Synthesis and Evaluation of 2-Amino-4H-Pyran-3-Carbonitrile Derivatives as Antitubercular Agents

Chunxia Chen¹*, Minghui Lu²*, Zhihui Liu³, Junting Wan², Zhengchao Tu², Tianyu Zhang²#, Ming Yan¹#
¹Institute of Drug Synthesis and Pharmaceutical Process, School of Pharmaceutical Sciences, Sun Yat-sen University, Guangzhou, China
²State Key Laboratory of Respiratory Diseases, Guangzhou Institutes of Biomedicine and Health, Chinese Academy of Science, Guangzhou, China
³Guangzhou Chest Hospital, Guangzhou, China
Email: ²zhang_tianyu@gibh.ac.cn, ¹yanming@mail.sysu.edu.cn

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ABSTRACT

A series of 2-amino-4H-pyran-3-carbonitrile derivatives were designed and synthesized. Their antitubercular activities were evaluated against autoluminescent Mycobacterium tuberculosis H37Ra and standard strain Mycobacterium tuberculosis H37Rv. No obvious antitubercular activities could be observed (MIC > 10 μg/mL). The results are in sharp contrast with the previously reported data.

Keywords: 2-Amino-4H-Pyran-3-Carbonitrile; Synthesis; Antitubercular Activity

1. Introduction

Tuberculosis (TB) is a chronic disease caused by Mycobacterium tuberculosis. It continues to be a serious threat for human health [1,2]. Every year about two million people die of this disease and almost eight million people get tuberculosis. The present antitubercular treatment typically requires the combination of at least two first-line drugs (rifampicin, isoniazid, ethambutol and pyrazinamide) for an extended period (6 - 12 months). The poor compliance to the rigid implementation of therapy leads to the emergence of multi-drug-resistant (MDR) and extensively drug-resistant (XDR) strains of Mycobacterium tuberculosis, which have brought new challenges for clinical treatment [3-6]. In addition, the rising incidence of TB and HIV co-infection makes the treatment more difficult.

The development of antitubercular agents with new action mechanism is an urgent task [7,8]. In recent ten years, a number of candidates have appeared with promising activities against sensitive and resistant Mycobacterium tuberculosis strains. In 2007, Perumal and co-workers reported 2-amino-pyranopyridine-3-carbonitriles as a new type of antitubercular agents (Scheme 1) [9]. Several compounds showed excellent antitubercular activity comparable with isoniazid. Recently Perumal, Srim and co-workers also found that 1,2,4-oxadiazoles derived from 2-amino-pyranopyridine-3-carbonitriles showed enhanced antitubercular activity (Scheme 1) [10]. These results strongly suggest that 2-amino-4H-pyran-3-carbonitrile is a new pharmacophore of antitubercular agents.

Recently we have developed efficient methods for the synthesis of homochiral 2-amino-4H-pyran-3-carbonitriles [11,12]. We are interested in the effect of chiral center of these compounds on the antitubercular activity. We are also interested in the further improvement of the antitubercular activity of 2-amino-4H-pyran-3-carbonitriles by structural modifications. In this paper, we report the synthesis of racemic and homochiral 2-amino-pyranopyridine-3-carbonitriles as well as their structural analogs. The antitubercular activity was evaluated in vitro against autoluminescent Mycobacterium tuberculosis H37Ra and standard strain Mycobacterium tuberculosis H37Rv.
2. Results and Discussion

2.1. Chemistry

Racemic 2-aminopyranopyridine-3-carbonitriles 2a-2g were prepared from dienones 1a-1g and malononitrile in the presence of piperidine. Generally excellent yields (92% - 99%) were obtained (Scheme 2).

To explore the effect of chiral centers in 2a-2g, homochiral (S)-2a, (S)-2d, (R)-2a, and (R)-2d were prepared. Excellent yields and enantioselectivities were achieved using chiral thiourea-tertiary amines as the catalysts (Scheme 3) [11].

2-Aminopyranopyridine-3-carbonitriles 2h-2n derived from monoenes 1h-1n were also prepared (Scheme 4) [12]. Cyclic enones 1h-1j reacted with malononitrile in the presence of triethylamine. The reaction of acyclic enones 1k-1n was achieved using piperidine as the catalyst. Generally products 2h-2n were obtained in good yields.

For a further understanding the effect of C (sp3) chiral structure in 2-aminopyranopyridine-3-carbonitriles, the compounds 4a and 4j with achiral pyridine structure were designed. The treatment of 2-amino-pyran 2a and 2j with ammonium acetate provided 4a and 4j in good yields (Scheme 5) [13].

2.2. Evaluation of Antitubercular Activity

The antituberculosis activity of racemic 2-amino-4H-pyran-3-carbonitriles 2a-2e, homochiral 2-amino-4H-pyran-3-carbonitriles (S)-2a, (S)-2d, (R)-2a, and (R)-2d were evaluated against autoluminescent M. tuberculosis H37Ra [14]. This screen model is fast and cost-efficient for the preliminary evaluation of antitubercular activity. Isoniazid and rifampicin were used as the positive control and the results are listed in Figure 1. The bacteria growth was conveniently monitored by the bioluminescence intensity. Unexpectedly all compounds including 2a and 2d did not showed obvious antitubercular activity.

We further examined the inhibitive activity of the compounds against standard strain M. tuberculosis H37Rv and the results are summarized in Table 1. Perumal and co-workers reported that compound 2a and 2d possess excellent antitubercular activities (MIC 0.97 and 0.92 μg/mL against H37Rv respectively) [9]. Our present study led to significantly different results. Racemic 2a, 2d and their homochiral enantiomers did not show obvious antitubercular activities (MIC > 10 μg/mL). Other structural analogs 2b, 2c, 2e-2g also appeared to be inefficient. The compounds 2h-2n and 4a, 4j with further structural diversities still showed disappointed antitubercular activities. These results brought the question about the reported antitubercular activity of 2-amino-4H-pyran-3-carbonitriles by Perumal and co-workers. Although a clear conclusion could not be achieved so far, the further examination of the reported data is highly desirable.

3. Conclusion

In conclusion, we designed and synthesized a series of 2-amino-4H-pyran-3-carbonitriles and their structural analogs. Homochiral 2-amino-4H-pyran-3-carbonitriles were also prepared via the organocatalytic enantioselective reaction. The antitubercular activities of these compounds were determined against autoluminescent M. tuberculosis H37Ra and standard strain M. tuberculosis H37Rv, however, no obvious inhibitive activities could be observed. The results are in sharp contrast with the previously reported data. Before the further attempt to develop 2-amino-4H-pyran-3-carbonitriles as potential antitubercular agents, the clarification of the contradictive activity data is required.

4. Experimental

1H and 13C NMR spectra were recorded on a Bruker Advance 400 MHz spectrometer as solutions in CDCl3. Chemical shifts in 1H NMR spectra are reported in parts per million (ppm, δ) downfield from the internal standard Me4Si (TMS, δ = 0 ppm). Chemical shifts in 13C NMR spectra are reported relative to the central line of the chloroform signal (δ = 77.0 ppm). The following abbreviations are used to designate chemical shift multiplicities: s = singlet, d = doublet, m = multiplet. High-resolution mass spectra were obtained with Shimadzu LCMS-IT-TOF mass spectrometer. Infrared (IR) spectra were recorded on a Bruker Tensor 37 spectrophotometer. Data are represented as follows: frequency of absorption (cm⁻¹), intensity of absorption (s = strong, m = medium, w = weak).
Scheme 2. Synthesis of 2-amino-4H-pyran-3-carbonitriles 2a-2g.

\[
\text{Ar-} + \text{CN-} \xrightarrow{\text{piperidine, EtOH, rt}} \text{NH}_2-\text{CN} \quad \text{92-99% yields}
\]

2a: Ar = 4-F-C_6H_4; X = CH_3N; 2b: Ar = 3-F-C_6H_4; X = CH_3N; 2c: Ar = 3,4-dif-C_6H_4; X = CH_3N; 2d: Ar = 4-Cl-C_6H_4; X = CH_3N; 2e: Ar = 4-F-C_6H_4; X = O; 2f: Ar = 4-F-C_6H_4; X = S; 2g: Ar = 4-F-C_6H_4; X = CH_2


\[
\text{Ar-} + \text{CN-} \xrightarrow{\text{3a or 3b (10 mol%)}} \text{NH}_2-\text{CN} \quad \text{94-96% yields} \quad \text{98-99% ee}
\]

3a 3b


ried out over silica gel (230 - 400 mesh), purchased from Qingdao Haiyang Chemical Co. Ltd. Melting points were recorded on an electrothermal digital melting point apparatus and were uncorrected. TLC analysis was performed on precoated silica gel GF254 slides, and visualised by either UV irradiation. Unless otherwise stated, all reagents were obtained from commercial sources and used as received. The solvents were used as commercial anhydrous grade without further purification. Enantiomeric excesses were determined by HPLC using a Daicel Chiralpak AD-H column (4.6 mm × 25 cm) and eluting with hexane/2-PrOH solution.
4.1. Typical Procedure for the Synthesis of Compounds 2a-2g

A mixture of (3E, 5E)-3,5-bis(4-fluorobenzylidene)-1-methylpiperidin-4-one 1a (66.1 mg, 0.2 mmol), malononitrile (19.8 mg, 0.3 mmol) and piperidine (17.0 mg, 0.2 mmol) in ethanol (2 mL) were stirred for 12 h at room temperature. The precipitate was filtered to provide 2a as a white solid.

4.1.1. (E)-2-Amino-8-(4-Fluorobenzylidene)-4-(4-Fluorophenyl)-6-Methyl-5,6,7,8-Tetrahydro-4H-Pyrano-[3,2-c]Pyridine-3-Carbonitrile (2a) [11]

White solid, yield 94%, mp 197°C - 198°C; 1H NMR (400 MHz, CDCl3): δ = 7.24 - 7.17 (4H, m, ArH), 7.08-7.02 (4H, m, ArH), 6.86 (1H, s, HC=O), 4.55 (2H, s, NH2), 4.03 (1H, s, CH), 3.52 (1H, d, J = 13.4 Hz, CH2), 3.36 (1H, d, J = 13.0 Hz, CH2), 2.95 (1H, d, J = 15.9 Hz, CH3), 2.72 (1H, d, J = 15.4 Hz, CH3), 2.28 (3H, s, CH3).

4.1.2. (E)-2-Amino-8-(3-Fluorobenzylidene)-4-(3-Fluorophenyl)-6-Methyl-5,6,7,8-Tetrahydro-4H-Pyrano-[3,2-c]Pyridine-3-Carbonitrile (2b) [9]

White solid, yield 92%, mp 169°C - 172°C; 1H NMR (400 MHz, CDCl3): δCDCl3 = 7.33 (2H, d, J = 5.9 Hz, ArH), 7.08 - 7.04 (1H, d, J = 7.6 Hz, ArH), 7.01 - 6.96 (4H, t, J = 19.2 Hz, ArH), 6.93 - 6.91 (1H, d, J = 9.6 Hz, ArH), 6.85 (1H, s, HC=C), 4.59 (2H, s, NH2), 4.04 (1H, s, CH), 3.55 (1H, d, J = 14.4 Hz, CH2), 3.36 (1H, d, J = 14.6 Hz, CH2), 2.97 (1H, d, J = 15.8 Hz, CH2), 2.74 (1H, d, J = 16.0 Hz, CH2), 2.29 (3H, s, CH3).

4.1.3. (E)-2-Amino-8-(3,4-Difluorobenzylidene)-4-(3,4-Difluorophenyl)-6-methyl-5,6,7,8-Tetrahydro-4H-Pyrano-[3,2-c]Pyridine-3-Carbonitrile (2c)

White solid, yield 92%, mp 207°C - 209°C; 1H NMR (400 MHz, CDCl3): δ = 158.77, 140.11, 139.09, 133.02, 127.90, 127.70, 123.78, 121.27, 119.07, 117.92, 117.75, 117.74, 117.67, 117.42, 117.25, 116.72, 112.66, 60.90, 55.13, 54.47, 44.90, 41.09; IR (KBr) v/cm⁻¹: 3484 (m), 2194 (s), 1681 (m), 1595 (m), 1301 (s), 1246 (s), 1159 (s), 1125 (m), 920 (w), 781 (s); HRMS (ESI) calcld for C23H17N3OF4+ [M + H]+: 428.1381, found: 428.1389.

4.1.4. (E)-2-Amino-8-(4-Chlorobenzylidene)-4-(4-Chlorophenyl)-6-Methyl-5,6,7,8-Tetrahydro-4H-Pyrano-[3,2-c]Pyridine-3-Carbonitrile (2d) [11]

White solid, yield 96%, mp 205°C - 207°C; 1H NMR (400 MHz, CDCl3): δ = 7.35 - 7.32 (4H, m, ArH), 7.21 -
4.1.5. (E)-2-Amino-8-(4-Fluorobenzylidene)-4-
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Table 1. Evaluation of antitubercular activity against M. tuberculosis H37Rv.

<table>
<thead>
<tr>
<th>Compounds</th>
<th>MIC (µg/mL)</th>
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<tbody>
<tr>
<td>2a</td>
<td>&gt;10</td>
</tr>
<tr>
<td>2b</td>
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<td>2c</td>
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<td>(S)-2d</td>
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<td>2h</td>
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</tr>
<tr>
<td>Isoniazid</td>
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</tr>
<tr>
<td>Rifampicin</td>
<td>&lt;0.25</td>
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7.13 (4H, m, ArH), 6.84 (1H, s, HC=C), 4.57 (2H, s, NH2), 4.02 (1H, s, CH), 3.51 (1H, d, J = 13.8 Hz, CH2), 3.35 (1H, d, J = 13.6 Hz, CH2), 2.94 (1H, d, J = 16.0 Hz, CH2), 2.72 (1H, d, J = 16.0 Hz, CH2), 2.27 (3H, s, CH3).

4.1.6. 2-Amino-8-(4-Fluorobenzylidene)-4-

4.1.7. (E)-2-Amino-8-(4-Fluorobenzylidene)-4-

4.2. Typical Procedure for the Synthesis of Homochiral Compounds (S)-2a, (S)-2d, (R)-2a, and (R)-2d

A mixture of (3E, 5E)-3,5-bis(4-fluorobenzylidene)-1-methylpyrroldine-4-one 1a (66.1 mg, 0.2 mmol), malononitrile (19.8 mg, 0.3 mmol) and 3a (9.0 mg, 0.02 mmol) in toluene (2 mL) were stirred for 28 h at room temperature. The white precipitate (S)-2a was collected by the centrifugation.

4.2.1. (S,E)-2-Amino-8-(4-Fluorobenzylidene)-4-

White solid, yield 99%, mp 211°C - 213°C; 1H NMR (400 MHz, CDCl3): δ = 7.24 - 7.08 (4H, m, ArH), 7.08 - 7.02 (4H, m, ArH), 6.83 (1H, s, HC=C), 4.55 (2H, s, NH2), 3.97 (1H, s, CH), 2.70 - 2.70 (1H, m, CH2), 2.60 - 2.56 (1H, m, CH2), 2.04 - 1.92 (2H, m, CH2), 1.74 - 1.52 (2H, m, CH2); HRMS (ESI) calcd for C23H18N2OF2+: 417.0843, found: 417.0843.

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Table 1. Evaluation of antitubercular activity against M. tuberculosis H37Rv.
254 nm, 0.8 mL/min), t_r (major) = 10.6 min, t_r (minor) = 7.5 min, 99%ee.

4.2.2. (R,E)-2-Amino-8-(4-Fluorobenzylidene)-4-(4-Fluorophenyl)-6-Methyl-5,6,7,8-Tetrahydro-4H-Pyrano-[3,2-c]Pyridine-3-Carbonitrile (R-2a) [11]
White solid, yield 94%, mp 197°C - 198°C; 1H NMR (400 MHz, CDCl3): δ = 7.24 - 7.17 (4H, m, ArH), 7.08 - 7.02 (4H, m, ArH), 6.86 (1H, s, NC=), 4.55 (2H, s, NH2), 4.03 (1H, s, CH), 3.52 (1H, d, J = 13.4 Hz, CH2), 3.36 (1H, d, J = 13.0 Hz, CH2), 2.95 (1H, d, J = 15.9 Hz, CH2), 2.72 (1H, d, J = 15.4 Hz, CH2), 2.28 (3H, s, CH3). Enantiomeric excess was determined by HPLC with a CHIRALPAK AD-H column (i-PrOH/hexane = 30:70, 254 nm, 0.8 mL/min), t_r (major) = 7.5 min, t_r (minor) = 10.6 min, 98%ee.

4.2.3. (S,E)-2-Amino-8-(4-Chlorobenzylidene)-4-(4-Chlorophenyl)-6-Methyl-5,6,7,8-Tetrahydro-4H-Pyrano-[3,2-c]Pyridine-3-Carbonitrile (S-2d) [11]
White solid, yield 96%, mp 205°C - 207°C; 1H NMR (400 MHz, CDCl3): δ = 7.35 - 7.32 (4H, m, ArH), 7.21 - 7.13 (4H, m, ArH), 6.84 (1H, s, HC=), 4.57 (2H, s, NH2), 4.02 (1H, s, CH), 3.51 (1H, d, J = 13.8 Hz, CH2), 3.35 (1H, d, J = 13.6 Hz, CH2), 2.94 (1H, d, J = 16.0 Hz, CH2), 2.72 (1H, d, J = 16.0 Hz, CH2), 2.27 (3H, s, CH3). Enantiomeric excess was determined by HPLC with a CHIRALPAK AD-H column (i-PrOH/hexane = 30:70, 254 nm, 0.8 mL/min), t_r (major) = 9.7 min, t_r (minor) = 7.6 min, 98%ee.

4.2.4. (R,E)-2-Amino-8-(4-Chlorobenzylidene)-4-(4-Chlorophenyl)-6-Methyl-5,6,7,8-Tetrahydro-4H-Pyrano-[3,2-c]Pyridine-3-Carbonitrile (R-2d) [11]
White solid, yield 96%, mp 205°C - 207°C; 1H NMR (400 MHz, CDCl3): δ = 7.35 - 7.32 (4H, m, ArH), 7.21 - 7.13 (4H, m, ArH), 6.84 (1H, s, HC=), 4.57 (2H, s, NH2), 4.02 (1H, s, CH), 3.51 (1H, d, J = 13.8 Hz, CH2), 3.35 (1H, d, J = 13.6 Hz, CH2), 2.94 (1H, d, J = 16.0 Hz, CH2), 2.72 (1H, d, J = 16.0 Hz, CH2), 2.27 (3H, s, CH3). Enantiomeric excess was determined by HPLC with a CHIRALPAK AD-H column (i-PrOH/hexane = 30:70, 254 nm, 0.8 mL/min), t_r (major) = 9.7 min, t_r (minor) = 7.6 min, 99%ee.

4.3. Typical Procedure for the Synthesis of Compounds 2h-2j
A mixture of (E)-2-(4-fluorobenzylidene)-3,4-dihy-dronaphthalen-1(2H)-one 1h (50.5 mg, 0.2 mmol), malononitrile (19.8 mg, 0.3 mmol) and triethylamine (20.2 mg, 0.2 mmol) in ethanol (2 mL) were stirred for 12 h at room temperature. The precipitate was filtered to provide 2h as a yellow solid.

4.3.1. 2-Amino-4-(4-Fluorophenyl)-5,6-Dihydro-4H-Benzo[h]Chromene-3-Carbonitrile (2h) [12]
Yellow solid, yield 94%, mp 184°C - 186°C; 1H NMR (400 MHz, CDCl3): δ = 7.46 (1H, d, J = 7.2 Hz, ArH), 7.26 - 7.19 (4H, m, ArH), 7.11 (1H, d, J = 6.8 Hz, ArH), 7.01 (2H, t, J = 8.2 Hz, ArH), 4.58 (2H, s, NH2), 4.07 (1H, s, CH), 2.81 (1H, dd, J = 15.9, 8.1 Hz, CH2), 2.69 (1H, dt, J = 15.7, 7.8 Hz, CH3), 2.16 (1H, dt, J = 15.9, 7.9 Hz, CH2), 2.09 - 1.96 (1H, m, CH2); HRMS (ESI) caledd for C20H15N2OF+ [M + Na]+: 341.1061, found: 341.1054.

4.3.2. 2-Amino-4-(4-Fluorophenyl)-4,5-Dihydropyran-3,2-c]Chromene-3-Carbonitrile (2l)
Yellow solid, yield 75%, mp 175°C - 177°C; 1H NMR (400 MHz, CDCl3): δ = 7.34 (1H, d, J = 7.6 Hz, ArH), 7.30 - 7.14 (3H, m, ArH), 7.05 (2H, dd, J = 12.0, 5.0 Hz, ArH), 6.96 (1H, t, J = 7.5 Hz, ArH), 6.80 (1H, d, J = 8.1 Hz, ArH), 4.67 (2H, s, NH2), 4.62 - 4.55 (1H, d, J = 13.6 Hz, CH2), 4.41 (1H, d, J = 13.7 Hz, CH2), 4.03 (1H, s, CH); 13C NMR (100 MHz, CDCl3): δ = 163.61, 161.17, 158.86, 154.14, 138.16, 136.81, 130.46, 129.55, 129.48, 121.36, 121.13, 116.58, 116.08, 116.00, 115.76, 104.75, 66.37, 60.87, 39.07; IR (KBr) v/cm-1: 3327 (m), 2195 (m), 1709 (s), 1656 (s), 1600 (s), 1536 (m), 1320 (s), 1157 (m), 1101 (w), 1036 (w), 836 (m), 755 (w); HRMS (ESI) caledd for C19H13N2O2F+ [M + Na]+: 343.0853, found: 343.0857.

4.3.3. 2-Amino-4-(4-Fluorophenyl)-4,5-Dihydrothiochromeno[4,3-b]Pyran-3-Carbonitrile (2j)
Yellow solid, yield 76%, mp 158°C - 160°C; 1H NMR (400 MHz, CDCl3): δ = 7.53 (1H, d, J = 16.8 Hz, ArH), 7.36 - 7.29 (2H, m, ArH), 7.28 - 7.14 (3H, m, ArH), 7.04 (2H, dd, J = 12.1, 4.9 Hz, ArH), 4.63 (2H, s, NH2), 4.13 (1H, s, CH), 3.29 (1H, d, J = 15.1 Hz, CH2), 3.09 (1H, d, J = 15.1 Hz, CH2); 13C NMR (100 MHz, CDCl3): δ = 163.63, 161.18, 158.63, 142.14, 137.53, 132.76, 129.77, 129.70, 129.11, 127.15, 125.58, 123.28, 119.20, 116.03, 115.81, 107.77, 60.99, 42.39, 27.00; IR (KBr) v/cm-1: 3475 (w), 2360 (w), 2197 (s), 2024 (w), 1692 (s), 1635 (w), 1598 (s), 1506 (w), 1407 (s), 1343 (w), 1258 (w), 1219 (w), 1121 (s), 848 (w), 728 (w); HRMS (ESI) caledd for C19H13N2O2S [M + Na]+: 359.0625, found: 359.0631.

4.4. Typical Procedure for the Synthesis of Compounds 2k-2n
A mixture of (E)-2-benzoyl-3-(4-fluorophenyl)acryloni-
itrile 1k (50.2 mg, 0.2 mmol), malononitrile (19.8 mg, 0.3 mmol), and piperidine (17.0 mg, 0.2 mmol) in toluene (2 mL) was stirred at room temperature for 24 h. After the solvent was evaporated, the residue was purified by flash column chromatography (ethyl acetate/petroleum ether = 1:5) to give 2k.

4.4.1. 2-Amino-4-(4-Fluorophenyl)-6-Phenyl-4H-Pyran-3,5,6-Dicarbonitrile (2k)  
Yellow solid, yield 85%, mp 61°C - 63°C; 1H NMR (400 MHz, CDCl3): δ = 7.23 - 7.13 (6H, m, ArH), 7.09 - 7.07 (2H, m, ArH), 7.10 (2H, ddd, J = 8.6, 5.0, 2.7 Hz, ArH), 4.89 (2H, s, NH2), 4.35 (1H, s, CH); 13C NMR (100 MHz, CDCl3): δ = 130.53, 130.50, 128.39, 127.84, 126.26, 116.94, 116.39, 116.12, 90.78, 90.92, 27.21; IR (KBr) ν/cm⁻¹: 3333 (w), 2198 (w), 1673 (s), 1602 (m), 1558 (s), 1506 (m), 1427 (m), 1341 (s), 1263 (w), 1081 (m), 743 (w); HRMS (ESI) calcd for C19H12N3O2FS⁺ [M + H]⁺: 389.1572, found: 389.1569.

4.4.2. 2-Amino-6-Methyl-4,5-Diphenyl-4H-Pyran-3-Carbonitrile (2l) [12]  
White solid, yield 78%, 1H NMR (400 MHz, CDCl3): δ = 7.26 - 7.13 (6H, m, ArH), 7.09 - 7.07 (2H, m, ArH), 6.90 - 6.87 (2H, m, ArH), 4.49 (2H, s, NH2), 4.18 (1H, s, CH), 1.80 (3H, s, CH3).

4.4.3. 2-Amino-4-(4-Chlorophenyl)-5,6-Diphenyl-4H-Pyran-3-Carbonitrile (2m) [12]  
White solid, yield 80%, 1H NMR (400 MHz, CDCl3): δ = 7.26 - 7.07 (12H, m, ArH), 6.84 - 6.82 (2H, m, ArH), 4.56 (2H, s, NH2), 4.35 (1H, s, CH).

4.4.4. 2-Amino-5,6-Diphenyl-4-(Thiophen-2-yl)-4H-Pyran-3-Carbonitrile (2n) [12]  
White solid, yield 86%, 1H NMR (400 MHz, CDCl3): δ = 7.25 - 7.10 (9H, m, ArH), 6.95 - 6.93 (2H, m, ArH), 6.87 - 6.85 (1H, m, ArH), 6.79 (1H, d, J = 2.8, Hz, ArH), 4.66 (1H, s, CH), 4.58 (2H, s, NH2).

4.5. Typical Procedure for the Synthesis of Compounds 4a and 4j

A mixture of compound 2a (39.1 mg, 0.1 mmol), AcONH4 (92 mg, 1.2 mmol), and AcOH (1.0 mL) in EtOAc (1.0 mL) was refluxed for 24 h. After cooled to room temperature, EtOAc (10 mL) was added. The mixture was washed with saturated aqueous NaHCO3 (10 mL) and brine (5 mL), and dried over anhydrous Na2SO4. After the solvent was evaporated under vacuum, the residue was purified by flash column chromatography (ethyl acetate/petroleum ether = 2:5) to give the products 4a.

4.5.1. (E)-2-Amino-8-(4-Fluorobenzylidene)-4-(4-Fluorophenyl)-6-Methyl-5,6,7,8-Tetrahydro-1,6-Naphthyridine-3-Carbonitrile (4a)  
Yellow solid, yield 80%, mp 208°C - 210°C; 1H NMR (400 MHz, CDCl3): δ = 8.02 (1H, d, J = 6.6 Hz, ArH), 7.51-7.11 (7H, m, ArH), 5.26 (2H, s, NH2), 3.65 (2H, s, CH2), 2.35 (3H, s, CH3); 13C NMR (100 MHz, CDCl3): δ = 164.50, 163.38, 162.20, 161.18, 157.36, 153.20, 152.20, 132.66, 131.78, 131.48, 131.40, 130.21, 130.13, 129.39, 116.55, 116.22, 116.00, 115.58, 115.37, 90.78, 55.84, 55.27, 45.52; IR (KBr) ν/cm⁻¹: 3427 (s), 2215 (w), 2024 (w), 1627 (m), 1602 (m), 1558 (s), 1506 (s), 1423 (w), 1224 (m), 1160 (w), 1103 (s), 917 (w), 829 (w), 558 (m); HRMS (ESI) calcd for C19H14FNN3O2S⁺ [M + H]⁺: 389.1572, found: 389.1569.

4.5.2. 2-Amino-4-(4-Fluorophenyl)-5H-Thiochromeno[4,3-b]Pyridine-3-Carbonitrile (4j)  
Yellow solid, yield 85%, mp 202°C - 204°C; 1H NMR (400 MHz, CDCl3): δ = 8.32 (1H, d, J = 6.6 Hz, ArH), 7.51-7.11 (7H, m, ArH), 5.26 (2H, s, NH2), 3.65 (2H, s, CH2); 13C NMR (100 MHz, CDCl3): δ = 164.51, 162.03, 158.00, 154.68, 151.51, 136.62, 133.51, 131.06, 130.57, 130.53, 130.50, 128.39, 127.84, 126.26, 116.94, 116.39, 116.17, 90.92, 27.21; IR (KBr) ν/cm⁻¹: 3446 (s), 2360 (w), 2213 (w), 2023 (w), 1626 (s), 1559 (m), 1427 (m), 1224 (w), 1074 (s), 843 (w), 767 (w), 546 (m); HRMS (ESI) calcd for C19H14FNN3S⁺ [M + H]⁺: 334.0804, found: 334.0804.

4.6. Evaluation of Antitubercular Activity

Autoluminescent M. tuberculosis H37Ra was constructed as previously reported [14] and was inoculated in a 50 mL centrifuge tube containing 5 mL 7H9 with 0.1% Tween80 and 10% ODAC, then incubated at 37°C with shaking. When the culture reached an OD600 nm of 0.7, the culture was diluted. 50 µL diluted H37Ra were inoculated in sterile 384 well plate. The RLU of each well should be between 8000 - 12000 and was recorded as the basic luminescence of Day 0. The test compounds and the positive drugs were added to the 384 well plate in triplicate by the Echo520 with the final concentration 1 µg/mL. The luminescent values were detected for the following three days. The data were analyzed with the Excel compared to the DMSO control to estimate the inhibition activity of the compounds.

The antitubercular activities against M. tuberculosis H37Rv were determined by standard agar dilution method [15].

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