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

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ABSTRACT

A series of 2-amino-4*H*-pyran-3-carbonitrile derivatives were designed and synthesized. Their antitubercular activities were evaluated against autoluminescent *M*. *tuberculosis* H37Ra and standard strain *M*. *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-4*H*-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 pyrazinaimde) 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-aminopyranopyridine-3-carbonitriles as a new type of antitubercular agents (**Scheme 1**) [9]. Several compounds showed excellent antitubercular activity comparable with isoniazid. Recently Perumal, Sriram and co-workers also found that 1,2,4-oxadiazoles derived from 2-aminopyranopyridine-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-4*H*-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-4*H*-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.

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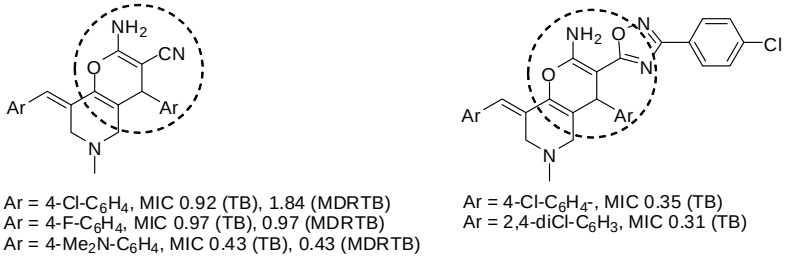


Ar = 4-Cl-C_6H_4, MIC 0.92 (TB), 1.84 (MDRTB)
Ar = 4-F-C_6H_4, MIC 0.97 (TB), 0.97 (MDRTB)
Ar = 4-Me_2N-C_6H_4, MIC 0.43 (TB), 0.43 (MDRTB)

Ar = 4-Cl-C_6H_4-, MIC 0.35 (TB)
Ar = 2,4-diCl-C_6H_3, MIC 0.31 (TB)

Scheme 1. 2-Amino-4*H*-pyran-3-carbonitrile and their derivatives with potent antitubercular activity.

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 **3a** and **3b** as the catalysts (**Scheme 3**) [11].

2-Aminopyranopyridine-3-carbonitriles **2h-2n** derived from monoenones **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 (sp^3) 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 antitubercular activity of racemic 2-amino-4*H*-pyran-3-carbonitriles **2a-2e**, homochiral 2-amino-4*H*-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 antitubercualr activity.

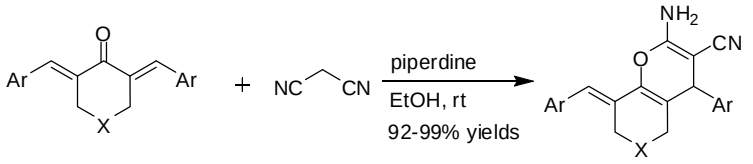
We further examined the inhibitive activity of the compounds against standard strain *M. tuberculosis* H37Rv and the results are summarized in **Table 1**. Perumal and co-workers reported that compound **2a** and **2d** possess excellent antitubercular activities (MIC 0.97 and 0.92 μg/mL against H37Rv 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-4*H*-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-4*H*-pyran-3-carbonitriles and their structural analogs. Homochiral 2-amino-4*H*-pyran-3-carbonitriles were also prepared via the organocatalytic enantioselctive reaction. The antitubercualr 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-4*H*-pyran-3-carbonitriles as potential antitubercular agents, the clarification of the contradictive activity data is required.

4. Experimentals

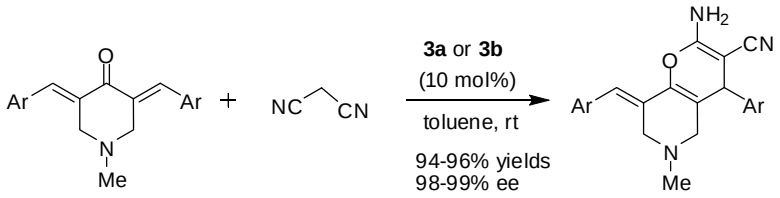
^1H and ^{13}C NMR spectra were recorded on a Bruker Advance 400 MHz spectrometer as solutions in $CDCl_3$. Chemical shifts in ^1H NMR spectra are reported in parts per million (ppm, δ) downfield from the internal standard Me_4Si (TMS, δ = 0 ppm). Chemical shifts in ^{13}C NMR spectra are reported relative to the central line of the chloroform signal (δ = 77.0 ppm). The following abbreviations are used to designate chemical shift mutiplicities: s = singlet, d = doublet, m = multiplet. High-resolution mass spectra were obtained with Shimadazu 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). The flash column chromatography was car-



1a-1g **2a-2g**

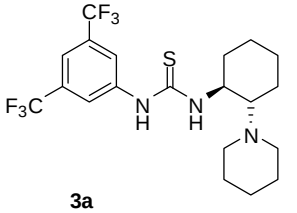
2a: Ar = 4-F-C_6H_4, X = CH_3N; **2b**: Ar = 3-F-C_6H_4, X = CH_3N;
2c: Ar = 3,4-diF-C_6H_3, X = CH_3N; **2d**: Ar = 4-Cl-C_6H_4, X = CH_3N;
2e: Ar = 4-F-C_6H_4, X = O; **2f**: Ar = 4-F-C_6H_4, X = S; **2g**: Ar = 4-F-C_6H_4, X = CH_2

Scheme 2. Synthesis of 2-amino-4*H*-pyran-3-carbonitriles 2a-2g.

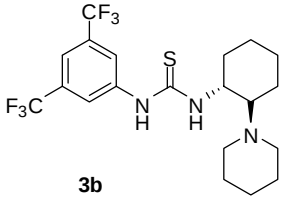


1a,1d

(*R*)-**2a, 2d** and (*S*)-**2a, 2d**

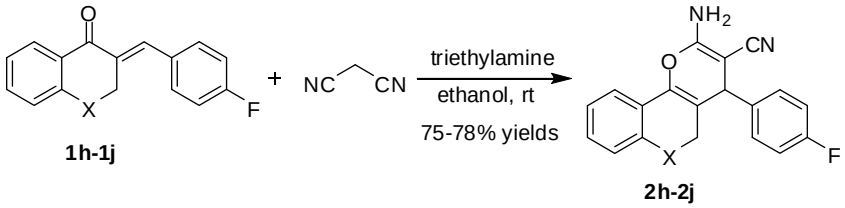


3a



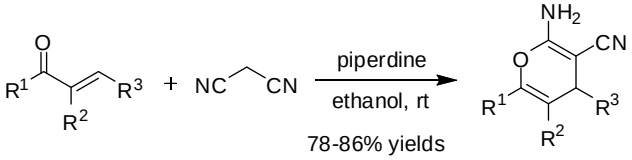
3b

Scheme 3. Synthesis of homochiral 2-amino-4*H*-pyran-3-carbonitriles.



1h-1j **2h-2j**

2h: X = CH_2; **2i**: X = O; **2j**: X = S

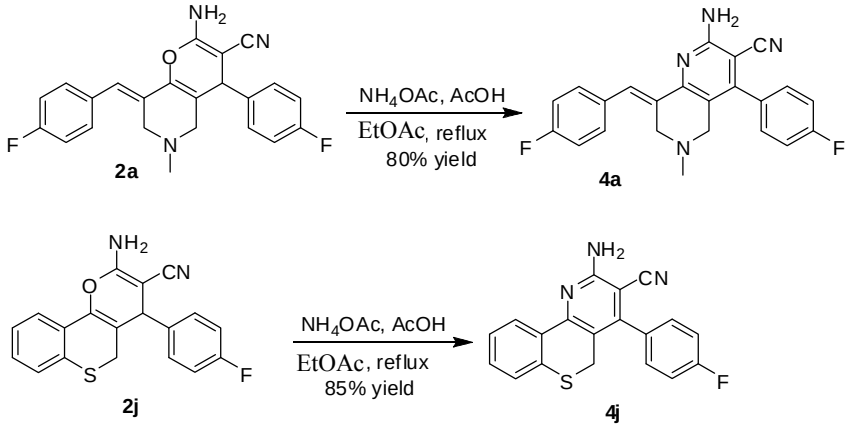


1k-1n **2k-2n**

2k: R^1 = Ph, R^2 = CN, R^3 = 4-F-C_6H_4; **2l**: R^1 = Me, R^2 = Ph, R^3 = Ph;
2m: R^1 = Ph, R^2 = Ph, R^3 =4-Cl-C_6H_4; **2n**: R^1 = Ph, R^2 = Ph, R^3 = 2-thiophenyl

Scheme 4. Synthesis of 2-amino-4*H*-pyran-3-carbonitriles 2h-2n derived from monoenones.

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.



Scheme 5. Synthesis of pyridine analogs 4a and 4j.

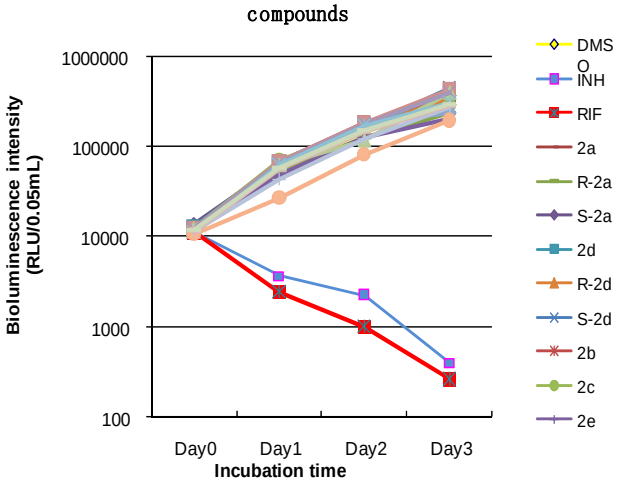


Figure 1. Inhibitive activity against autoluminescent *M. tuberculosis* H37Ra, (INH = isoniazid, RIF = rifampicin. All the compounds were tested at the concentration of 10 μg/mL).

4.1. Typical Procedure for the Synthesis of Compounds 2a-2g

A mixture of (3*E*, 5*E*)-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; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.24 - 7.17 (4H, m, ArH), 7.08-7.02 (4H, m, ArH), 6.86 (1H, s, HC=C), 4.55 (2H, s, NH_2), 4.03 (1H, s, CH), 3.52 (1H, d, J = 13.4 Hz, CH_2), 3.36 (1H, d, J = 13.0 Hz, CH_2), 2.95 (1H, d, J = 15.9 Hz, CH_2), 2.72 (1H, d, J = 15.4 Hz, CH_2), 2.28 (3H, s, CH_3).

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; ^{1}H NMR (400 MHz, $CDCl_3$): $\delta CDCl_3$ = 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, NH_2), 4.04 (1H, s, CH), 3.55 (1H, d, J = 14.4 Hz, CH_2), 3.36 (1H, d, J = 14.6 Hz, CH_2), 2.97 (1H, d, J = 15.8 Hz, CH_2), 2.74 (1H, d, J = 16.0 Hz, CH_2), 2.29 (3H, s, CH_3).

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; ^{1}H NMR (400 MHz, CDCl3): δ = 7.19 - 6.94 (6H, m, ArH), 6.80 (1H, s, HC=C), 4.63 (2H, s, NH_2), 4.01 (1H, s, CH), 3.50 (1H, d, J = 13.8 Hz, CH_2), 3.34 (1H, d, J = 14.0 Hz, CH_2), 2.96 (1H, d, J = 16.1 Hz, CH_2), 2.72 (1H, d, J = 15.8 Hz, CH_2), 2.30 (3H, s, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 158.77, 140.11, 139.09, 133.02, 127.70, 125.36, 123.78, 121.27, 119.07, 117.92, 117.75, 117.74, 117.60, 117.42, 117.25, 116.72, 112.66, 60.09, 55.13, 54.47, 44.90, 41.09; IR (KBr) ν/cm^{-1}: 3484 (m), 2194 (s), 1681 (m), 1644 (m), 1595 (m), 1517 (s), 1396 (w), 1264 (w), 1110 (m), 910 (w), 785 (w); HRMS (ESI) calcd for $C_{23}H_{17}N_3OF_4^+$ [M + H]$^+$: 428.1381, found: 428.1389.

4.1.4. (E)-2-Amino-8-(4-Chlorobenzylidene)-4-(4-Chlorophe-nyl)-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; ^{1}H NMR (400 MHz, CDCl3): δ = 7.35 - 7.32 (4H, m, ArH), 7.21 -

Table 1. Evaluation of antitubercular activity against *M. tuberculosis* H37Rv.

Compounds	MIC (μg/mL)
2a	>10
2b	>10
2c	>10
2d	>10
2e	>10
2f	>10
2g	>10
(*R*)-2a	>10
(*R*)-2d	>10
(*S*)-2a	>10
(*S*)-2d	>10
2h	>10
2i	>10
2j	>10
2k	>10
2l	>10
2m	>10
2n	>10
4a	>10
4j	>10
Isoniazid	0.03
Rifampicin	<0.25

7.13 (4H, m, ArH), 6.84 (1H, s, HC=C), 4.57 (2H, s, NH_2), 4.02 (1H, s, CH), 3.51 (1H, d, J = 13.8 Hz, CH_2), 3.35 (1H, d, J = 13.6 Hz, CH_2), 2.94 (1H, d, J = 16.0 Hz, CH_2), 2.72 (1H, d, J = 16.0 Hz, CH_2), 2.27(3H, s, CH_3).

4.1.5. (E)2-Amino-8-(4-Fluorobenzylidene)-4-(4-Fluorophenyl)-4,5,7,8-Tetrahydropyrano [4,3-b]Pyran-3-Carbonitrile (2e)

While solid, yield 99%, mp 221˚C - 223˚C; ^{1}H NMR (400 MHz, DMSO-d6): δ = 7.44 - 7.05 (8H, m, ArH), 6.92 (1H, s, HC=C), 6.90 (2H, s, NH_2), 4.59 (1H, d, J = 14.0 Hz, CH_2), 4.48 (1H, d, J = 13.9 Hz, CH_2), 4.17 (1H, d, J = 15.5, CH_2), 4.15 (1H, s, CH), 3.72 (1H, d, J = 15.6 Hz, CH_2); ^{13}C NMR (100 MHz, DMSO): δ = 162.50, 160.08, 159.70, 139.17, 138.07, 131.72, 131.02, 130.99, 129.42, 129.30, 126.09, 120.38, 120.15, 115.61, 115.55, 115.40, 115.34, 112.96, 65.12, 64.90, 55.77; IR (KBr) ν/cm^{-1}: 3472 (m), 3312 (w), 2220 (s), 1685 (m), 1660 (m), 1603 (s), 1508 (s), 1414 (w), 1391 (w), 1264 (w), 1228 (s), 1155 (w), 1098 (m), 883 (w), 838 (w), 780 (w), 744 (m); HRMS (ESI) calcd for $C_{22}H_{16}N_2O_2F_2^+$ [M + Na]$^+$: 401.1072, found: 401.1065.

4.1.6. 2-Amino-8-(4-Fluorobenzylidene)-4-(4-Fluorophenyl)-4,5,7,8-Tetrahydrothiopyrano [4,3-b]Pyran-3-Carbonitrile (2f)

White solid, yield 98%, mp 205˚C - 207˚C; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.25 - 7.21 (4H, m, ArH), 7.10 - 7.03 (4H, m, ArH), 6.93 (1H, s, HC=C), 4.53 (2H, s, NH_2), 4.03 (1H, s, CH), 3.58 (2H, q, J = 9.0 Hz, CH_2), 3.04 (2H, q, J = 12.0 Hz, CH_2); ^{13}C NMR (100 MHz, DMSO-d6): δ = 159.65, 143.42, 141.39, 135.84, 129.13, 128.74, 128.51, 127.51, 127.39, 127.16, 126.35, 124.39, 120.23, 114.04, 55.97, 43.21, 27.20, 27.15; IR (KBr) ν/cm^{-1}: 3452 (m), 3355 (m), 2360 (s), 2192 (m), 2024 (w), 1673 (s), 1627 (w), 1599 (w), 1506 (s), 1410 (m), 1233 (m), 1126 (m), 881 (w), 850 (w); HRMS (ESI) calcd for $C_{22}H_{16}N_2OF_2S^+$ [M + Na]$^+$: 417.0844, found: 417.0843.

4.1.7. (E)-2-Amino-8-(4-Fluorobenzylidene)-4-(4-Fluorophenyl)-5,6,7,8-Tetrahydro-4H-Chromene-3-Carbonitrile (2g) [10]

White solid, yield 99%, mp 211˚C - 213˚C: ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.25 - 7.20 (4H, m, ArH), 7.06 - 7.02 (4H, m, ArH), 6.83 (1H, s, HC=C), 4.56 (2H, s, NH_2), 3.97 (1H, s, CH), 2.74 - 2.70 (1H, m, CH_2), 2.60 - 2.56 (1H, m, CH_2), 2.04 - 1.92 (2H, m, CH_2), 1.74 - 1.52 (2H, m, CH_2); HRMS (ESI) calcd for $C_{23}H_{18}N_2OF_2^+$ [M + Na]$^+$: 399.1279, found: 399.1261.

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

A mixture of (3*E*, 5*E*)-3,5-bis(4-fluorobenzylidene)-1-methylpiperidin-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 centrifugalization.

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

White solid, yield 94%, mp 197˚C - 198˚C; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.24 - 7.17 (4H, m, ArH), 7.08-7.02 (4H, m, ArH), 6.86 (1H, s, HC=C), 4.55 (2H, s, NH_2), 4.03 (1H, s, CH), 3.52 (1H, d, J = 13.4 Hz, CH_2), 3.36 (1H, d, J = 13.0 Hz, CH_2), 2.95 (1H, d, J = 15.9 Hz, CH_2), 2.72 (1H, d, J = 15.4 Hz, CH_2), 2.28 (3H, s, CH_3). 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) = 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; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.24 - 7.17 (4H, m, ArH), 7.08 - 7.02 (4H, m, ArH), 6.86 (1H, s, HC=C), 4.55 (2H, s, NH_2), 4.03 (1H, s, CH), 3.52 (1H, d, J = 13.4 Hz, CH_2), 3.36 (1H, d, J = 13.0 Hz, CH_2), 2.95 (1H, d, J = 15.9 Hz, CH_2), 2.72 (1H, d, J = 15.4 Hz, CH_2), 2.28 (3H, s, CH_3). 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-Chlorophe-nyl)-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; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.35 - 7.32 (4H, m, ArH), 7.21-7.13 (4H, m, ArH), 6.84 (1H, s, HC=C), 4.57 (2H, s, NH_2), 4.02 (1H, s, CH), 3.51 (1H, d, J = 13.8 Hz, CH_2), 3.35 (1H, d, J = 13.6 Hz, CH_2), 2.94 (1H, d, J = 16.0 Hz, CH_2), 2.72 (1H, d, J = 16.0 Hz, CH_2), 2.27 (3H, s, CH_3). 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-Chlorophe-nyl)-6-Methyl-5,6,7,8-Tetrahydro-4H-Pyr-ano[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=C), 4.57 (2H, s, NH_2), 4.02 (1H, s, CH), 3.51 (1H, d, J = 13.8 Hz, CH_2), 3.35 (1H, d, J = 13.6 Hz, CH_2), 2.94 (1H, d, J = 16.0 Hz, CH_2), 2.72 (1H, d, J = 16.0 Hz, CH_2), 2.27 (3H, s, CH_3). 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) = 7.6 min, t_r (minor) = 9.7 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(2*H*)-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; ^{1}H NMR (400 MHz, $CDCl_3$): 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, NH_2), 4.07 (1H, s, CH), 2.81 (1H, dd, J = 15.9, 8.1 Hz, CH_2), 2.69 (1H, dt, J = 15.7, 7.8 Hz, CH_2), 2.16 (1H, dt, J = 15.9, 7.9 Hz, CH_2), 2.09 - 1.96 (1H, m, CH_2); HRMS (ESI) calcd for $C_{20}H_{15}N_2OF^+$ $[M + Na]^+$: 341.1061, found: 341.1054.

4.3.2. 2-Amino-4-(4-Fluorophenyl)-4,5-Dihydropyrano[3,2-c]Chromene-3-Carbonitrile (2i)

Yellow solid, yield 75%, mp 175˚C - 177˚C; ^{1}H NMR (400 MHz, $CDCl_3$): 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, NH_2), 4.62 - 4.55 (1H, d, J = 13.6 Hz, CH_2), 4.41 (1H, d, J = 13.7 Hz, CH_2), 4.03 (1H, s, CH); ^{13}C NMR (100 MHz, $CDCl_3$): 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) ν/cm^{-1}: 3327 (m), 2195 (m), 1709 (s), 1656 (s), 1600 (s), 1506 (m), 1356 (m), 1230 (s), 1157 (m), 1101 (w), 1036 (w), 836 (m), 755 (w); HRMS (ESI) calcd for $C_{19}H_{13}N_2O_2F^+$ $[M + Na]^+$: 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; ^{1}H NMR (400 MHz, $CDCl_3$): 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, NH_2), 4.13 (1H, s, CH), 3.29 (1H, d, J = 15.1 Hz, CH_2), 3.09 (1H, d, J = 15.1 Hz, CH_2); ^{13}C NMR (100 MHz, $CDCl_3$): 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) ν/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) calcd for $C_{19}H_{13}N_2OFS^+$ $[M + Na]^+$: 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-Dicarbonitrile (2k)

Yellow solid, yield 85%, mp 61˚C - 63˚C; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.74 (2H, dd, J = 7.0, 1.5 Hz, ArH), 7.56 - 7.40 (3H, m, ArH), 7.38 - 7.27 (2H, m, ArH), 7.10 (2H, ddd, J = 8.6, 5.0, 2.7 Hz, ArH), 4.89 (2H, s, NH_2), 4.35 (1H, s, CH); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 163.98, 161.48, 157.86, 157.70, 136.49, 131.96, 129.71 , 129.60, 129.51, 128.81, 127.80, 117.76, 116.83, 116.34, 116.12, 90.70, 59.91, 40.11; IR (KBr) ν/cm^{-1}: 3333 (w), 2198 (w), 1673 (s), 1602 (w), 1508 (m), 1401 (m), 1341 (s), 1263 (w), 1081 (m), 743 (w); HRMS (ESI) calcd for $C_{19}H_{12}N_3OF^+$ $[M + Na]^+$: 340.0857, found: 340.0891.

4.4.2. 2-Amino-6-Methyl-4,5-Diphenyl-4H-Pyran-3-Carbonitrile (2l) [12]

White solid, yield 78%, ^{1}H NMR (400 MHz, CDCl3): δ = 7.23 - 7.13 (6H, m, ArH), 7.09 - 7.07 (2H, m, ArH), 6.90 - 6.87 (2H, m, ArH), 4.49 (2H, s, NH_2), 4.18 (1H, s, CH), 1.80 (3H, s, CH_3).

4.4.3. 2-Amino-4-(4-Chlorophenyl)-5,6-Diphenyl-4H-Pyran-3-Carbonitrile (2m) [12]

White solid, yield 80%, ^{1}H NMR (400 MHz, CDCl3): δ = 7.26 - 7.07 (12H, m, ArH), 6.84 - 6.82 (2H, m, ArH), 4.56 (2H, s, NH_2), 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%, ^{1}H 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, NH_2).

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

A mixture of compound **2a** (39.1 mg, 0.1 mmol), $AcONH_4$ (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 $NaHCO_3$ (10 mL) and brine (5 mL), and dried over anhydrous Na_2SO_4. 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; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.02 (1H, s, HC=C), 7.31 - 7.10 (8H, m, ArH), 5.15 (2H, s, NH_2), 3.61 (2H, s, CH_2), 3.27 (2H, s, CH_2), 2.35 (3H, s, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): 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 $C_{23}H_{18}N_4F_2^+$ $[M + H]^+$: 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; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.32 (1H, d, J = 6.6 Hz, ArH), 7.51-7.11 (7H, m, ArH), 5.26 (2H, s, NH_2), 3.65 (2H, s, CH_2); ^{13}C NMR (100 MHz, $CDCl_3$): 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 $C_{19}H_{12}N_3FS^+$ $[M + H]^+$: 334.0809, 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 OD_{600} 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 or 10 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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