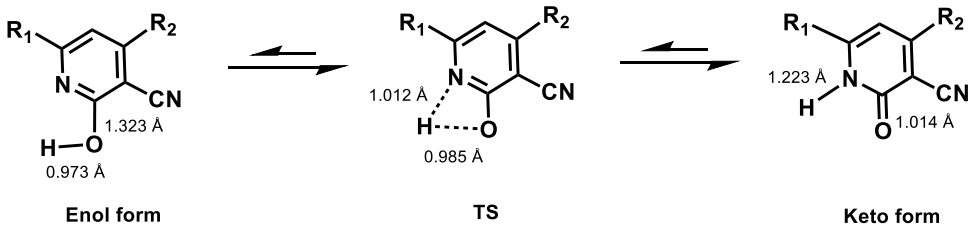


Fig. 3 Optimized structures of 2-ONN tautomers and their transition state (TS) using DFT-ωB97X-D level of theory

Table 1 Geometrical optimization of compound **3**'s tautomers and their TS

Tautomer	Energy (au)	Relative energy (KJ/mol)	E HOMO (eV)	E LUMO (eV)	Boltzmann weight
A1	−995.714616	0.00	−7.93	−1.83	0.989
A2	−995.710322				
A3	−995.710341	11.22	−8.03	−0.08	0.011

The centers for electrophilic attack in **3** are N^1 and O^2 positions (Fig. 4).

The intrinsic low solubility of the diaryl-2-ONN derivatives in aqueous and most of the organic solvents creates a barrier for those entities to show biological activity. Branching of the diaryl-2-ONN derivatives with either transient or permanent solubilizing groups could overcome their low solubility problem. Glycosylation of small molecules, especially biologically active compounds, with specific mono- and disaccharides has been utilized to modulate their water solubility or their cellular uptake, and hence their pharmacological characteristics [52, 53]. In view of that, the 2-ONN derivative **3** was glycosylated with three glycosyl bromides under basic conditions (Table 2). Treatment of **3** with peracetylated α-D-glucopyranosyl bromide [54] in the presence of K_2CO_3 in DMF at room temperature gave corresponding *N*-glucoside derivative **4** in 57% yield along with traces of the corresponding *O*-glycosyl derivative. In a similar manner, the *N*-lactoside derivative **6** was prepared by coupling of **3** with peracetylated 1-α-D-lactosyl bromide [55] in 51% yield (Table 2). The N^1-glycosidation in **4** and **6** was confirmed by the presence of the amide $\nu_{C=O}$ signals in the range of 1644–1641 cm^{-1} in their IR spectra. The diaxial coupling between the anomeric proton and the H-2′ ($J_{1',2'} = 7.86–8.82$ Hz) confirms the β-configuration at the anomeric position of the 2-ONN glycosides (**4–6**). The observed preference of *N*-glycosylation over *O*-glycosylation coincides with the DFT conclusion of the predominance of the keto form of the 2-ONN derivative 3, despite the steric hindrance at the C-6 position. Treatment of the *N*-glycosides **4** and **6** with triethylamine (TEA) in H_2O/MeOH gave the corresponding free *N*-glycosides **5** and **7**, respectively, in good yields. Branching at the amide moiety 2-ONN derivatives with acyclic sugar mimics, alkyl or substituted alkyl derivatives, has resulted in better biological activity compared with the native 2-ONN [42]. Base-catalyzed coupling of **3** with 4-bromobutyl acetate gave a separable mixture of *N*-butyl acetate derivative **8** and the corresponding *O*-butyl acetate derivative **10** in good yield (Table 2). Treatment of **3** with 2-bromomethoxyethyl

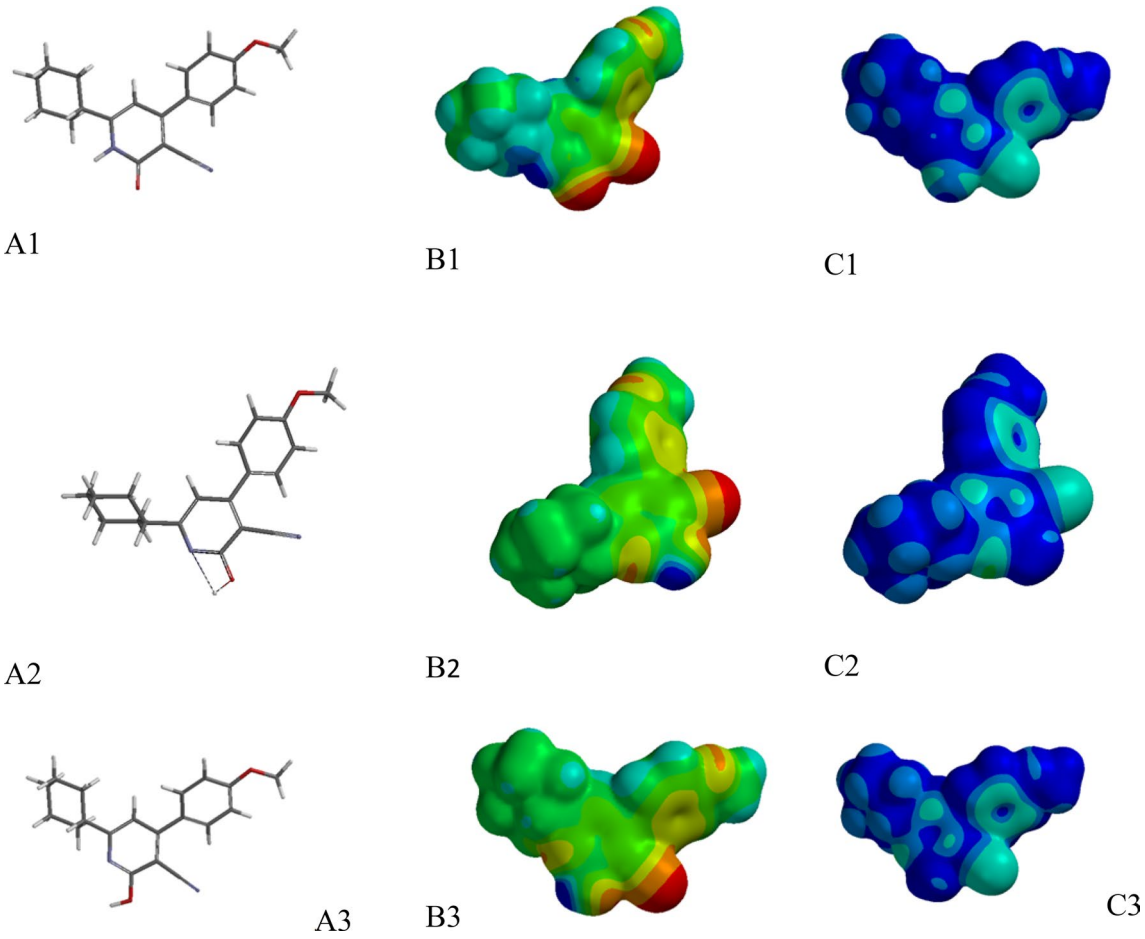


Fig. 4 **A1**, **A3** Minimized energy for the tautomers of the 2-ONN derivative **3**. **B1**, **B2** Electrostatic potential map for the tautomers of the 2-ONN derivative **3**. **C1**, **C2** Local ionization map for the tautomers of the 2-ONN derivative **3**

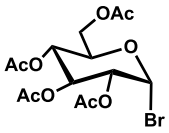
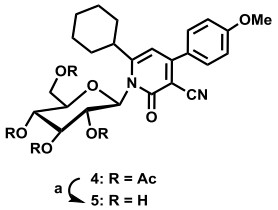
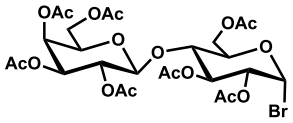
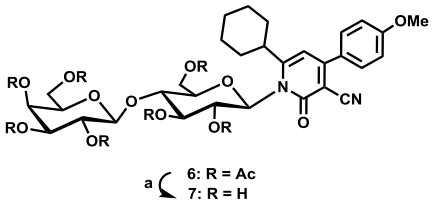
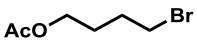
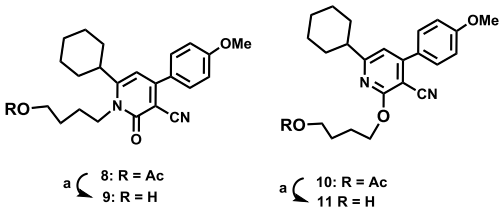
acetate gave a mixture of the corresponding *N*-acyclic nucleoside mimic **12** and the corresponding *O*-alkyl derivative **14** in good isolated yields (Table 2). The site of alkylation in the above mentioned reactions is determined by IR and ^{13}C-NMR spectroscopy. For instance, the *N*-alkylation in **12** is supported by the presence of $\nu_{C=O}$ signal (1647 cm^{-1}) in its IR spectrum and 170.3 ppm (C2) signal in its ^{13}C-NMR spectrum. Deacetylation of **8**, **10, 12,** and **14** was performed with Et_3N in MeOH/H_2O to give **9**, **11, 13,** and **15**, respectively, in good yields. Treatment of **3** with propargyl bromide under (K_2CO_3/acetone/reflux) conditions gave a separable mixture of the *N*- and *O*-propargyl derivatives **16** and **17** in good yields (Table 2). In a similar manner, treatment of **3** with allyl bromide under the same conditions gave a separable mixture of *N*-allyl and *O*-allyl derivatives **18** and **19**, respectively, in good yields (Table 2). On the other hand, treatment of **3** with epichlorohydrin under the same conditions (K_2CO_3/acetone/reflux) none of the *O*-alkyl derivative was isolated, but the *N*-alkyl derivative **20** was isolated in 42% yield (Table 2).

Biology

Evaluation of antimicrobial activities

The new synthesized 2-ONN derivatives were screened in vitro for antibacterial activity against the Gram-positive bacteria: *Staphylococcus aureus* ATCC 6538 and *M. luteus* ATCC 10240 and Gram-negative bacteria: *P. aeruginosa* ATCC 9027 and *Escherichia coli* ATCC 10536. The antifungal activity of the 2-ONN derivatives was assessed against *Candida albicans* ATCC 10231 and *Aspergillus niger* ATCC 16404. Agar-diffusion method [42] was used to assess the antibacterial and antifungal activity of the tested compounds. The diameter of inhibition zone (IZ) of the bacterial and fungal growth in mm was used to assess the preliminary antimicrobial activity of the tested compounds [42].

Table 2 Glycosylation and alkylation of the 2-ONN derivative **3**

Entry	Coupling agent	Product[b,c]	Yield %
1			**4**: 57 [b] **5**: 90
2			**6**: 49 [b] **7**: 87
3			**8**: 34 [c] **9**: 80 **10**48 [c] **11**: 83

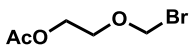
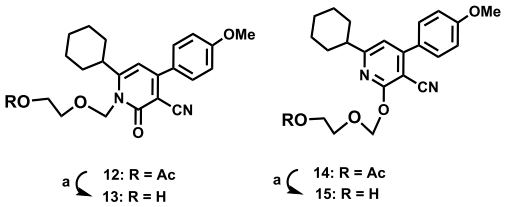
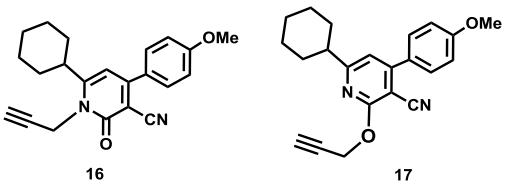
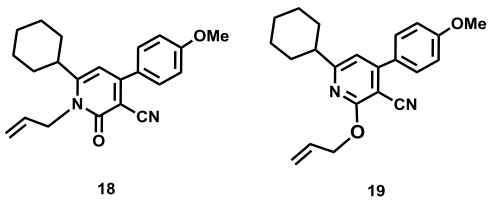
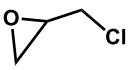
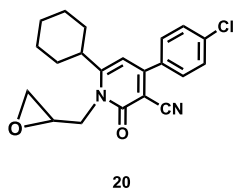
Concentration of the tested samples was 4 mg/mL, ND—not determined

The parent 2-ONN derivative **3** showed significant activity against the Gram-negative bacteria: *E. coli* ATCC-10536 and *P. aeruginosa* ATCC-9027, while a moderate activity against the Gram-positive bacteria: *S. aureus* ATCC-6538 and *M. luteus* ATCC-10242, was observed (Table 3). Additionally, a significant antifungal activity was observed with the 2-ONN derivative **3**, and its *N*-glucoside derivatives **4** and **5** against *Candida albicans* and *Aspergillus niger* (Table 3). It is notable that weak to moderate antibacterial activity is observed with *O*-alkyl derivatives **11**, **17**, and **19** and the *N*-oxiranylmethyl derivative **20**. The antifungal activity of selected number of the newly synthesized compounds was evaluated against *Candida albicans* ATCC 10231 and *Aspergillus niger* ATCC 16404. The parent compound **3**, *N*-glucosyl acetate derivative **4**, its free derivative **5**, and *N*-(2-hydroxyethoxy)methyl derivative **13** showed superior antifungal activity against both strains compared with nystatin. The data observed with compounds **4**, **5**, **11**, **13**, **16–20** imply that branching of the parent 2-ONN derivative **3** with solubilizing groups affects the antimicrobial activity.

Conclusions

A one-pot four-component reaction was utilized to synthesize the 6-cyclohexyl-4-methoxy-2-ONN derivative **3** in good yield. DFT calculations of tautomeric forms of 3 show the predominance of the 2-keto form, (A1) over the enol form (A2). Branching of the amide moiety of **3** by glycosylation gave the corresponding *N*-glycosides in good yields. Alkylation of **3** with several alkyl halides gave separable mixtures of the corresponding *N*- and *O*-alkyl derivatives. Significant antibacterial activity was observed with **13**, **16**, and **18** against *S. aureus* ATCC-6538 and *M. luteus* ATCC-10242, while the parent 2-ONN derivative **3** showed good activity against *E. coli*. On the other hand, a significant antifungal activity was observed in 2-ONN derivative **3**, its *N*-glucoside derivatives **4** and **5** against *Candida albicans*

Table 2 (continued)

4			**12**: 27 [c] **13**: 83 **14**: 42 [c] **15**: 87
5			**16**: 30 [c] **17**: 29 [c]
6			**18**: 32 [c] **19**: 39 [c]
7			**20**:42[c]

[a]Et3N, MeOH, H2O, r.t.

[b]K2CO3, DMF, r.t.

[c]K2CO3, acetone, reflux

Table 3 Antibacterial and antifungal activity of selected 2-ONNs derivatives

Compd.	Diameter of inhibition zone (mm)					
	S. aureus ATCC 6538	*M. luteus* ATCC 10240	*P. aeruginosa* ATCC 9027	*E. coli* ATCC 10536	*Candida albicans* ATCC 10231	*Aspergillus niger* ATCC 16404
3	25	23	28	30	30	32
4	30	34	22	23	30	32
5	20	24	20	20	31	33
6	29	30	20	19	20	21
7	21	20	20	20	21	20
8	26	29	20	20	25	28
9	30	31	22	23	29	31
10	ND	ND	ND	ND	32	35
11	21	24	20	20	18	20
13	37	39	20	22	30	33
15	31	29	20	20	ND	ND
16	34	33	22	19	27	29
17	21	22	20	21	ND	ND
18	36	39	21	22	30	32
19	22	19	20	18	ND	ND
20	24	25	20	23	ND	ND
Cefotaxime	31	28	32	34		
Nystatin					25	20
DMF						

Concentration of the tested samples was 4 mg/mL, *ND*—not determined

and *Aspergillus niger*. The results of this study provide hits for further optimizations for the discovery of potent antimicrobial agents.

Experimental results and protocols

Material, instruments, and general considerations

Starting materials and reagents were purchased from Acros-Organics, Alpha Aesar, and Sigma-Aldrich. 4-Bromobutyl acetate [56] and 2-bromomethoxyethyl acetate [57, 58] were prepared according to the literature procedures. Reaction's progress was monitored by TLC analysis using aluminum-backed plates pre-coated with Merck silica gel 60 F254. Column chromatography was carried out on Agela Technologies flash silica 40–60 mesh. Melting points were recorded on Electrothermal IA 9100 apparatus and were uncorrected. IR spectra (KBr disk) were recorded on either a Pye Unicam Sp 3-300 or a Shimadzu FTIR 8101 PC infrared spectrometer. ^{1}H and ^{13}C-NMR spectra were recorded on Bruker 400 MHz spectrometer. Chemical shifts (δ) were reported in ppm and were referenced to TMS as a standard (0.00 ppm). Chemical shifts for ^{13}C-NMR were referenced relative to DMSO (39.50 ppm). Elemental microanalysis was recorded on PerkinElmer 240 analyzer. Analyses indicated by the symbols of the elements were within 0.4% of the theoretical values.

Chemistry

6-Cyclohexyl-4-(4-methoxyphenyl)-2-oxo-1,2-dihydropyridine-3-carbonitrile (3)

A mixture of 4-methoxybenzaldehyde (6.80 g, 0.05 mol), cyclohexyl methyl ketone (6.31 g, 0.05 mol), and ammonium acetate (30.8 g, 0.4 mol) in ethanol (150 mL) was refluxed for 48 h. The reaction mixture was cooled to room temperature, and precipitate was filtered off, washed with ethanol, and dried under vacuum. The precipitate was recrystallized from a mixture of acetic acid/ethanol (1:5 v:v) to give **3** (11.56 g, 75%) as a pale yellow crystals: m.p. 242–244 °C; IR (KBr): 3286 cm^{-1} (NH), 2036 cm^{-1} (CN) and 1647 cm^{-1} (C=O, amide); ^{1}H-NMR (DMSO-d_6) δ 1.23–1.84 (11H, m, cyclohexyl), 3.83 (3H, s, OCH_3), 6.26 (1H, s, H-5, pyridone), 7.07 (2H, d, Ar–H, $J = 8.7$ Hz),

7.60 (2H, d, Ar–H,J = 8.7 Hz), 12.36 (1H, s, NH); ^{13}C-NMR (DMSO-d_6)δ25.03, 25.66, 30.80, 41.64, 55.39, 88.9, 104.0, 114.2, 116.9, 128.2, 129.8, 159.6, 159.8, 161.0, 161.8; Anal. Calcd for $C_{19}H_{20}N_2O_2$ (308.37): C, 74.00; H, 6.54; N, 9.08. Found: C, 74.09; H, 6.55; N, 9.09.

1-(2′,3′,4′,6′-Tetra-O-acetyl-β-D-glucopyranosyl)-6-cyclohexyl-4-(4-methoxyphenyl)-2-oxo-1,2-dihydropyridine-3-carbonitrile (4)

A mixture of **3** (1 g, 3.24 mmol) and anhydrous K_2CO_3 (0.45 g, 3.27 mmol) in dry DMF (10 mL) was stirred for 30 min. at 0 °C. 1-Bromo-2,3,4,6-tetra-*O*-acetyl-D-glucopyranose [54] (1.23 g, 3.0 mmol) was added to reaction mixture in portions, and the mixture was stirred overnight at room temperature. Aqueous 1 *N* NH_4Cl (5 mL) was added, and the solvents were evaporated under reduced pressure. The residue was partitioned between EtOAc and H_2O; the organic phase was dried (Na_2SO_4) and evaporated under reduced pressure. The residue was purified by a silica gel column chromatography (eluate: 10% EtOAc/hexanes) to give **4** (1.18 g,57%yield) as a white powder: m.p. 184–186 °C; IR (KBr) 2228 cm^{-1} (CN) and broad band at 1754 cm^{-1} (C=O, acetoxy and amide); ^{1}H-NMR (DMSO-d_6) δ 1.30–1.85 (10H, m, cyclohexyl), 1.97, 1.98, 2.00, 2.02 (12H, 4 s, 4C$\underline{H}_3$CO), 2.74 (1H, t, H1-cyclohexyl, J = 2.80 Hz), 3.83 (3H, s, OC$\underline{H}_3$), 4.01 (1H, d, H-6′, $J_{6',5'}$ = 4.96 Hz, $J_{6',6''}$ = 11.71 Hz), 4.18 (1H, dd, H-6″,$J_{6'',5'}$ = 5.60 Hz, $J_{6'',6'}$ = 5.60 Hz), 4.32 (1H, m, H-5′), 5.03 (1H, t, H-3′,$J_{3',2'}$ = 9.60 Hz, $J_{3',4'}$ = 10.0 Hz), 5.17 (1H, t, H-2′,$J_{2',3'}$ = 4.80 Hz,$J_{2',1'}$ = 8.39 Hz), 5.60 (1H, t, H-4′,$J_{4',5'}$ = 9.6 Hz, $J_{4',3'}$ = 9.6 Hz), 6.48 (1H, d, H-1′, $J_{1',2'}$ = 8.39 Hz), 7.12 (1H, d, Ar–H,J = 8.8 Hz,), 7.21 (1H, s, pyridone-H-5), 7.64 (1H, d, Ar–H, J = 8.8 Hz);^{13}C-NMR (DMSO-d_6)δ 20.18, 20.24, 20.27, 20.32, 25.36, 25.67, 25.72, 31.25, 55.34, 61.85, 68.13, 70.06, 71.45, 72.02, 91.22, 93.24, 114.2, 114.3, 116.7, 127.5, 130.0, 155.5, 160.7, 161.7, 168.3, 168.7, 169.2, 169.5, 169.8; Anal. Calcd for $C_{33}H_{38}N_2O_{11}$ (638.66): C, 62.06; H, 6.00; N, 4.39. Found: C, 62.07; H, 6.01; N, 4.37.

6-Cyclohexyl-1-(β-D-glucopyranosyl)-4-(4-methoxyphenyl)-2-oxo-1,2-dihydropyridine-3-carbonitrile (5)

A mixture of **4** (0.63 g, 1.0 mmol), Et_3N (1 mL), and H_2O (0.1 mL) in MeOH (5 mL) was stirred overnight at room temperature. The volatiles were evaporated under reduced pressure. The residue was repeatedly co-evaporated with MeOH until complete removal of Et_3N, and the residue was crystallized from 95% ethanol gave **5** (0.42 g, 90% yield) as a white powder: m.p. 246–248 °C; IR (KBr) 3427 cm^{-1} (4OH), 2217 cm^{-1} (CN) and 1644 cm^{-1} (C=O, amide); ^{1}H-NMR (DMSO-d_6-D_2O) δ1.16–1.84 (10H, m, cyclohexyl), 2.60 (1H, t, cyclohexyl-H-1, J = 2.82 Hz), 3.1–3.5 (6H, m, H-6′, H-6″, H-5′, H-4′, H-3′ and H-2′), 3.83 (3H, s, OC$\underline{H}_3$), 6.27 (1H, d, H-1′, $J_{1',2'}$ = 8.21 Hz), 6.98 (1H, s, pyridone-H-5), 7.08 (2H, d, Ar–H, J = 8.84 Hz), 7.61 (2H, d, Ar–H, J = 8.84 Hz); Anal. Calcd for $C_{25}H_{30}N_2O_7$ (470.51): C, 63.82; H, 6.43; N, 5.95. Found: C, 63.80; H, 6.42; N, 5.96.

1-(2′,3′,4′,6′-Tetra-O-acetyl-β-D-galactopyranosyl-(1 → 4)-(2′,3′,6′-tri-O-acetyl-β-D-glucopyranosyl)-6-cyclohexyl-4-(4-methoxyphenyl)-2-oxo-1,2-dihydropyridine-3-carbonitrile (6)

A mixture of **3** (1 g, 3.24 mmol) and anhydrous K_2CO_3 (0.45 g, 3.27 mmol) in dry DMF (10 mL) was stirred for 30 min. at 0 °C. 1-Bromo-2′,3′,4′,6′-tetra-*O*-acetyl-*β*-D-galactopyranosyl-(*1* → *4*)-(2′,3′,6′-tri-*O*-acetyl-*β*-D-glucopyranose [55] (1.85 g, 3.0 mmol) was added to the reaction mixture in portions, and the mixture was stirred for 14 h at room temperature. Aqueous 1 *N* NH_4Cl (5 mL) was added, and the solvents were evaporated under reduced pressure. The residue was partitioned between EtOAc and H_2O; the organic phase was dried (Na_2SO_4) and evaporated under reduced pressure. The residue was partitioned between EtOAc and H_2O; the organic phase was separated, dried (Na_2SO_4), and evaporated under reduced pressure. The residue was purified by a silica gel column chromatography to give **6** (1.47 g, yield 49%) as a pale yellow powder: m.p.150–152 °C. IR (KBr) 2227 cm^{-1} (CN) and broad band at 1752 cm^{-1} (C=O, acetoxy), 1644 cm^{-1} (C=O, amide) amidic groups); ^{1}H-NMR (DMSO-d_6) δ 1.12–1.85 (10H, m, cyclohexyl), 1.94, 1.96, 1.97, 2.01, 2.03, 2.05, 2.06 (21H, 7 s, 7C$\underline{H}_3$CO), 2.73 (1H, t, cyclohexyl-H-1,J = 2.86 Hz), 3.83 (3H, s, OC$\underline{H}_3$), 4.03–4.08 (3H, m, H-2′b, H-6′a and H-6′b), 4.15 (1H, dd, H-6″a, $J_{6''a,6'a}$ = 11.49, $J_{6''a,5'a}$ = 5.61 Hz), 4.26 (1H, m, H-5′b), 5.03 (1H, dd, H-6″b,$J_{6''b,5'b}$ = 6.32 Hz), 5.07 (1H, m, H-5′a), 5.19 (1H, dd, H-1′b, $J_{1'b,2'b}$ = 7.86 Hz), 5.22 (1H, dd, H-4′b,$J_{4'b,3'b}$ = 3.42, $J_{4'b,5'b}$ = 3.82 Hz), 5.36 (1H, dd, H-2′a, $J_{2'a,1'a}$ = 8.97, $J_{2'a,3'a}$ = 8.40 Hz), 5.38 (1H, dd, H-4′a, $J_{4'a,3'a}$ = 9.15, $J_{4'a,5'a}$ = 6.90 Hz), 5.64 (1H, d, H-3′b,$J_{3'b,4'b}$ = 3.45 Hz), 6.44 (1H, d, H-3′a, $J_{3'a,2'a}$ = 6.40 Hz), 6.56 (1H, d, H-1′a,$J_{1'a,2'a}$ = 8.82 Hz), 7.12 (1H, d, Ar–H, J = 7.99 Hz), 7.23 (1H, s,pyridone-H-5), 7.63 (1H, d, Ar–H, J = 8.39 Hz); Anal. Calcd for $C_{45}H_{54}N_2O_{19}$ (926.91): C, 58.31; H, 5.87; N, 3.02. Found: C, 58.30; H, 5.88; N, 3.02.

1-(β-D-galactopyranosyl-(1 → 4)-(β-D-glucopyranosyl)-6-cyclohexyl-4-(4-methoxyphenyl)-2-oxo-1,2-dihydropyridine-3-carbonitrile (**7**). A mixture of **6** (0.92 g, 1.0 mmol), Et_3N (1 mL), and H_2O (0.1 mL) in MeOH (5 mL) was stirred overnight at room temperature. The volatiles were evaporated under reduced pressure. The residue was repeatedly co-evaporated with MeOH until complete removal of Et_3N, and the residue was crystallized from 95% ethanol to give **7** (0.55 g, 87% yield) as a pale yellow

powder: m.p. 220–222 °C; IR (KBr) 3412 cm^{-1} (7OH), 2224 cm^{-1} (CN) and 1641 cm^{-1} (C=O, amide); ^{1}H-NMR (DMSO-d_6/D_2O) δ 1.16–1.89 (10H, m, cyclohexyl), 2.83 (1H, t, H-1cyclohexyl, J = 2.83 Hz), 3.85 (3H, s, OC$\underline{H}_3$), 3.94–4.12 (3H, m, H-2′b, H-6′a and H-6′b), 4.16 (1H, dd, H-6″a, $J_{6''a,6'a}$ = 11.59, $J_{6''a,5'a}$ = 5.64 Hz), 4.22 (1H, m, H-5′b), 4.56 (1H, dd, H-6″b, $J_{6''b,5'b}$ = 6.35 Hz), 4.98 (1H, m, H-5′a), 5.14 (1H, dd, H-1′b, $J_{1'b,2'b}$ = 7.76 Hz), 5.20 (1H, dd, H-4′b, $J_{4'b,3'b}$ = 3.62, $J_{4'b,5'b}$ = 3.86 Hz), 5.31 (1H, dd, H-2′a, $J_{2'a,1'a}$ = 8.87 Hz, $J_{2'a,3'a}$ = 8.44 Hz), 5.34 (1H, dd, H-4′a, $J_{4'a,3'a}$ = 9.05, $J_{4'a,5'a}$ = 6.56 Hz), 5.62 (1H, d, H-3′b, $J_{3'b,4'b}$ = 3.55 Hz), 6.24 (1H, d, H-3′a, $J_{3'a,2'a}$ = 6.43 Hz), 6.76 (1H, d, H-1′a, $J_{1'a,2'a}$ = 8.80 Hz), 7.15 (1H, d, Ar–H, J = 7.99 Hz), 7.25 (1H, s, pyridone-H-5), 7.69 (1H, d, Ar–H, J = 8.39 Hz); Anal. Calcd for $C_{31}H_{40}N_2O_{12}$ (632.66): C, 58.85; H, 6.37; N, 4.43. Found: C, 58.86; H, 6.37; N, 4.43.

4-(3-Cyano-6-cyclohexyl-4-(4-methoxyphenyl)-2-oxopyridin-1(2H)-yl)butyl acetate (8) and 4-((3-Cyano-6-cyclohexyl-4-(4-methoxyphenyl)pyridin-2-yl)oxy)butyl acetate (**10**). A mixture of **3** (1 g, 3.24 mmol) with 4-bromobutyl acetate (0.58 g, 3.0 mmol) was added to dry acetone (10 mL) in the presence of anhydrous K_2CO_3 (0.45 g, 3.27 mmol); the reaction mixture was heated for 15 h at reflux temperature. The reaction mixture was cooled to room temperature, and the insoluble were removed by filtration. The filtrate was evaporated under reduced pressure and then the formed solid product was separated by silica gel chromatography (eluate; $CH_2Cl_2$to 5% EtOAc/CH_2Cl_2) to give **8** (0.44 g, 32% yield) as a pale yellow oil and **10** (0.65 g, 48% yield) as a pale yellow oil. Spectral and analytical data for **8**: IR (KBr): 2219 cm^{-1} (CN), 1734 cm^{-1} (C=O, acetoxy) and 1644 cm^{-1} (C = O, amide);^{1}H NMR (DMSO-d_6)δ1.13–1.86 (14H, m, cyclohexyl, C$\underline{H}_{2b}$, and C$\underline{H}_{2c}$), 2.01 (3H, s, COC$\underline{H}_3$), 2.55 (1H, t, H-1cyclohexyl, J = 2.82 Hz), 3.83 (3H, s, OC$\underline{H}_3$), 4.07 (t, 1H,C$\underline{H}_{2a}$, J = 5.60, 5.95 Hz), 4.46 (t, 1H,C$\underline{H}_{2d}$, J = 7.93, 12.81 Hz), 6.41 (s, 1H, H-5 pyridone), 7.09 (2H, d, Ar–H, J = 8.00 Hz), 7.63 (2H, d, Ar–H, J = 9.19 Hz); Anal. Calcd for $C_{25}H_{30}N_2O_4$ (422.52): C, 71.07; H, 7.16; N, 6.63. Found: C, 71.08; H, 7.19; N, 6.66. Spectral and analytical data for **10**: IR (KBr)2223 cm^{-1} (CN) and 1734 cm^{-1} (C=O, acetoxy); ^{1}H-NMR (DMSO-d_6) δ1.12–1.87 (14H, m, cyclohexyl, C$\underline{H}_{2b}$, and C$\underline{H}_{2c}$), 1.97 (3H, s, COC$\underline{H}_3$), 2.62 (1H, t, cyclohexyl-H-1, J = 2.81 Hz), 3.81 (3H, s, OC$\underline{H}_3$), 4.05 (1H, t, C$\underline{H}_{2a}$, J = 6.01 Hz), 4.45 (1H, t, C$\underline{H}_2$(d), J = 5.62 Hz), 7.03 (1H, s, pyridone-H-5), 7.07 (2H, d, Ar–H, J = 8.69 Hz), 7.58 (2H, d, Ar, J = 8.69 Hz);Anal. Calcd. for $C_{25}H_{30}N_2O_4$ (422.52): C, 71.07; H, 7.16; N, 6.63. Found: C, 71.09; H, 7.15; N, 6.64.

6-Cyclohexyl-1-(4-hydroxybutyl)-4-(4-methoxyphenyl)-2-oxo-1,2-dihydropyridine-3-carbonitrile (9). A mixture of **8** (0.5 g, 1.18 mmol), Et_3N (1 mL), and H_2O (0.1 mL) in MeOH (5 mL) was stirred overnight at room temperature. The volatiles were evaporated under reduced pressure. The residue was repeatedly co-evaporated with MeOH until complete removal of Et_3N, and the residue was purified by silica gel column chromatography (eluate 10% MeOH/CH_2Cl_2) to give **9** (0.36 g, 80% yield) as a pale yellow syrup: IR (KBr) 3428 cm^{-1} (OH), 2221 cm^{-1} (CN) and 1642 cm^{-1} (C=O, amide); ^{1}H-NMR (DMSO-d_6/D_2O) δ 1.22–1.87 (14H, m, cyclohexyl, and C$\underline{H}_{2b}$, and C$\underline{H}_{2c}$), 2.68 (1H, t, H1-cyclohexyl, J = 2.80 Hz), 3.44 (2H, t, C$\underline{H}_{2d}$, J = 6.42 Hz), 3.81 (3H, s, OC$\underline{H}_3$), 4.43 (2H, t, C$\underline{H}_{2a}$, J = 6.82 Hz), 7.02 (1H, s, H-5 pyridone), 7.08 (2H, d, Ar–H, J = 7.64 Hz), 7.59 (2H, d, Ar–H, J = 7.64 Hz); Anal. Calcd for $C_{23}H_{28}N_2O_3$ (380.48): C, 72.60; H, 7.42; N, 7.36. Found: C, 72.63; H, 7.45; N, 7.35.

6-Cyclohexyl-2-(4-hydroxybutoxy)-4-(4-methoxyphenyl) nicotinonitrile (**11**). A mixture of **10** (0.42 g, 1.0 mmol), Et_3N (1 mL), and H_2O (0.1 mL) in MeOH (5 mL) was stirred overnight at room temperature. The volatiles were evaporated under reduced pressure. The residue was repeatedly co-evaporated with MeOH until complete removal of Et_3N, and the residue was purified by a silica gel column chromatography (eluate: 7% MeOH/CH_2Cl_2) to give **11** (0.31 g, 83% yield) as a colorless syrup: IR (KBr) 3426 cm^{-1} (OH) and 2222 cm^{-1} (CN); ^{1}H-NMR (DMSO-d_6) δ 1.33–1.89 (14 H, m, cyclohexyl, C$\underline{H}_{2b}$, and C$\underline{H}_{2c}$), 2.68 (1H, t, H-1cyclohexyl, J = 2.80 Hz), 3.08 (1H, d, exchangeable with D_2O, OH, J = 7.21 Hz), 3.43 (2H, t, C$\underline{H}_{2d}$, J = 5.62, J = 6.40 Hz), 3.83 (3H, s, OC$\underline{H}_3$), 4.43 (2H, t, C$\underline{H}_{2a}$), J = 6.42, J = 6.01 Hz), 7.04 (1H, s, H-5pyridone), 7.08 (2H, d, Ar–H, J = 8.46 Hz), 7.60 (2H, d, Ar–H, J = 8.46 Hz); Anal. Calcd for $C_{23}H_{28}N_2O_3$ (380.48): C, 72.60; H, 7.42; N, 7.36. Found: C, 72.65; H, 7.44; N, 7.39.

2-((3-Cyano-6-cyclohexyl-4-(4-methoxyphenyl)-2-oxopyridin-1(2H)-yl)methoxy)ethyl acetate (**12**) *and 2-(((3-Cyano-6-cyclohexyl-4-(4-methoxyphenyl)pyridin-2-yl)oxy)methoxy)ethyl acetate* (**14**). A mixture of **3** (1 g, 3.24 mmol) with (2-acetoxyethoxy)methyl bromide (0.59 g, 3.0 mmol) was added to dry acetone (10 mL) in the presence of anhydrous K_2CO_3 (0.45 g, 3.27 mmol); the reaction mixture was heated for 15 h under reflux temperature. The reaction mixture was cooled to room temperature, and the inorganic residue was removed by filtration. The solvent was evaporated under reduced pressure and then the residue was purified by silica gel chromatography (eluate: $CH_2Cl_2$to 3% EtoAc in CH_2Cl_2) to give **12** (0.37 g, 27% yield) as a colorless oil and **14** (0.57 g, 42% yield) as a pale yellow syrup. Spectral and analytical data for **12**: IR (KBr) 2222 cm^{-1} (CN), 1735 cm^{-1} (C=O, acetoxy) and 1647 cm^{-1} (C=O, amide); ^{1}H-NMR (DMSO-d_6) δ 1.58–1.84 (10H, m, cyclohexyl), 2.01 (3H, s, COC$\underline{H}_3$), 2.26 (1H, t, H1-cyclohexyl-, J = 2.84 Hz), 3.79 (3H, s, OC$\underline{H}_3$), 4.06 (3H, t, C$\underline{H}_2$(c), J = 4.81, 6.00 Hz), 4.47 (3H, t, C$\underline{H}_2$(d), J = 5.63, 4.81 Hz), 4.60 (2H, s, CH_2(a)), 7.05 (1H, s, H5-pyridone), 7.63 (2H, d, Ar–H, J = 8.82 Hz), 7.90 (2H, d, Ar–H,

$J = 8.82$ Hz); ^{13}C-NMR (DMSO-d_6) δ 18.80, 20.67, 24.10, 25.15, 28.84, 30.62, 60.32, 63.32, 66.91, 99.63, 116.0, 120.2, 127.9, 128.1, 129.3, 130.8, 133.2, 134.5, 159.4, 170.3; Anal. Calcd for $C_{24}H_{28}N_2O_5$ (424.49): C, 67.91; H, 6.65; N, 6.60. Found: C, 67.94; H, 6.63; N, 6.60. Spectral and analytical data for **14**: IR (KBr)2222 cm^{-1} (CN) and 1740 cm^{-1} (C=O, acetoxy); ^{1}H-NMR (DMSO-d_6) δ 1.47–1.76 (10H, m, cyclohexyl), 1.98 (3H, s, COC$\underline{H}_3$), 2.65 (1H, t, cyclohexyl-H-1, $J = 2.85$ Hz), 3.83 (3H, s, OC$\underline{H}_3$), 4.05 (2H, t, C$\underline{H}_2$(c), $J = 6.42$ Hz), 4.44 (2H, t, C$\underline{H}_{2d}$, $J = 5.91$ Hz), 4.64 (2H, s, C$\underline{H}_2$(a)), 7.03 (1H, s, pyridone-H-5), 7.08 (2H, d, Ar–H, $J = 8.83$ Hz), 7.62 (2H, d, Ar–H, $J = 8.83$ Hz); Anal. Calcd for $C_{24}H_{28}N_2O_5$ (424.49): C, 67.91; H, 6.65; N, 6.60. Found: C, 67.89; H, 6.64; N, 6.60.

6-Cyclohexyl-1-((2-hydroxyethoxy)methyl)-4-(4-methoxyphenyl)-2-oxo-1,2-dihydropyridine-3-carbonitrile (**13**). A mixture of **12** (0.42 g, 1.0 mmol), Et_3N (1 mL), and H_2O (0.1 mL) in MeOH (5 mL) was stirred overnight at room temperature. The volatiles were evaporated under reduced pressure. The residue was repeatedly co-evaporated with MeOH until complete removal of Et_3N, and the residue was crystallized from 95% ethanol to give **13** (0.32 g, 84% yield) as a pale yellow powder: m.p. 60–62 °C; IR (KBr) 3420 cm^{-1} (OH), 2222 cm^{-1} (CN) and 1644 cm^{-1} (C=O, amide); ^{1}H-NMR (DMSO-d_6/D_2O) δ 1.55–1.86 (10H, m, cyclohexyl), 2.26 (1H, d, H1-cyclohexyl, $J = 2.62$ Hz), 3.31 (3H, s, OC$\underline{H}_3$), 3.47 (2H, t, C$\underline{H}_{2d}$, $J = 5.60$ Hz), 4.14 (2H, t, C$\underline{H}_{2c}$, $J = 6.52$ Hz), 4.49 (2H, s, C$\underline{H}_{2a}$), 7.03–7.34 (5H, m, Ar–H); Anal. Calcd for $C_{22}H_{26}N_2O_4$ (382.45): C, 69.09; H, 6.85; N, 7.32. Found: C, 69.06; H, 6.87; N, 7.32.

6-Cyclohexyl-2-((2-hydroxyethoxy)methoxy)-4-(4-methoxyphenyl)-nicotinonitrile (**15**). A mixture of **14** (0.42 g, 1.0 mmol), Et_3N (1 mL), and H_2O (0.1 mL) in MeOH (5 mL) was stirred overnight at room temperature. The volatiles were evaporated under reduced pressure. The residue was repeatedly co-evaporated with MeOH until complete removal of Et_3N, and the residue was purified by a silica gel column chromatography (eluate: 6% MeOH in CH_2Cl_2) to give **15** (0.33 g, 87% yield) as a colorless syrup: IR (KBr) 3428 cm^{-1} (OH) and 2222 cm^{-1} (CN); ^{1}H-NMR (DMSO-d_6) δ1.24–1.89 (10H, m, cyclohexyl), 2.69 (1H, t, cyclohexyl-H-1, $J = 2.83$ Hz), 3.45 (2H, t, C$\underline{H}_2$(d), $J = 5.6$ Hz), 3.75 (2H, t, C$\underline{H}_2$(c), $J = 4.43$ Hz), 3.83 (3H, s, OC$\underline{H}_3$), 4.45 (2H, s, C$\underline{H}_2$(a)), 4.87 (1H, t, OH, $J = 4.65$ Hz, exchange with D_2O), 7.06 (1H, s, H5-pyridine-), 7.09 (2H, d, Ar–H, $J = 8.00$ Hz), 7.61 (2H, d, Ar–H, $J = 7.69$ Hz); Anal. Calcd. for $C_{22}H_{26}N_2O_4$ (382.45): C, 69.09; H, 6.85; N, 7.32. Found: C, 69.11; H, 6.88; N, 7.30.

6-Cyclohexyl-4-(4-methoxyphenyl)-2-oxo-1-(prop-2-yn-1-yl)-1,2-dihydropyridine-3-carbonitrile (**16**) *and 6-Cyclohexyl-4-(4-methoxyphenyl)-2-(prop-2-yn-1-yloxy) nicotinonitrile* (**17**). A mixture of **3** (2 g, 6.48 mmol) with propargyl bromide (0.7 g, 6.5 mmol) was added to dry acetone (20 mL) in the presence anhydrous K_2CO_3 (0.9 g, 6.5 mmol); the reaction mixture was heated under reflux for 15 h. The inorganic residue was removed by filtration; the solvent was evaporated under reduced pressure and then the formed solid product was separated by silica gel column chromatography (eluate: CH_2Cl_2 to 1% MeOH/CH_2Cl_2) to give **16** (0.67 g, 30% yield) as a yellow oil and **17** (0.65 g, 29% yield) as pale yellow oil. Spectral and analytical data for **16**: IR (KBr) 2216 cm^{-1} (CN) and 1641 cm^{-1} (C=O, amide); ^{1}H-NMR (DMSO-d_6) δ 1.14–1.96 (10 H, m, cyclohexyl), 2.90 (1H, t, H-1-cyclohexyl, $J = 2.81$ Hz), 3.34 (1H, s, ≡C-$\underline{H}$), 3.84 (3H, s, OC$\underline{H}_3$), 4.99 (2H, d, *N*-C$\underline{H}_2$, $J = 2.41$ Hz), 6.46 (1H, s, H-5-pyridone), 7.09 (2H, d, Ar–H, $J = 8.69$ Hz), 7.65 (2H, d, Ar–H, $J = 9.01$ Hz); Anal. Calcd for $C_{22}H_{22}N_2O_2$ (346.42): C, 76.28; H, 6.40; N, 8.09. Found: C, 76.26; H, 6.44; N, 8.08. Spectral and analytical data for **17**: IR (KBr) 2222 cm^{-1} (CN); ^{1}H-NMR (DMSO-d_6) δ 1.22–1.93 (10 H, m, cyclohexyl), 2.72 (1H, t, H-1-cyclohexyl, $J = 2.78$ Hz), 3.51 (1H, s, ≡C-$\underline{H}$), 3.84 (3H, s, OC$\underline{H}_3$), 5.14 (2H, d, OC$\underline{H}_2$, $J = 2.42$ Hz), 7.09–7.12 (3H, m, Ar–H and H5-pyridone), 7.61 (2H, d, Ar–H, $J = 8.69$ Hz); Anal. Calcd for $C_{22}H_{22}N_2O_2$ (346.42): C, 76.28; H, 6.40; N, 8.09. Found: C, 76.25; H, 6.45; N, 8.06.

1-Allyl-6-cyclohexyl-4-(4-methoxyphenyl)-2-oxo-1,2-dihydropyridine-3-carbonitrile (**18**) and *2-(Allyloxy)-6-cyclohexyl-4-(4-methoxyphenyl)nicotinonitrile* (**19**): A mixture of **3** (1 g, 3.24 mmol) with allyl bromide (0.36 g, 3.0 mmol) was added to dry acetone (10 mL) in the presence anhydrous K_2CO_3 (0.45 g, 3.27 mmol); the reaction mixture was heated under reflux for 15 h. The inorganic residue was removed by filtration; the solvent was evaporated under reduced pressure and then the formed solid product was separated by silica gel column chromatography (eluate: CH_2Cl_2 to 5% EtOAc/CH_2Cl_2) to give **18** (0.37 g, 32% yield) as a yellow powder and **19** (0.39 g, 39%yield) as a white powder. Spectral and analytical data for **19**: m.p. 90–92 °C; IR (KBr) 2213 cm^{-1} (CN) and 1644 cm^{-1} (C=O, amide); ^{1}H-NMR (DMSO-d_6) δ 1.22–1.91 (10H, m, cyclohexyl), 2.70 (1H, t, cyclohexyl-H-1, $J = 2.39$ Hz), 2.84 (3H, s, OC$\underline{H}_3$), 4.98 (2H, d, C$\underline{H}_2$(a), $J = 4.49$ Hz), 5.31 (2H, d, Hc', $J = 10.49$ Hz), 5.43 (2H, d, Hc, $J = 17.41$ Hz), 6.12 (1H, m, Hb), 7.06 (1H, s, pyridone-H-5), 7.09 (2H, d, Ar–H, $J = 8.69$), 7.60 (2H, d, Ar–H, $J = 8.69$); Anal. Calcd for $C_{22}H_{24}N_2O_2$ (348.44): C, 75.83; H, 6.94; N, 8.04. Found: C, 75.85; H, 6.95; N, 8.03. Spectral and analytical data for **18**: m.p. 60–62 °C; IR (KBr) 2220 cm^{-1} (CN); ^{1}H-NMR (DMSO-d_6) δ 1.15–1.86 (10H, m, cyclohexyl), 2.72 (1H, t, cyclohexyl-H-1, $J = 2.49$ Hz), 3.85 (3H, s, OC$\underline{H}_3$), 4.79 (2H, d, C$\underline{H}_2$(a), $J = 4.49$ Hz), 5.06 (1H, d, Hc, $J = 17.40$ Hz), 5.19 (1H, d, Hc', $J = 10.79$ Hz), 5.92–6.05 (1H, m, Hb), 6.42 (1H, s, pyridone-H-5), 7.09 (2H, d, Ar–H, $J = 8.69$ Hz), 7.64 (2H, d, Ar–H, $J = 9.01$ Hz); Anal. Calcd for $C_{22}H_{24}N_2O_2$

(348.44): C, 75.83; H, 6.94; N, 8.04. Found: C, 75.80; H, 6.92; N, 8.07.

6-Cyclohexyl-4-(4-methoxyphenyl)-1-(oxiran-2-ylmethyl)-2-oxo-1,2-dihydropyridine-3-carbonitrile (**20**). A mixture of **3** (1 g, 3.24 mmol) and anhydrous K_2CO_3 (0.45 g, 3.27 mmol) in dry acetone (10 mL) was added to epichlorohydrin (0.27 g, 3.0 mmol), and the mixture was heated under reflux for 15 h. The inorganic residue was removed by filtration; the solvent was evaporated under reduced pressure and then the formed solid product was purified by silica gel column chromatography (eluate: 1% MeOH in CH_2Cl_2) to give **20** (0.47 g, 42%) as a colorless syrup, IR (KBr) 2222 cm^{-1} (CN) and 1608 cm^{-1} (C=O, amide); ^{1}H-NMR (DMSO-d_6) δ 1.14–1.84 (10H, m, cyclohexyl), 2.51 (1H, t, cyclohexyl, J = 2.81 Hz), 3.83 (3H, s, OCH_3), 4.33–4.36 (1H, m, Hc'-oxiran ring), 4.44 (1H, t, Hc-oxiran ring, J = 4.82 Hz), 4.49–4.60 (2H, m, Ha, and Hb), 5.44 (1H, m, Ha'), 7.04 (1H, s, H5-pyridone), 7.11 (2H, d, Ar–H, J = 9.29 Hz), 7.57 (2H, d, Ar–H, 8.69 Hz); Anal. Calcd for $C_{22}H_{24}N_2O_3$ (364.44): C, 72.50; H, 6.64; N, 7.69. Found: C, 72.55; H, 6.66; N, 7.68.

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