An efficient one-pot synthesis and in vitro antimicrobial activity of new pyridine derivatives bearing the tetrazoloquinoline nucleus

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Abstract
A new series of 2-amino-3-cyano-4-tetrazoloquinolinylpyridine derivatives has been synthesized by the one-pot cyclocondensation reaction of a tetrazolo[1,5-a]quinoline-4-carbaldehyde, malononitrile, a heterocyclic/aromatic methyl ketone and ammonium acetate. All the synthesized compounds were subjected to in vitro antimicrobial screening against a panel of pathogenic strains of bacteria and fungi. Some of the compounds were found to be equipotent or more potent than commercial antibiotics as evident from the results.

Keywords: 2-Amino-3-cyano-4-tetrazoloquinolinylpyridine, tetrazolo[1,5-a]quinoline-4-carbaldehyde, one-pot synthesis, antimicrobial activity, MIC

Introduction

Many naturally occurring and synthetic compounds bearing pyridine scaffold possess interesting biological properties.¹ In association with those, 2-amino-3-cyanopyridine derivatives have been identified as IKK-β inhibitors² along with its importance and utility as intermediates in preparing variety of heterocyclic compounds.³ Consequently, the synthesis of 2-amino-3-cyanopyridine derivatives keeps on attracting much interest in organic chemistry. Various routes for the synthesis of 2-amino-3-cyanopyridine derivatives have been reported using two-component as well as three-component reactions.⁴-⁷ Tu and coworkers have reported a facile synthesis of 2-amino-3-cyanopyridine derivatives in a one-pot reaction using aromatic aldehyde, methyl ketone, malononitrile and ammonium acetate.⁴ A literature survey shows that a number of pyridine derivatives have been synthesized using various aldehydes but not a single reference have been found where tetrazolo[1,5-a]quinoline-4-carbaldehyde is used. We wish to report herein this heterocyclic aldehyde which is biologically active⁸-¹⁰ with a view to obtaining more active heterocyclic system containing two biologically active moieties quinoline¹¹-¹³ and pyridine¹⁴,¹⁵ together. The most suitable protocol for the synthesis of functionalized organic compounds would be a one-pot reaction due to the fact that the synthesis can be performed without the
isolation of the intermediates, without discharging any functional groups in short reaction time.\textsuperscript{16} Hence, in the present investigation, we report an efficient one-pot multicomponent synthesis of 2-amino-3-cyanopyridine derivatives having tetrazoloquinoline nucleus which have also been recognized as promising new scaffold to endow good biological properties\textsuperscript{17,18} such as anti-inflammatory and antimicrobial activity.\textsuperscript{19-22}

**Results and Discussion**

In the present study, an effort has been made to undertake the synthesis of 2-amino-6-het/aryl-4-(7-(un)-substituted-(tetrazolo[1,5-a]quinolin-4-yl))pyridine-3-carbonitriles through a one step process. For this purpose, the required tetrazolo[1,5-a]quinoline derivatives 1\textsuperscript{a-c} were prepared from 2-chloro-3-formylquinoline and sodium azide by our known literature process.\textsuperscript{23} The target compounds 4\textsuperscript{a-x} were prepared in moderate to good yield (52-77\%) by the reaction of tetrazolo[1,5-a]quinoline-4-carbaldehyde 1\textsuperscript{a-c}, malononitrile 2, (het)aryl methyl ketone 3\textsuperscript{a-h} and ammonium acetate in absolute alcohol (Scheme 1). The formation of compounds 4\textsuperscript{a-x} may proceed via imine formed from ketone and ammonium acetate, imine reacts with alkylidenemalononitrile formed from Knoevenagel condensation of aldehyde and malononitrile, followed by cycloaddition, isomerization and aromatization to afford the 2-amino-3-cyano-4-tetrazoloquinolinylpyridine derivatives 4\textsuperscript{a-x}. The identity of the product was determined by IR, \textsuperscript{1}H NMR, and \textsuperscript{13}C NMR spectral studies. The IR spectrum of compound 4\textsuperscript{a} exhibited absorption at 3410 cm\textsuperscript{-1} (asymmetric N-H stretching) and 3314 cm\textsuperscript{-1} (symmetric N-H stretching) for –NH\textsubscript{2}, 2214 cm\textsuperscript{-1} for –CN, 3015 cm\textsuperscript{-1} for (aromatic C-H stretching) and 1400 to 1600 cm\textsuperscript{-1} for (C=C aromatic and C=N stretching of pyridine). The \textsuperscript{1}H NMR spectra of compound 4\textsuperscript{a} showed the absence of the aldehyde proton, moreover singlets at $\delta$ 7.06 ppm and multiplets at $\delta$ 7.20-8.71 ppm appeared for amine and aromatic protons respectively. The \textsuperscript{13}C NMR spectrum is in good agreement with the structure assigned. All the aromatic carbons of compounds 4\textsuperscript{a} showed signals around $\delta$ 115.7–154.3 ppm in the \textsuperscript{13}C NMR spectra. The signal at $\delta$ 96.0 ppm is assigned to carbon attached with carbonitrile. Besides, the structure of the compound was well confirmed by its mass spectral studies. Mass spectra of compound 4\textsuperscript{i} and 4\textsuperscript{l} gave molecular ion peak at \textit{m/z} 378 (M+1) and \textit{m/z} 368 (M+1) corresponding to molecular formula $C_{22}H_{15}N_{7}$ and $C_{20}H_{13}N_{7}O$ respectively (Scheme 1). The elemental analysis values are in good agreement with theoretical data. Similarly, all these compounds were characterized on the basis of spectral studies. All the compounds were screened for their antibacterial and antifungal activity.
Antimicrobial activity

The *in vitro* antimicrobial activity of all the synthesized compounds was carried out by broth microdilution method. Mueller Hinton broth was used as nutrient medium to grow and dilute the compound suspension for the test bacteria and Sabouraud Dextrose broth used for fungal nutrition. Inoculum size for test strain was adjusted to $10^8$ CFU [Colony Forming Unit] per milliliter by comparing the turbidity. The strains employed for the activity were procured from [MTCC – Micro Type Culture Collection] Institute of Microbial Technology, Chandigarh.

The compounds 4a-x were screened for their antibacterial activity against *Bacillus subtilis* (MTCC 441), *Clostridium tetani* (MTCC 449), *Streptococcus pneumoniae* (MTCC 1936), *Escherichia coli* (MTCC 443), *Salmonella typhi* (MTCC 98), *Vibrio cholerae* (MTCC 3906) as well as antifungal activity against *Aspergillus fumigatus* (MTCC 3008) and *Candida albicans* (MTCC 227). DMSO was used as vehicle to get desired concentration of compounds to test upon microbial strains. The lowest concentration, which showed no visible growth after spot subculture was considered as MIC for each compound. The standard antibiotics used for
comparison in the present study were ampicillin for evaluating antibacterial activity as well as griseofulvin and nystatin for antifungal activity. The protocols are summarized in (Table 1).

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Ampi.: Ampicillin, Grise.: Griseofulvin, Nyst.: Nystatin

An examination of the data (Table 1) reveals that amongst all the synthesized compounds 4a-x, compound 4o exhibited excellent activity against Gram positive bacteria *Streptococcus pneumoniae* and Gram negative bacteria *Vibrio cholerae* while compounds 4m and 4w are found to be highly active against Gram negative bacteria *Escherichia coli* and *Salmonella typhi* respectively as compared to standard antibiotic ampicillin.

Compounds 4m, 4n, 4q, 4r, 4t and 4w are found to be more potent as compared to standard antibiotic ampicillin against Gram positive bacteria *Bacillus subtilis*. In case of Gram positive bacteria *Clostridium tetani*, compounds 4b, 4c, 4g, 4k, 4l, 4s and 4v are found to be more potent than ampicillin.
Antifungal study revealed that compounds 4d, 4g, 4j, 4r, 4t and 4v are more potent as compared to standard fungicidal griseofulvin against Candida albicans. Most of the compounds were not found sufficiently potent to inhibit Aspergillus fumigatus.

Conclusions

A series of some new 2-amino-3-cyano-4-tetrazoloquinolinylpyridine derivatives has been synthesized through a facile one-pot multicomponent reaction. This synthetic strategy allows the construction of relatively complicated nitrogen containing heterocyclic system as well as the introduction of various aromatic and heteroaromatic substitutions into 4- and 6- positions of pyridine. It can be concluded from Table 2 that compound 4o having methyl group on tetrazoloquinoline nucleus and 3-pyridyl substitution on pyridine is highly active against Streptococcus pneumoniae as well as Vibrio cholerae. From the activity data, it is worth mentioning that minor change in molecular configuration of these compounds profoundly influences the activity.

Experimental Section

General. All the reagents were obtained commercially and used with further purification. All melting points were taken in open capillaries and are uncorrected. The monitoring of the progress of all reactions and homogeneity of the synthesized compounds was carried out by TLC. TLC was run using TLC aluminum sheets silica gel 60 F254 (Merck). Elemental analysis (% C, H, N) was carried out by Perkin Elmer 2400 CHN elemental analyzer at Sophisticated Instrumentation Centre for Applied Research & Training (SICART), Vallabh Vidyanagar. IR spectra were recorded on a Shimadzu FTIR 8401 spectrophotometer in KBr. 1H NMR and 13C NMR spectra were recorded on a Bruker Avance 400 MHz spectrometer using solvent peak as internal standard. Mass spectra were scanned on a Shimadzu LCMS 2010 spectrometer.

General procedure for the synthesis of 2-amino-6-hetaryl-4-(7-(un)-substituted-tetrazolo[1,5-a]quinolin-4-yl))pyridine-3-carbonitriles 4a-x

7-(Un)substituted-tetrazolo[1,5-a]quinoline-4-carbaldehyde 1a-c (5 mmole), malononitrile 2 (5 mmole), (het)aryl methyl ketone 3a-h (5 mmole), ammonium acetate (40 mmole) and absolute alcohol (15 ml) were charged in a 50 ml round bottom flask. Then, the reaction mixture was refluxed for 2 to 2.5 hr. Progress of reaction was monitored by the TLC. After the completion of reaction, the reaction mixture was cooled to room temperature and stirred for 0.5 hr. The resulting solid was collected by filtration and washed well with absolute alcohol to obtain the pure solid sample of product 4a-x.
2-Amino-6-phenyl-4-(tetrazolo[1,5-a]quinolin-4-yl)pyridine-3-carbonitrile 4a. Yield 72%, m.p. 264 °C, Anal. Calcd. for C_{21}H_{13}N_{7}: C 69.41, H 3.60, N 26.98% Found: C 69.32, H 3.56, N 26.72%. IR (KBr, cm\(^{-1}\)): 3410, 3314 (NH\(_2\)), 2214 (CN), 3015 (Ar-C-H). \(^1\)H NMR (400 MHz, DMSO-\(d_6\)) \(\delta\): 7.20-8.71 (m, 11H, Ar-H), 7.06 (s, 1H, NH\(_2\)). \(^13\)C NMR (400 MHz, DMSO-\(d_6\)) \(\delta\): 96.0 (C-CN), 115.7, 116.2, 116.7, 120.0, 122.5, 123.9, 128.9, 129.2, 130.1, 130.4, 130.6, 133.0, 134.9, 137.7, 143.1, 146.9, 150.4, 154.3 (Ar-C).

2-Amino-6-(4-methylphenyl)-4-(tetrazolo[1,5-a]quinolin-4-yl)pyridine-3-carbonitrile 4b. Yield 65%, m.p. 276 °C, Anal. Calcd. for C_{22}H_{15}N_{7}: C 70.01, H 4.00, N 25.97% Found: C 69.72, H 3.92, N 25.91%. IR (KBr, cm\(^{-1}\)): 3405, 3312 (NH\(_2\)), 2210 (CN), 3005 (Ar-C-H). \(^1\)H NMR (400 MHz, DMSO-\(d_6\)) \(\delta\): 2.39 (s, 3H, CH\(_3\)), 7.03 (s, 1H, NH\(_2\)), 7.18-8.71 (m, 10H, Ar-H). \(^13\)C NMR (400 MHz, DMSO-\(d_6\)) \(\delta\): 21.3 (CH\(_3\)), 96.0 (C-CN), 115.8, 116.8, 119.9, 122.5, 124.0, 129.1, 130.4, 133.0, 134.8, 138.3, 140.7, 143.0, 146.4, 146.8, 150.5, 154.4, 160.4, 166.9 (Ar-C).

2-Amino-6-(4-methoxyphenyl)-4-(tetrazolo[1,5-a]quinolin-4-yl)pyridine-3-carbonitrile 4c. Yield 66%, m.p. 256-259 °C, Anal. Calcd. for C_{22}H_{15}N_{7}O: C 67.16, H 3.84, N 24.92% Found: C 67.13, H 3.71, N 25.01%. IR (KBr, cm\(^{-1}\)): 3400, 3310 (NH\(_2\)), 2180 (CN), 3000 (Ar-C-H). \(^1\)H NMR (400 MHz, DMSO-\(d_6\)) \(\delta\): 3.97 (s, 3H, OCH\(_3\)), 7.10 (s, 1H, NH\(_2\)), 7.30-8.51 (m, 10H, Ar-H). \(^13\)C NMR (400 MHz, DMSO-\(d_6\)) \(\delta\): 56.3 (OCH\(_3\)), 95.8 (C-CN), 116.8, 117.5, 119.0, 123.8, 124.9, 128.1, 131.2, 133.0, 134.6, 138.4, 141.7, 143.4, 146.4, 146.6, 151.5, 154.7, 161.4, 166.0 (Ar-C).

2-Amino-6-(2-furyl)-4-(tetrazolo[1,5-a]quinolin-4-yl)pyridine-3-carbonitrile 4d. Yield 76%, m.p. 273 °C, Anal. Calcd. for C_{19}H_{11}N_{7}O: C 64.56, H 3.13, N 27.74% Found: C 64.62, H 3.11, N 27.67%. IR (KBr, cm\(^{-1}\)): 3416, 3314 (NH\(_2\)), 2190 (CN), 3005 (Ar-C-H). \(^1\)H NMR (400 MHz, DMSO-\(d_6\)) \(\delta\): 94.7 (C-CN), 116.4, 119.2, 121.7, 124.4, 128.5, 129.6, 130.1, 134.1, 136.5, 137.7, 138.4, 142.5, 146.4, 148.5, 148.7, 151.4, 153.5, 154.5 (Ar-C).

2-Amino-6-(2-thienyl)-4-(tetrazolo[1,5-a]quinolin-4-yl)pyridine-3-carbonitrile 4e. Yield 68%, m.p. 243-245 °C, Anal. Calcd. for C_{19}H_{11}N_{7}S: C 61.77, H 3.00, N 26.54% Found: C 61.66, H 2.98, N 26.49%. IR (KBr, cm\(^{-1}\)): 3414, 3314 (NH\(_2\)), 2190 (CN), 3016 (Ar-C-H). \(^1\)H NMR (400 MHz, DMSO-\(d_6\)) \(\delta\): 93.8 (C-CN), 115.4, 117.2, 120.7, 123.4, 126.9, 129.0, 130.1, 133.5, 136.5, 137.0, 137.4, 141.5, 144.8, 147.5, 148.4, 150.4, 153.8, 155.1 (Ar-C).

2-Amino-6-(2-pyridyl)-4-(tetrazolo[1,5-a]quinolin-4-yl)pyridine-3-carbonitrile 4f. Yield 77%, m.p. 265 °C, Anal. Calcd. for C_{20}H_{12}N_{8}: C 65.92, H 3.32, N 30.75% Found: C 66.08, H 3.24, N 30.61%. IR (KBr, cm\(^{-1}\)): 3416, 3330 (NH\(_2\)), 2234 (CN), 3028 (Ar-C-H). \(^1\)H NMR (400 MHz, DMSO-\(d_6\)) \(\delta\): 7.42 (s, 2H, NH\(_2\)), 7.52-8.61 (m, 9H, Ar-H). \(^13\)C NMR (400 MHz, DMSO-\(d_6\)) \(\delta\): 98.1 (C-CN), 116.4, 117.6, 122.7, 124.4, 125.5, 128.8, 130.9, 132.1, 135.8, 136.6, 137.2, 142.7, 146.7, 148.2, 148.6, 150.3, 152.2, 157.9, 158.5 (Ar-C).

2-Amino-6-(3-pyridyl)-4-(tetrazolo[1,5-a]quinolin-4-yl)pyridine-3-carbonitrile 4g. Yield 69%, m.p. 270-273 °C, Anal. Calcd. for C_{20}H_{12}N_{8}: C 65.92, H 3.32, N 30.75% Found: C 66.02, H 3.29, N 30.66%. IR (KBr, cm\(^{-1}\)): 3410, 3334 (NH\(_2\)), 2216 (CN), 3020 (Ar-C-H). \(^1\)H NMR (400
MHz, DMSO-$d_6$): $\delta$ 7.22 (s, 2H, NH$_2$), 7.46-9.07 (m, 10H, Ar-H). $^{13}$C NMR (400 MHz, DMSO-$d_6$): $\delta$: 98.9 (C-CN), 115.4, 118.2, 120.7, 123.4, 126.5, 128.0, 130.0, 133.1, 135.5, 136.3, 138.5, 143.5, 147.4, 148.5, 148.9, 151.3, 153.2, 156.1, 158.2 (Ar-C).

2-Amino-6-(4-pyridyl)-4-(tetrazolo[1,5-a]quinolin-4-yl)pyridine-3-carbonitrile 4h. Yield 72%, m.p. 258-260 °C, Anal. Calcd. for C$_{20}$H$_{12}$N$_8$: C 65.92, H 3.32, N 30.75% Found: C 66.04, H 3.35, N 30.67%. IR (KBr, cm$^{-1}$): 3400, 3336 (NH$_2$), 2208 (CN), 3005 (Ar-C-H). $^1$H NMR (400 MHz, DMSO-$d_6$): $\delta$: 7.30 (s, 2H, NH$_2$), 7.45-8.91 (m, 10H, Ar-H). $^{13}$C NMR (400 MHz, DMSO-$d_6$): $\delta$: 97.0 (C-CN), 119.5, 121.4, 123.0, 126.7, 128.6, 129.7, 131.4, 136.4, 138.7, 141.0, 143.1, 145.5, 146.9, 148.4, 149.5, 151.2, 153.4, 154.4 (Ar-C).

2-Amino-6-phenyl-4-(7-methyl-(tetrazolo[1,5-a]quinolin-4-yl))pyridine-3-carbonitrile 4i. Yield 71%, m.p. 274 °C, Anal. Calcd. for C$_{22}$H$_{15}$N$_7$: C 70.01, H 4.00, N 25.97% Found: C 69.94, H 3.92, N 25.89%. IR (KBr, cm$^{-1}$): 3400, 3310 (NH$_2$), 2214 (CN), 3032 (Ar-C-H). $^1$H NMR (400 MHz, DMSO-$d_6$): $\delta$: 2.50 (s, 3H, CH$_3$), 7.03-8.60 (m, 10H, Ar-H). $^{13}$C NMR (400 MHz, DMSO-$d_6$): $\delta$: 21.3 (CH$_3$), 96.0 (C-CN), 115.8, 119.4, 121.5, 126.4, 126.9, 128.2, 130.9, 132.1, 134.4, 136.5, 138.8, 140.5, 144.9, 147.8, 149.3, 150.5, 153.4, 154.4 (Ar-C), MS: (M+1) 378.

2-Amino-6-(4-methylphenyl)-4-(7-methyl-(tetrazolo[1,5-a]quinolin-4-yl))pyridine-3-carbonitrile 4j. Yield 72%, m.p. 264 °C, Anal. Calcd. for C$_{23}$H$_{17}$N$_7$: C 70.57, H 4.37, N 25.04% Found: C 70.42, H 3.92, N 25.01%. IR (KBr, cm$^{-1}$): 3405, 3300 (NH$_2$), 2240 (CN), 3026 (Ar-C-H). $^1$H NMR (400 MHz, DMSO-$d_6$): $\delta$: 2.39 (s, 3H, CH$_3$), 2.45 (s, 3H, CH$_3$), 7.03 (s, 2H, NH$_2$), 7.18-8.71 (m, 9H, Ar-H). $^{13}$C NMR (400 MHz, DMSO-$d_6$): $\delta$: 21.3 (CH$_3$), 23.4 (CH$_3$), 96.0 (C-CN), 115.8, 119.9, 122.5, 124.0, 128.9, 130.4, 130.6, 133.0, 133.3, 138.8, 139.9, 140.7, 145.0, 146.4, 146.8, 150.5, 154.4, 160.4 (Ar-C).

2-Amino-6-(4-methoxyphenyl)-4-(7-methyl-(tetrazolo[1,5-a]quinolin-4-yl))pyridine-3-carbonitrile 4k. Yield 70%, m.p. 243 °C, Anal. Calcd. for C$_{23}$H$_{17}$N$_7$O: C 67.80, H 4.20, N 24.06% Found: C 67.77, H 4.11, N 24.00%. IR (KBr, cm$^{-1}$): 3436, 3310 (NH$_2$), 2210 (CN), 3005 (Ar-C-H). $^1$H NMR (400 MHz, DMSO-$d_6$): $\delta$: 2.50 (s, 3H, CH$_3$), 3.97 (s, 3H, OCH$_3$), 6.87 (s, 2H, NH$_2$), 7.03-8.62 (m, 9H, Ar-H). $^{13}$C NMR (400 MHz, DMSO-$d_6$): $\delta$: 21.3 (CH$_3$), 56.3 (OCH$_3$), 96.4 (C-CN), 117.1, 121.9, 124.4, 126.7, 128.3, 128.9, 129.1, 132.7, 133.3, 136.0, 137.4, 138.6, 141.5, 143.0, 144.2, 145.8, 148.0, 150.4 (Ar-C).

2-Amino-6-(2-furyl)-4-(7-methyl-(tetrazolo[1,5-a]quinolin-4-yl))pyridine-3-carbonitrile 4l. Yield 73%, m.p. 278-280 °C, Anal. Calcd. for C$_{20}$H$_{13}$N$_7$O: C 65.38, H 3.56, N 26.68% Found: C 65.25, H 3.44, N 26.65%. IR (KBr, cm$^{-1}$): 3410, 3336 (NH$_2$), 2214 (CN), 3026 (Ar-C-H). $^1$H NMR (400 MHz, DMSO-$d_6$): $\delta$: 2.50 (s, 3H, CH$_3$), 6.79 (s, 2H, NH$_2$), 7.03-8.62 (m, 9H, Ar-H). $^{13}$C NMR (400 MHz, DMSO-$d_6$): $\delta$: 21.3 (CH$_3$), 56.3 (OCH$_3$), 96.4 (C-CN), 117.1, 121.9, 124.4, 126.7, 128.3, 128.9, 129.1, 132.7, 133.3, 136.0, 137.4, 138.6, 141.5, 143.0, 144.2, 145.8, 148.0, 150.4 (Ar-C), MS: (M+1) 368.

2-Amino-6-(2-thienyl)-4-(7-methyl-(tetrazolo[1,5-a]quinolin-4-yl))pyridine-3-carbonitrile 4m. Yield 66%, m.p. 264-267 °C, Anal. Calcd. for C$_{20}$H$_{13}$N$_7$S: C 62.64, H 3.41, N 25.57% Found: C 62.60, H 3.39, N 25.42%. IR (KBr, cm$^{-1}$): 3400, 3316 (NH$_2$), 2230 (CN), 3005 (Ar-C-H). $^1$H NMR (400 MHz, DMSO-$d_6$): $\delta$: 2.47 (s, 3H, CH$_3$), 6.84 (s, 2H, NH$_2$), 6.96-8.54 (m, 8H,
Ar-H). $^{13}$C NMR (400 MHz, DMSO-$d_6$) $\delta$: 21.6 (CH$_3$), 94.1 (C-CN), 114.1, 118.4, 122.6, 124.0, 126.0, 127.9, 128.7, 130.4, 131.1, 134.2, 135.1, 136.0, 137.5, 138.9, 141.0, 141.7, 142.4, 143.8 (Ar-C).

2-Amino-6-(2-pyridyl)-4-(7-methyl-(tetrazolo[1,5-a]quinolin-4-yl))pyridine-3-carbonitrile 4n. Yield 70%, m.p. 252 °C, Anal. Calcd. for C$_{21}$H$_{14}$N$_8$: C 66.65, H 3.72, N 29.61% Found: C 65.70, H 3.64, N 29.58%. IR (KBr, cm$^{-1}$): 3430, 3326 (NH$_2$), 2230 (CN), 3016 (ArC-H). $^1$H NMR (400 MHz, DMSO-$d_6$): $\delta$ 2.21 (s, 3H, CH$_3$), 6.94 (s, 2H, NH$_2$), 7.23-8.78 (m, 9H, Ar-H).

$^{13}$C NMR (400 MHz, DMSO-$d_6$) $\delta$: 21.9 (CH$_3$), 94.8 (C-CN), 115.6, 118.3, 121.4, 123.7, 125.5, 127.4, 130.0, 133.4, 133.9, 134.7, 135.6, 137.2, 139.0, 140.4, 144.6, 148.3, 152.0, 153.4, 154.9 (Ar-C).

2-Amino-6-(3-pyridyl)-4-(7-methyl-(tetrazolo[1,5-a]quinolin-4-yl))pyridine-3-carbonitrile 4o. Yield 54%, m.p. 270-273 °C, Anal. Calcd. for C$_{21}$H$_{14}$N$_8$: C 66.65, H 3.72, N 29.61% Found: C 65.68, H 3.69, N 29.48%. IR (KBr, cm$^{-1}$): 3400, 3330 (NH$_2$), 2216 (CN), 3000 (ArC-H). $^1$H NMR (400 MHz, DMSO-$d_6$): $\delta$ 2.25 (s, 3H, CH$_3$), 7.04 (s, 2H, NH$_2$), 7.20-8.70 (m, 9H, Ar-H).

$^{13}$C NMR (400 MHz, DMSO-$d_6$) $\delta$: 22.1 (CH$_3$), 95.6 (C-CN), 117.1, 119.3, 122.9, 123.0, 126.5, 128.4, 130.5, 133.0, 133.9, 134.5, 136.7, 137.0, 139.3, 141.5, 144.0, 148.1, 152.3, 154.2, 155.0 (Ar-C).

2-Amino-6-(4-pyridyl)-4-(7-methyl-(tetrazolo[1,5-a]quinolin-4-yl))pyridine-3-carbonitrile 4p. Yield 68%, m.p. 267 °C, Anal. Calcd. for C$_{21}$H$_{14}$N$_8$: C 66.65, H 3.72, N 29.61% Found: C 65.61, H 3.80, N 29.55%. IR (KBr, cm$^{-1}$): 3408, 3338 (NH$_2$), 2236 (CN), 3018 (ArC-H). $^1$H NMR (400 MHz, DMSO-$d_6$): $\delta$ 2.20 (s, 3H, CH$_3$), 7.12 (s, 2H, NH$_2$), 7.26-8.77 (m, 9H, Ar-H).

$^{13}$C NMR (400 MHz, DMSO-$d_6$) $\delta$: 21.8 (CH$_3$), 94.2 (C-CN), 117.1, 119.3, 122.9, 123.0, 126.5, 128.4, 130.5, 133.0, 133.9, 134.5, 136.7, 137.0, 139.3, 141.5, 144.0, 148.1, 152.3, 153.4, 154.9 (Ar-C).

2-Amino-6-phenyl-4-(7-methoxy-(tetrazolo[1,5-a]quinolin-4-yl))pyridine-3-carbonitrile 4q. Yield 62%, m.p. 243-245 °C, Anal. Calcd. for C$_{22}$H$_{15}$N$_7$O: C 67.16, H 3.84, N 24.92% Found: C 67.10, H 3.74, N 24.89%. IR (KBr, cm$^{-1}$): 3440, 3322 (NH$_2$), 2232 (CN), 3028 (Ar-C-H). $^1$H NMR (400 MHz, DMSO-$d_6$): $\delta$ 3.84 (s, 3H, OCH$_3$), 7.01 (s, 1H, NH$_2$), 7.16-8.31 (m, 10H, Ar-H).

$^{13}$C NMR (400 MHz, DMSO-$d_6$) $\delta$: 56.8 (OCH$_3$), 96.0 (C-CN), 115.7, 116.2, 116.7, 120.0, 122.5, 123.9, 128.9, 129.2, 130.1, 130.4, 130.6, 133.0, 134.9, 137.7, 143.1, 146.9, 150.4, 154.3 (Ar-C).

2-Amino-6-(4-methylphenyl)-4-(7-methoxy-(tetrazolo[1,5-a]quinolin-4-yl))pyridine-3-carbonitrile 4r. Yield 70%, m.p. 274-276 °C, Anal. Calcd. for C$_{23}$H$_{17}$N$_7$O: C 67.80, H 4.20, N 24.06% Found: C 67.71, H 4.13, N 23.00%. IR (KBr, cm$^{-1}$): 3400, 3310 (NH$_2$), 2210 (CN), 2990 (Ar-C-H). $^1$H NMR (400 MHz, DMSO-$d_6$): $\delta$ 2.50 (s, 3H, CH$_3$), 3.86 (s, 3H, OCH$_3$), 6.91 (s, 2H, NH$_2$), 7.08-8.68 (m, 9H, Ar-H).

$^{13}$C NMR (400 MHz, DMSO-$d_6$) $\delta$: 21.4 (CH$_3$), 55.9 (OCH$_3$), 97.2 (C-CN), 118.1, 120.9, 123.1, 126.7, 128.0, 128.9, 129.6, 133.1, 133.3, 136.4, 138.4, 138.6, 141.9, 143.0, 144.1, 145.3, 149.0, 152.4 (Ar-C).

2-Amino-6-(4-methoxyphenyl)-4-(7-methoxy-(tetrazolo[1,5-a]quinolin-4-yl))pyridine-3-carbonitrile 4s. Yield 52%, m.p. 292 °C, Anal. Calcd. for C$_{23}$H$_{17}$N$_7$O$_2$: C 65.24, H 4.04, N 23.15% Found: C 65.30, H 4.00, N 23.11%. IR (KBr, cm$^{-1}$): 3420, 3312 (NH$_2$), 2230 (CN), 3020.
(ArC-H). $^1$H NMR (400 MHz, DMSO-$d_6$): δ 3.90 (s, 3H, OCH$_3$), 3.97 (s, 3H, OCH$_3$), 6.98 (s, 1H, NH$_2$), 7.12-8.70 (9H, m, Ar-H). $^{13}$C NMR (400 MHz, DMSO-$d_6$) δ: 54.9 (OCH$_3$), 56.3 (OCH$_3$), 95.8 (C-CN), 117.4, 121.9, 123.3, 123.6, 126.7, 128.9, 129.9, 129.6, 130.3, 133.4, 133.6, 136.9, 138.4, 139.6, 141.0, 143.5, 144.6, 145.3 (Ar-C).

2-Amino-6-(2-furyl)-4-(7-methoxy-(tetrazolo[1,5-a]quinolin-4-yl))pyridine-3-carbonitrile 4t. Yield 70%, m.p. 266-268 °C, Anal. Calcd. for C$_{20}$H$_{13}$N$_7$O$_2$: C 62.66, H 3.41, N 25.57% Found: C 62.60, H 3.39, N 25.52%. IR (KBr, cm$^{-1}$): 3440, 3326 (NH$_2$), 2200 (CN), 3035 (ArC-H). $^1$H NMR (400 MHz, DMSO-$d_6$): δ 3.76 (s, 3H, OCH$_3$), 6.98 (s, 2H, NH$_2$), 7.41-8.94 (m, 8H, Ar-H). $^{13}$C NMR (400 MHz, DMSO-$d_6$) δ: 56.1 (OCH$_3$), 96.3 (C-CN), 116.0, 117.8, 119.2, 120.2, 122.2, 124.4, 126.9, 129.3, 131.3, 134.1, 136.0, 140.4, 143.8, 144.2, 146.9, 148.9, 151.3 (Ar-C).

2-Amino-6-(2-thienyl)-4-(7-methoxy-(tetrazolo[1,5-a]quinolin-4-yl))pyridine-3-carbonitrile 4u. Yield 71%, m.p. 271-273 °C, Anal. Calcd. for C$_{20}$H$_{13}$N$_7$OS: C 60.14, H 3.28, N 24.54% Found: C 60.10, H 3.30, N 25.61%. IR (KBr, cm$^{-1}$): 3430, 3320 (NH$_2$), 2220 (CN), 3040 (ArC-H). $^1$H NMR (400 MHz, DMSO-$d_6$): δ 3.45 (s, 3H, OCH$_3$), 7.21 (s, 2H, NH$_2$), 7.35-8.83 (m, 8H, Ar-H). $^{13}$C NMR (400 MHz, DMSO-$d_6$) δ: 55.3 (OCH$_3$), 96.8 (C-CN), 117.8, 118.8, 119.2, 120.7, 123.2, 124.9, 126.5, 129.0, 133.3, 134.5, 136.0, 138.4, 141.4, 143.5, 144.2, 146.0, 148.2, 151.3 (Ar-C).

2-Amino-6-(2-pyridyl)-4-(7-methoxy-(tetrazolo[1,5-a]quinolin-4-yl))pyridine-3-carbonitrile 4v. Yield 60%, m.p. 276 °C, Anal. Calcd. for C$_{21}$H$_{14}$N$_8$O: C 63.95, H 3.57, N 28.41% Found: C 64.02, H 3.60, N 28.38%. IR (KBr, cm$^{-1}$): 3410, 3318 (NH$_2$), 2214 (CN), 3000 (ArC-H). $^1$H NMR (400 MHz, DMSO-$d_6$): δ 3.64 (s, 3H, OCH$_3$), 7.21 (s, 2H, NH$_2$), 7.33-8.71 (m, 9H, Ar-H). $^{13}$C NMR (400 MHz, DMSO-$d_6$) δ: 55.4 (OCH$_3$), 96.7 (C-CN), 117.7, 118.0, 119.4, 120.8, 123.1, 125.9, 127.8, 129.0, 131.5, 135.4, 138.7, 140.1, 142.7, 145.6, 146.8, 148.1, 149.0, 150.4, 153.2 (Ar-C).

2-Amino-6-(3-pyridyl)-4-(7-methoxy-(tetrazolo[1,5-a]quinolin-4-yl))pyridine-3-carbonitrile 4w. Yield 58%, m.p. 244-247 °C, Anal. Calcd. for C$_{21}$H$_{14}$N$_8$O: C 63.95, H 3.57, N 28.41% Found: C 63.88, H 3.57, N 28.41%. IR (KBr, cm$^{-1}$): 3416, 3310 (NH$_2$), 2228 (CN), 3020 (Ar-C-H). $^1$H NMR (400 MHz, DMSO-$d_6$): δ 3.60 (s, 3H, OCH$_3$), 7.21 (s, 2H, NH$_2$), 7.33-8.71 (m, 9H, Ar-H). $^{13}$C NMR (400 MHz, DMSO-$d_6$) δ: 56.9 (OCH$_3$), 97.2 (C-CN), 115.7, 117.0, 118.6, 120.2, 124.4, 126.0, 128.8, 129.7, 131.8, 136.4, 138.0, 140.4, 142.5, 143.0, 146.5, 148.8, 149.4, 151.0, 152.8 (Ar-C).

2-Amino-6-(4-pyridyl)-4-(7-methoxy-(tetrazolo[1,5-a]quinolin-4-yl))pyridine-3-carbonitrile 4x. Yield 70%, m.p. 258-260 °C, Anal. Calcd. for C$_{21}$H$_{14}$N$_8$O: C 63.95, H 3.57, N 28.41% Found: C 63.91, H 3.44, N 28.39%. IR (KBr, cm$^{-1}$): 3446, 3318 (NH$_2$), 2220 (CN), 3024 (Ar-C-H). $^1$H NMR (400 MHz, DMSO-$d_6$): δ 3.85 (s, 3H, OCH$_3$), 7.04 (s, 2H, NH$_2$), 7.14-8.81 (m, 9H, Ar-H). $^{13}$C NMR (400 MHz, DMSO-$d_6$) δ: 56.1 (OCH$_3$), 97.4 (C-CN), 120.4, 122.4, 125.3, 126.9, 128.3, 130.2, 131.0, 132.2, 133.3, 135.9, 137.4, 138.6, 141.6, 142.3, 144.8, 145.5, 147.5 (Ar-C).
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References