FeF$_3$-catalyzed MCR in PEG-400: ultrasound assisted synthesis of N-substituted 2-aminopyridines

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**General methods:** Unless stated otherwise, solvents and chemicals were obtained from commercial sources and were used without further purification. Reactions were monitored by thin layer chromatography (TLC) on silica gel plates (60 F254), visualizing with ultraviolet light or iodine spray. Flash chromatography was performed on silica gel (100-200 mesh) using hexane and ethyl acetate. $^1$H and $^{13}$C NMR spectra were determined in CDCl$_3$ solution by using 400 or 100 MHz spectrometers, respectively. Proton chemical shifts (δ) are relative to tetramethylsilane (TMS, δ = 0.00) as internal standard and expressed in ppm. Spin multiplicities are given as s (singlet), d (doublet), t (triplet) and m (multiplet) as well as b (broad). Coupling constants (J) are given in hertz. Infrared spectra were recorded on a FT-IR spectrometer. Melting points were determined using melting point B-540 apparatus and are uncorrected. HRMS was determined using waters LCT premier XETOF ARE-047 apparatus. Reactions were performed using a laboratory ultrasonic bath Bandelin SONOREX™ SUPER RK 514 BH model producing irradiation of 35 kHz.

**Typical procedure for the synthesis of 5a:** To a mixture of acetophenone (1a, 150 mg, 1.25 mmol), benzaldehyde (2a, 130mg, 1.25 mmol), o-toluidine (3a, 134 mg, 1.25 mmol), and malononitrile (4, 82 mg, 1.25 mmol) in PEG-400 (0.3 mL) was added FeF$_3$ (14.1mg, 10 mol%) at room temperature. The mixture was then stirred at 60 ºC under ultrasound irradiation in open air for 3h (the reaction was monitored by TLC). After completion of the reaction the mixture was diluted with EtOAc (10 mL) and washed with cold water (2 x 5 mL). The organic layer was collected, dried over anhydrous Na$_2$SO$_4$, filtered and concentrated under low vacuum. The residue was purified by column chromatography over silica gel (100-200 mesh) using EtOAc-hexane to give the desired product.
**Table S-1.** Ultrasound assisted synthesis of \(N\)-substituted 2-aminopyridines (5) via \(\text{FeF}_3\) catalyzed MCR.

\[
\begin{array}{cccccc}
\text{Entry} & \text{Acetophenone (1)} & \text{Aldehyde (2)} & \text{Aniline (3)} & \text{Product (5)} & \text{Yield (\%)} \\
1 & \text{Acetophenone 1a} & \text{Bezaldehyde 2a} & \text{o-Toludine 3a} & \text{5a} & 92 \\
2 & 1a & 2a & \text{Aniline 3b} & \text{5b} & 90 \\
3 & 1a & 2a & \text{2,3-Dimethyl aniline 3c} & \text{5c} & 92 \\
4 & 1a & \text{4-Methoxy bezaldehyde 2b} & \text{4-Flouro aniline 3d} & \text{5d} & 89 \\
5 & 1a & \text{Thiophene-2-aldehyde 2c} & 3b & \text{5e} & 90 \\
\end{array}
\]
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</table>
Spectral data

4,6-Diphenyl-2-(o-tolylamino)nicotinonitrile (5a)

![Chemical Structure](image)

Off white solid, mp:194-196 °C; $^1$H NMR (400 MHz, CDCl$_3$): $\delta$ 8.13 (d, $J = 7.6$ Hz, 1H), 8.03-8.00 (m, 2H), 7.69-7.66 (m, 2H), 7.58-7.52 (m, 3H), 7.46-7.43 (m, 3H), 7.33-7.27 (m, 4H), 7.14 (t, $J = 7.2$ Hz, 1H), 2.40 (s, 3H); $^{13}$C NMR (100 MHz, CDCl$_3$):$\delta$ 159.6, 156.6, 153.1, 148.3, 143.1, 138.3, 137.4, 130.6, 130.2, 129.8, 129.0 (2C), 128.7 (2C), 128.2 (2C), 127.3 (2C), 126.5, 124.3 (2C), 122.7, 111.1, 89.5, 18.0; IR (KBr, cm$^{-1}$): 3334, 2963, 2214, 1601, 1582, 1491; HRMS (ESI) ([M] $^+$1) calcd for C$_{25}$H$_{20}$N$_3$O: 362.1657, found: 362.1669.

4,6-Diphenyl-2-(phenylamino)nicotinonitrile (5b)

![Chemical Structure](image)

Pale yellow solid, mp: 213-215°C; $^1$H NMR (400 MHz, CDCl$_3$): $\delta$ 8.08 (dd, $J_{1,2} = 2.4$ Hz, $J_{1,3} = 8.0$ Hz, 2H), 7.77 (d, $J = 7.2$ Hz, 2H), 7.67-7.65 (m, 2H), 7.57-7.47 (m, 6H), 7.43 (t, $J = 7.6$ Hz, 2H), 7.33 (s, 1H), 7.27-7.26 (m, 1H), 7.16 (t, $J = 7.6$ Hz, 1H); $^{13}$C NMR (100 MHz, CDCl$_3$):$\delta$ 158.9, 156.5, 155.4, 138.9, 137.8, 136.9, 130.3, 129.9, 128.9 (2C), 128.9 (2C), 128.8(2C), 128.1 (2C), 127.4(2C), 123.5, 120.5 (2C), 117.0, 111.4, 90.0; IR (KBr, cm$^{-1}$): 3335, 2215, 1602, 1582, 1497; HRMS (ESI) ([M] $^+$1) calcd for C$_{24}$H$_{18}$N$_3$: 348.1501, found: 348.1514.

2-((2,3-Dimethylphenyl)amino)-4,6-diphenylnicotinonitrile (5c)

![Chemical Structure](image)

Off white solid, mp: 172-174°C; $^1$H NMR (400 MHz, CDCl$_3$): $\delta$ 7.99 – 7.95 (m, 2H), 7.80 (d, $J =$
8.0Hz, 1H), 7.69-7.65 (m, 2H), 7.57-7.51 (m, 3H), 7.43-7.40 (m, 3H), 7.29 (s, 1H), 7.22 (t, J = 8.0 Hz, 1H), 7.09-7.05 (m, 2H), 2.37 (s, 3H), 2.28 (s, 3H); $^{13}$C NMR (100 MHz, CDCl$_3$): δ 158.8, 157.4, 155.3, 137.8, 137.4, 137.1, 136.8, 130.1, 129.8, 129.6, 128.9 (2C), 128.7 (2C), 128.2 (2C), 127.3 (2C), 126.6, 125.5, 121.8, 117.2, 110.8, 89.5, 20.7, 14.0; IR (KBr, cm$^{-1}$): 3395, 2965, 2209, 1603, 1586, 1447; HRMS (ESI) ([M$^+$]+1) calcld for C$_{26}$H$_{22}$N$_3$: 376.1814, found: 376.1801.

2-((4-Fluorophenyl)amino)-4-(4-methoxyphenyl)-6-phenylnicotinonitrile (5d)

Yellow solid, mp: 169-172°C; $^1$H NMR (400 MHz, CDCl$_3$): δ 8.13 (d, J = 8.0 Hz, 1H), 8.03-8.01 (m, 2H), 7.65-7.61 (m, 2H), 7.52 (t, J = 8.0 Hz, 3H), 7.39 (d, J = 8.4 Hz, 3H), 7.03-6.94 (m, 4H), 3.85 (s, 3H); $^{13}$C NMR (100 MHz, CDCl$_3$): δ 161.5, 158.0, 159.1 & 157.1 (d, $^1$J$_{C,F}$ = 241.9 Hz), 155.1, 149.0, 144.5, 133.3,130.9, 130.8 (2C), 129.6 & 129.5 (d, $^3$J$_{C,F}$ =6.1 Hz) (2C), 128.9 (2C), 127.3 (2C), 127.1, 115.6, 115.3 (d, $^2$J$_{C,F}$ =22.9 Hz) (2C), 114.7 (2C), 114.4, 113.6, 89.6, 55.5; IR (KBr, cm$^{-1}$): 3336, 2952, 2931, 2216, 1619, 1582, 1498,1210; HRMS (ESI) ([M$^+$]+1) calcld for C$_{25}$H$_{19}$N$_3$OF: 396.1512, found: 396.1513.

6-Phenyl-2-(phenylamino)-4-(thiophen-2-yl)nicotinonitrile (5e)

Pale yellow solid, mp:186-188 °C; $^1$H NMR (400 MHz, CDCl$_3$): δ 8.07 (dd, $J_{1,2}$ = 2.4 Hz, $J_{1,3}$= 7.6 Hz, 2H), 7.89 – 7.88 (m, 1H), 7.75 (d, J = 7.6 Hz, 2H), 7.56 (d, J = 5.2 Hz, 1H), 7.52 – 7.47 (m, 3 H), 7.43 – 7.41 (m, 3H), 7.28 – 7.26 (m, 1H), 7.24 – 7.22 (m, 1H), 7.16 (t, J = 7.6 Hz, 1H); $^{13}$C NMR (100 MHz, CDCl$_3$): δ 159.2, 156.9, 146.9, 138.8, 138.7, 137.8, 130.4, 130.3, 128.9,
128.8 (2C), 128.7, 128.5 (2C), 127.4 (2C), 123.6, 120.7 (2C), 117.5, 110.4, 90.8; IR (KBr, cm<sup>-1</sup>): 3328, 2216, 1603, 1549, 1497; HRMS (ESI) ([M] <sup>+</sup>1) calcd for C<sub>22</sub>H<sub>16</sub>N<sub>3</sub>S: 354.1065, found: 354.1068.

**4-Isobutyl-6-phenyl-2-(o-tolylamino)nicotinonitrile (5f)**

![Chemical structure](image)

Off White color solid, mp: 131-133 °C; <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>): δ 8.14 (d, <em>J</em> = 8.0 Hz, 1H), 7.98 (dd, <em>J</em><sub>1,2</sub> = 3.2 Hz, <em>J</em><sub>1,3</sub> = 4.8 Hz, 2H), 7.58 – 7.49 (m, 3H), 7.44 (d, <em>J</em> = 3.2 Hz, 2H), 7.09 – 7.08 (m, 3H), 2.72 (d, <em>J</em> = 7.6 Hz, 2H), 2.37 (s, 3H), 2.17-2.11 (m, 1H), 1.04 (d, <em>J</em> = 6.4 Hz, 6H); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>): δ 158.5, 157.0, 156.0, 139.1, 139.0, 138.0, 130.6, 130.5, 128.7 (2C), 127.3 (2C), 126.4 (2C), 122.3, 116.7, 111.7, 91.4, 43.9, 29.6, 22.4 (2C), 18.0; IR (KBr, cm<sup>-1</sup>): 3316, 2952, 2864, 2216, 1619, 1582, 1498; HRMS (ESI) ([M] <sup>+</sup>1) calcd for C<sub>23</sub>H<sub>24</sub>N<sub>3</sub>: 342.1970, found: 342.1984.

**6-(4-Methoxyphenyl)-4-phenyl-2-(o-tolylamino)nicotinonitrile (5g)**

![Chemical structure](image)

Brown color solid, mp: 199-201°C; <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>): δ 8.12 (d, <em>J</em> = 8.0 Hz, 1H), 8.0 (d, <em>J</em> = 8.8 Hz, 2H), 7.68 - 7.65 (m, 2H), 7.57 - 7.50 (m, 3H), 7.33 – 7.27 (m, 2H), 7.25 (s, 1H), 7.14 – 7.10 (m, 2H), 6.97 (d, <em>J</em> = 8.0 Hz, 2H), 3.87 (s, 3H), 2.40 (s, 3H); <sup>13</sup>C NMR (100 MHz, CDCl<sub>3</sub>): δ 160.0, 157.4, 156.4, 155.8, 141.8, 137.7, 136.6, 136.3, 130.6, 129.7, 128.9 (2C), 128.9 (2C), 128.2 (2C), 126.4 (2C), 124.3, 122.3, 114.1 (2C), 110.3, 90.8, 55.3, 18.0; IR (KBr, cm<sup>-1</sup>): 3423, 2962, 2923, 2200, 1598, 1483, 1168; HRMS (ESI) ([M] <sup>+</sup>1) calcd for C<sub>26</sub>H<sub>22</sub>N<sub>3</sub>O: 392.1763, found: 392.1747.
4-Isobutyl-6-phenyl-2-(phenylamino)nicotinonitrile (5h)

White solid, mp: 124-126°C; $^1$H NMR (400 MHz, CDCl$_3$): $\delta$ 8.03 (dd, $J_{1,2} = 2.0$ Hz, $J_{1,3} = 8.0$ Hz, 2H), 7.74 (d, $J = 8.0$ Hz, 2H), 7.50 - 7.43 (m, 3H), 7.41 (t, $J = 8.0$ Hz, 2H), 7.12-7.09 (m, 3H), 2.71 (d, $J = 6.8$ Hz, 2H), 2.13-2.06 (m, 1H), 1.03 (d, $J = 6.8$ Hz, 6H); $^{13}$C NMR (100 MHz, CDCl$_3$): $\delta$ 158.4, 157.0, 156.1, 139.0, 138.0, 130.1, 128.9 (2C), 128.7 (2C), 127.3 (2C), 123.3, 120.3 (2C), 116.5, 112.0, 91.0, 44.0, 29.7, 22.3 (2C); IR (KBr, cm$^{-1}$): 3335, 2953, 2863, 2216, 1619, 1582, 1498; HRMS (ESI) ([M]$^+$) calcd for C$_{22}$H$_{22}$N$_3$: 328.1814, found: 328.1810.

4-(4-Nitrophenyl)-6-phenyl-2-(phenylamino)nicotinonitrile (5i)

Orange solid, mp: 208-210 °C; $^1$H NMR (400 MHz, CDCl$_3$): $\delta$ 8.43 (d, $J = 8.4$ Hz, 2H), 8.07 (d, $J = 3.6$ Hz, 2H), 7.84 (d, $J = 8.8$ Hz, 2H), 7.75 (d, $J = 8.4$ Hz, 2H), 7.51-7.42 (m, 5H), 7.31-7.26 (m, 2H), 7.21-7.18 (m, 1H); $^1$H NMR (400 MHz, D$_2$O exchange): $\delta$ 8.43 (d, $J = 8.0$ Hz, 2H), 8.07 (d, $J = 4.0$ Hz, 2H), 7.84 (d, $J = 8.8$ Hz, 2H), 7.75 (d, $J = 8.0$ Hz, 2H), 7.52-7.49 (m, 3H), 7.45-7.43 (m, 2H), 7.31 (s, 1H), 7.22-7.16 (m, 1H); $^{13}$C NMR (100 MHz, CDCl$_3$): $\delta$ 159.6, 156.6, 153.1, 148.3, 143.1, 138.5, 137.4, 130.7, 129.4 (2C), 129.0, 128.9 (2C), 127.4 (2C), 124.5 (2C), 124.0 (2C), 120.9 (2C), 116.3, 110.9, 89.5; IR (KBr, cm$^{-1}$): 3335, 2217, 1607, 1579, 1514, 1498, 1352; HRMS (ESI) ([M]$^+$) calcd for C$_{24}$H$_{17}$N$_4$O$_2$: 393.1352, found: 393.1349.
2-((4-Cyanophenyl)amino)-4-(4-methoxyphenyl)-6-phenyl nicotinonitrile (5j)

Off white color solid, mp: 157-159 °C; $^1$H NMR (400 MHz, CDCl$_3$): $\delta$ 7.97-7.90 (m, 3H), 7.65-7.60 (m, 2H), 7.51 (t, $J = 7.2$ Hz, 3H), 7.38 (d, $J = 8.0$ Hz, 3H), 7.02-6.93 (m, 4H), 3.92 (s, 3H); $^{13}$C NMR (100 MHz, CDCl$_3$): $\delta$ 161.7, 159.8, 158.7, 148.1, 137.4, 135.7, 134.1 (2C), 133.4, 129.1 (2C), 128.8 (2C), 128.6 (2C), 128.3, 128.0 (2C), 115.0, 114.5 (2C), 111.9, 111.7, 108.7, 89.6, 55.2; IR (KBr, cm$^{-1}$): 3394, 2913, 2254, 2223, 1606, 1572, 1449, 1178; HRMS (ESI) ([M] $^+$1) calcd for C$_{26}$H$_{19}$N$_4$O: 403.1559, found: 403.1560.

2-((4-Bromophenyl)amino)-6-phenyl-4-(thiophen-2-yl) nicotinonitrile (5k)

Brown color solid, mp: 196-198°C; $^1$H NMR (400 MHz, CDCl$_3$): $\delta$ 8.04 (dd, $J_{1,2}= 3.2$ Hz, $J_{1,3}= 8.0$ Hz, 2H), 7.89 (dd, $J_{1,2}= 2.8$ Hz, $J_{1,3}= 8.0$ Hz, 1H), 7.65-7.63 (m, 2H), 7.57-7.49 (m, 7H), 7.44 (s, 1H), 7.24-7.22 (m, 1H); $^{13}$C NMR (100 MHz, CDCl$_3$): $\delta$ 159.2, 156.9, 149.3, 146.9, 137.9, 137.6, 131.8 (2C), 130.5, 129.1, 128.9 (2C), 128.5 (2C), 127.3 (2C), 122.3 (2C), 117.9, 116.3, 110.7, 88.1; IR (KBr, cm$^{-1}$): 3343, 2218, 1620, 1573, 1490; HRMS (ESI) ([M] $^+$1) calcd for C$_{22}$H$_{15}$N$_3$SBr: 432.0170, found: 432.0199.
6-(4-Chlorophenyl)-2-((2,3-dimethylphenyl)amino)-4-phenylnicotinonitrile (5l)

![Chemical Structure](image)

Off white solid, mp: 152-154°C; ^1H NMR (400 MHz, CDCl₃): δ 7.91 (dd, J₁₂ = 2.0 Hz, J₁₃ = 8.8 Hz, 2H), 7.72 - 7.65 (m, 3H), 7.57 - 7.51 (m, 3H), 7.40 (dd, J₁₂ = 2.0 Hz, J₁₃ = 6.8 Hz, 2H), 7.24 (s, 1H), 7.21 (t, J = 8.0 Hz, 1H), 7.09-7.06 (m, 2H), 2.37 (s, 3H), 2.27 (s, 3H); ^13C NMR (100 MHz, CDCl₃): δ 157.5, 157.4, 155.5, 141.8, 137.5, 136.9, 136.7, 136.3, 136.2, 129.9, 129.0 (2C), 128.9 (2C), 128.5 (2C), 128.1 (2C), 126.8, 125.5, 121.9, 117.0, 110.5, 89.8, 20.7, 14.0; IR (KBr, cm⁻¹): 3307, 2916, 2218, 1583, 1569, 1487; HRMS (ESI) ([M⁺] + 1) calcd for C₂₆H₂₁N₃Cl: 410.1424, found: 410.1431.

4-(4-Bromophenyl)-6-phenyl-2-(o-tolylamino)nicotinonitrile (5m)

![Chemical Structure](image)

Pale yellow color solid, mp: 158-160 °C; ^1H NMR (400 MHz, CDCl₃): δ 8.11 (d, J = 8.0 Hz, 1H), 8.02-7.98 (m, 2H), 7.70 (dd, J₁₂ = 1.6 Hz, J₁₂ = 8.4 Hz, 2H), 7.57-7.50 (m, 4H), 7.46 (t, J = 4.0 Hz, 2H), 7.33 - 7.27 (m, 3H), 7.15-7.12 (m, 1H), 2.39 (s, 3H); ^13C NMR (100 MHz, CDCl₃): δ 158.2, 155.3, 153.4, 143.3, 137.2, 135.2, 132.3, 132.2 (2C), 130.5, 129.9 (2C), 129.1, 128.9 (2C), 127.3 (2C), 126.5, 124.5, 122.2, 122.0, 115.5, 112.9, 89.7, 18.0; IR (KBr, cm⁻¹): 3446, 2916, 2235, 1658, 1608, 1486; HRMS (ESI) ([M⁺] + 1) calcd for C₂₅H₁₉N₃Br: 440.0762, found: 440.0753.

4-(Furan-2-yl)-6-phenyl-2-(o-tolylamino)nicotinonitrile (5n)

![Chemical Structure](image)

Brown color solid, mp: 147-149 °C; ^1H NMR (400 MHz, CDCl₃): δ 8.10 (d, J = 8.0 Hz, 1H), 8.04
– 8.02 (m, 2H), 7.67 (d, J = 7.2 Hz, 2H), 7.53 (d, J = 4.0 Hz, 1H), 7.47-7.44 (m, 3H), 7.31-7.28
(m, 2H), 7.13 (t, J = 7.6 Hz, 2H), 6.65 (dd, J1,2 = 1.6 Hz, J1,3 = 3.6 Hz, 1H), 2.39 (s, 3H); 13C NMR
(100 MHz, CDCl3): δ 158.7, 155.6, 153.1, 146.6, 144.5, 138.8, 138.3, 130.6, 130.2, 128.7 (2C),
127.3 (2C), 126.5, 124.3, 124.2, 120.7, 116.5, 113.1, 112.6, 106.2, 86.0, 18.0; IR (KBr, cm⁻¹):

6-(4-Cyanophenyl)-2-((2,3-dimethylphenyl)amino)-4-phenylnicotinonitrile (5o)

Brown color solid, mp: 198-200 °C; 1H NMR (400 MHz, CDCl3): δ 8.06 (d, J = 8.8 Hz, 2H), 7.69-
7.64 (m, 4H), 7.58-7.55 (m, 2H), 7.39-7.29 (m, 3H), 7.22 (t, J = 7.6 Hz, 1H), 7.12-6.92 (m, 2H),
2.37 (s, 3H), 2.27 (s, 3H); 13C NMR (100 MHz, CDCl3): δ 157.4, 156.4, 155.8, 141.8, 137.7,
136.6, 136.3, 132.4 (2C), 130.1, 129.2, 129.0 (2C), 128.1 (2C), 127.4 (2C), 127.1, 125.6, 122.1,
118.5, 116.7, 113.3, 111.2, 90.8, 20.7, 14.0; IR (KBr, cm⁻¹): 3308, 2938, 2228, 2204, 1606, 1583,

2-((2,3-Dimethylphenyl)amino)-6-phenyl-4-propylnicotinonitrile (5p)

Brown gummy solid; 1H NMR (400 MHz, CDCl3): δ 7.93 (d, J = 4.0 Hz, 2H), 7.78 (d, J = 8.0 Hz,
1H), 7.41-7.40 (m, 3H), 7.16-7.11 (m, 2H), 7.04-7.02 (m, 1H), 6.90 (s, 1H), 2.82 (t, J = 7.6 Hz,
2H), 2.35 (s, 3H), 2.26 (s, 3H), 1.82-1.76 (m, 2H), 1.07 (t, J = 7.2 Hz, 3H); 13C NMR (100 MHz,
CDCl3): δ 158.6, 157.0, 156.1, 139.0, 138.1, 130.6, 129.0 (2C), 128.9, 127.6 (2C), 126.6, 125.5,
123.3, 120.0, 115.3, 112.1, 89.5, 42.4, 29.6, 20.9, 13.8, 13.6; IR (KBr, cm⁻¹): 3316, 2916, 2864,
6-(Furan-2-yl)-4-phenyl-2-(o-tolylamino) nicotinonitrile (5q)

![Chemical structure](image)

Pale yellow solid, mp: 166-168 °C; ^1H NMR (400 MHz, CDCl₃): δ 8.07 (d, J = 8.0 Hz, 1H), 7.61 - 7.59 (m, 2H), 7.47 - 7.45 (m, 4H), 7.22 -7.18 (m, 2H), 7.02 – 6.95 (m, 3H), 6.66 (t, J = 7.6 Hz, 1H), 6.47 (dd, J₁₂ = 2.0 Hz, J₁₃ = 3.6 Hz, 1H), 2.31(s, 3H); ^13C NMR (100 MHz, CDCl₃):δ 156.8, 155.2, 152.9, 150.1, 144.5, 137.1, 136.8, 130.5, 129.8, 129.3, 128.9(2C), 128.1(2C), 126.4, 124.2, 122.2, 117.1, 112.4, 112.1,109.1, 89.4, 18.0; IR (KBr, cm⁻¹) 3431, 2201, 1602, 1541,1478, 1012; ESI-MS (M+1); 352.1.

2-(3-Oxo-1,3-diphenylpropyl)malononitrile (6)

![Chemical structure](image)

White solid, mp 122- 124 °C; ^1H NMR (400 MHz, CDCl₃): δ 7.98 (d, J = 7.6 Hz, 2H), 7.64-7.51 (m, 1H), 7.49-7.39 (m, 7H), 4.65 (d, J = 4.8 Hz, 1H), 3.98-3.93 (m, 1H), 3.70-3.61 (m, 2H); IR (KBr, cm⁻¹) 2901, 2255, 1682, 1449, 1186; MS (ESI) (M+NH₃); 292.10.

3-(4-Chlorophenyl)-5-phenyl-2,6-dicyanoanilines (7)

![Chemical structure](image)

Off white solid; mp: 246-248°C; ^1H NMR (400 MHz, CDCl₃): δ 7.58 (d, J = 8.0 Hz, 2H), 7.53 – 7.47 (m, 7H), 6.86 (s, 1H), 5.39 (s, 2H); IR (KBr): 3466, 3365, 2214, 1499 cm⁻¹

2-(4-Methoxybenzylidene)malononitrile (8)
Pale yellow solid, mp 114-116 °C; \(^1\)H NMR (400 MHz, CDCl\(_3\)): 7.92 (d, \(J = 8.0\) Hz, 2H), 7.65 (s, 1H), 7.03 (d, \(J = 8.0\) Hz, 2H), 3.91 (s, 3H); IR (KBr, cm\(^{-1}\)) 2984, 2222, 1605, 1456.

**Procedure for the scale up of compound 5i**

To a mixture of acetophenone (1a, 4.0 g, 0.033 mmol), 4-nitrobenzaldehyde (2e, 5.03 g, 0.033 mmol), aniline (3b, 3.1 g, 0.033 mmol), and malononitrile 4, 2.2 g, 0.033 mmol) in PEG-400 (8 mL) was added FeF\(_3\) (0.375 g, 10 mol%) at room temperature. The mixture was then stirred at 60 °C under ultrasound irradiation in open air for 3h (the reaction was monitored by TLC). After completion of the reaction the mixture was diluted with EtOAc (60 mL) and filtered to separate the catalyst. The filtrate was collected, washed with cold water (2 x 30 mL), dried over anhydrous Na\(_2\)SO\(_4\), filtered and concentrated under low vacuum. The residue was purified by column chromatography over silica gel (100-200 mesh) using EtOAc-hexane to give the desired product 5i (11.75 g, 90%).

**Recovery of catalyst:** The filtered catalyst was collected, dried under vacuum below 50 °C and reused for the next cycle.

**Pharmacology**

*Materials and Methods*

**Cells and Reagents:** HEK 293 and Sf9 cells were obtained from ATCC (Washington D.C., USA). HEK 293 cells were cultured in DMEM supplemented with 10% fetal bovine serum (Invitrogen Inc., San Diego, CA, USA). Sf9 cells were routinely maintained in Grace’s supplemented medium (Invitrogen) with 10% FBS. RAW 264.7 cells (murine macrophage cell line) were obtained from ATCC and routinely cultured in RPMI 1640 medium with 10% fetal bovine serum (Invitrogen Inc.). cAMP was purchased from SISCO Research Laboratories (Mumbai, India). PDElight HTS cAMP phosphodiesterase assay kit was procured from Lonza (Basel, Switzerland).

**PDE4B protein production and purification**

PDE4B cDNA was sub-cloned into pFAST Bac HTB vector (Invitrogen) and transformed into
DH10Bac (Invitrogen) competent cells. Recombinant bacmids were tested for integration by PCR analysis. Sf9 cells were transfected with bacmid using Lipofectamine 2000 (Invitrogen) according to manufacturer’s instructions. Subsequently, P3 viral titer was amplified, cells were infected and 48 h post infection cells were lysed in lysis buffer (50 mM Tris-HCl pH 8.5, 10 mM 2-Mercaptoethanol, 1 % protease inhibitor cocktail (Roche), 1 % NP40). Recombinant His-tagged PDE4B protein was purified as previously described elsewhere (Wang et al., 1997). Briefly, lysate was centrifuged at 10,000 rpm for 10 min at 4°C and supernatant was collected. Supernatant was mixed with Ni-NTA resin (GE Life Sciences) in a ratio of 4:1 (v/v) and equilibrated with binding buffer (20 mM Tris-HCl pH 8.0, 500 mM-KCl, 5 mM imidazole, 10 mM 2-mercaptoethanol and 10 % glycerol) in a ratio of 2:1 (v/v) and mixed gently on rotary shaker for 1 hour at 4°C. After incubation, lysate-Ni-NTA mixture was centrifuged at 4,500 rpm for 5 min at 4°C and the supernatant was collected as the flow-through fraction. Resin was washed twice with wash buffer (20 mM Tris-HCl pH 8.5, 1 M KCl, 10 mM 2-Mercaptoethanol and 10% glycerol). Protein was eluted sequentially twice using elution buffers (Buffer I: 20 mM Tris-HCl pH 8.5, 100 mM KCl, 250 mM imidazole, 10 mM 2-mercaptoethanol, 10% glycerol, Buffer II: 20 mM Tris-HCl pH 8.5, 100 mM KCl, 500 mM imidazole, 10 mM 2-mercaptoethanol, 10% glycerol). Eluates were collected in four fractions and analyzed by SDS-PAGE. Eluates containing PDE4B protein were pooled and stored at -80°C in 50% glycerol until further use.

**PDE4B enzymatic assay**

The inhibition of PDE4B enzyme was measured using PDElight HTS cAMP phosphodiesterase assay kit (Lonza) according to manufacturer’s recommendations. Briefly, 10 ng of PDE4B enzyme was pre-incubated either with DMSO (vehicle control) or compound for 15 min before incubation with the substrate cAMP (5 µM) for 1 h. The reaction was halted with stop solution followed by incubation with detection reagent for 10 minutes in dark. Luminescence values (RLUs) were measured by a Multilabel plate reader (Perklin Elmer 1420 Multilabel counter). The percentage of inhibition was calculated using the following formula:

\[
\% \text{ inhibition} = \frac{(\text{RLU of vehicle control} - \text{RLU of inhibitor})}{\text{RLU of vehicle control}} \times 100
\]

**Docking studies**

To understand the binding affinity and molecular interactions of compounds in the binding pocket of PDE4B the molecular docking simulations were carried out using GRIP method of docking in
Biopredicta module of Vlife MDS (Molecular Design Suite) 4.6.

**Docking Method:** The PDE4B protein in complex with rolipram obtained from Protein Data Bank (PDB ID: 1XMY) was used as the receptor for docking. The protein structure was visualized and pre-processed with Dock Prep tool of UCSF. Ligand geometries were optimized by energy minimization using Merck Molecular Force Field MMFF94 and Gasteiger-Marsili charges for the atoms till a gradient of 0.001 kcal/mol/Å° was reached, maintaining the template structure rigid during the minimization. The active site pocket of the co-crystallized ligand was selected for docking. The GRIP batch docking and subsequent scoring were performed using the default parameters of the Biopredicta program. The following parameters were followed in the standard docking protocol, number of placements: 30, rotation angle: 30°, exhaustive docking method, scoring function: PLP score.

**Results:** The GRIP docking employs PLP (Piecewise Linear Pair wise Potential) scoring function for protein ligand interactions which includes hydrogen bonding, steric interactions, van der Waals interactions, hydrophobic interactions and electrostatic interactions. Post docking analysis involved evaluation of interaction energies between each ligand and PDE4B protein for best ligand pose inside the receptor as PLP score. The PLP scores of compounds were compared with the reference Rolipram (Table S-2). For consensus docking results and to validate the accuracy, molecular simulations were also done with SWISSDOCK web server and ΔG values were generated (Table S-2).

| **Table S-2.** The PLP scores of compounds and the reference compound rolipram and ΔG values of compounds. |
|---------------------------------|-----------------|
| **Vlife MDS**                 |                 |
| **Compound**                  | **PLP score**   |
| Rolipram                      | -59.35 kcal/mol |
| 5d                             | -89.26 kcal/mol |
| 5j                             | -91.51 kcal/mol |
| **SWISSDOCK**                 |                 |
| **Compound**                  | **ΔG**          |
|                                |                 |


Molecular Interactions: The H-bonds and hydrophobic interactions were analyzed post docking simulations and the results showed good binding modes in the active site of PDE4B. The molecular interactions summary of top-ranked docking poses of compounds 5d and 5j with PDE4B are listed in Table S-3.

Table S-3. H-bonds and hydrophobic interactions of compounds with PDE4B

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Hydrogen bonds</th>
<th>Hydrophobic bonds</th>
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<tbody>
<tr>
<td>5d</td>
<td>CYS432</td>
<td>SER429, MET431</td>
</tr>
<tr>
<td>5j</td>
<td>PRO430</td>
<td>SER429, GLN284</td>
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</tbody>
</table>

The compound 5d and 5j showed good binding affinity as compared to standard drugs which revealed that the nature of the substituent and substitution pattern on the basic ring may have a considerable impact on the PDE4B activity of the synthesized compounds. Docking studies demonstrated that both the compounds were binding in the solvent filled side pocket residues in the active site of PDE4B. The binding interactions of compounds with PDE4B are shown in Figure S-1 and S-2. Dotted white bond showing H-bond interactions and yellow bonds shows hydrophobic interactions with binding site residues. Compounds and protein are represented by sticks and colored according to the atom type.
Figure S-1. Binding interaction of compound 5d with PDE4B receptor

Figure S-2. Binding interaction of compound 5j with PDE4B receptor
Copies of spectra

4,6-Diphenyl-2-(o-tolylamino)nicotinonitrile
Elemental Composition Report

Single Mass Analysis
Tolerance = 5.0 PPM / DBE: min = -1.5, max = 100.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 2

Monoisotopic Mass, Even Electron ions
96 formula(s) evaluated with 1 results within limits (up to 10 closest results for each mass)
Elements Used:
C: 0-30  H: 0-30  N: 0-5  O: 0-2  Br: 0-1

C283/MALO1/056
151016005 18 (0.339) Cm (17:18)

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4,6-Diphenyl-2-(phenylamino)nicotinonitrile
Elemental Composition Report

Single Mass Analysis
Tolerance = 5.0 PPM  DBE: min = -1.5, max = 100.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 2

Monoisotopic Mass, Even Electron Ions
48 formula(s) evaluated with 1 results within limits (up to 10 closest results for each mass)
Elements Used:
C: 0-30  H: 0-30  N: 0-5  O: 0-2
C26S/MALCOV058
151016006.19 (0.352) Cm (18.16)

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<td>C24 H18 N3</td>
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Minimum:  5.0  5.0  100.0
Maximum:  5.0  5.0  -1.5

1: TOF MS ES+ 8.539+004
2-((2,3-Dimethylphenyl)amino)-4,6-diphenylnicotinonitrile
**Elemental Composition Report**

**Single Mass Analysis**
Tolerance = 5.0 PPM / DBE: min = -1.5, max = 100.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 2

Monoisotopic Mass, Even Electron Ions
47 formula(e) evaluated with 1 results within limits (up to 10 closest results for each mass)

Elements Used:
C: 0-30  H: 0-30  N: 0-5  O: 0-2
C26H22N3

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<td>1.9</td>
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2-((4-Fluorophenyl)amino)-4-(4-methoxyphenyl)-6-phenylnicotinonitrile
Elemental Composition Report

Single Mass Analysis
Tolerance = 5.0 PPM / DBE: min = -1.5, max = 100.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 2

Monoisotopic Mass, Even Electron Ions
56 formula(e) evaluated with 1 results within limits (up to 10 closest results for each mass)
Elements Used:
C: 0-27 H: 0-30 N: 0-5 O: 0-2 F: 0-1
C29H34F6N10O8 22 (0.412) Cm (21:22)

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<td>0.3</td>
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<td>1.6</td>
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6-Phenyl-2-(phenylamino)-4-(thiophen-2-yl)nicotinonitrile
Elemental Composition Report

Single Mass Analysis
Tolerance = 5.0 PPM / DBE: min = -1.5, max = 100.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 2

Monoisotopic Mass, Even Electron Ions
20 formula(e) evaluated with 1 results within limits (all results (up to 1000) for each mass)
Elements Used:
C: 0.25  H: 0.17  N: 0.5  S: 0.2
C383H76O12S3
188122065 54 (1.003) Cm (51.54-79.87x0.500)

Minimum:
Maximum:
Mass  Calc. Mass  mDa  PPM  DBB  i-FIT  Formula
354.1068  354.1065  0.3  0.0  16.5  6.4  C22 H16 N3 S
4-Isobutyl-6-phenyl-2-(o-tolylamino)nicotinonitrile
Elemental Composition Report

Single Mass Analysis
Tolerance = 5.0 PPM  /  DBE: min = -1.5, max = 100.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 2

Monoisotopic Mass, Even Electron Ions
46 formula(s) evaluated with 1 results within limits (up to 10 closest results for each mass)
Elements Used:
C: 0-30  H: 0-30  N: 0-5  O: 0-2
C293H2A01N1O54

Mass  Calc. Mass  mDa  PPM  DBE  i-FIT  Formula
342.1984  342.1970  1.4  4.1  13.5  25.2  C23 H24 N3
6-(4-Methoxyphenyl)-4-phenyl-2-(o-tolylamino)nicotinonitrile
Elemental Composition Report

Single Mass Analysis
Tolerance = 5.0 PPM / DBE: min = -1.5, max = 100.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 2

Monoisotopic Mass, Even Electron Ions
53 formula(e) evaluated with 1 results within limits (up to 10 closest results for each mass)
Elements Used:
C: 0-27  H: 0-30  N: 0-5  O: 0-2  F: 0-1
C283/MAL01065
151016009 73 (1.356) Cm (73:74)

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<td>C26 H22 N3 O</td>
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</table>
4-Isobutyl-6-phenyl-2-(phenylamino)nicotinonitrile
**Elemental Composition Report**

**Single Mass Analysis**
- Tolerance = 10.0 PPM / DBE: min = -1.5, max = 100.0
- Element prediction: Off
- Number of isotope peaks used for i-FIT = 2

Monoisotopic Mass, Even Electron Ions
- 48 formula(s) evaluated with 1 results within limits (up to 10 closest results for each mass)

Elements Used:
- C: 0-27
- H: 0-30
- N: 0-5
- O: 0-2

**Spectral Data**

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<td>C22 H22 N3</td>
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Minimum: 5.0  Maximum: 100.0

1: TOF MS, ES+ 1.77e+005
4-(4-Nitrophenyl)-6-phenyl-2-(phenylamino)nicotinonitrile
4-(4-Nitrophenyl)-6-phenyl-2-(phenylamino)nicotinonitrile

D$_2$O exchange
Elemental Composition Report

Single Mass Analysis
Tolerance = 10.0 PPM / DRF: min = -1.5, max = 100.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 2

Monoisotopic Mass, Even Electron Ions
25 formula(c)s evaluated with 1 results within limits (up to 10 closest results for each mass)
Elements Used:
C: 0.27  H: 0.30  N: 0.5  O: 0.2
C22H31NO7 C1510160122 0.412 Cm [22:24:11:13x0.500]

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2-((4-Cyanophenyl)amino)-4-(4-methoxyphenyl)-6-phenylnicotinonitrile
Elemental Composition Report

Single Mass Analysis
Tolerance = 5.0 PPM  /  DBE: min = -1.5, max = 100.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 2

Monoisotopic Mass, Even Electron Ions
24 formula(e) evaluated with 1 results within limits (up to 10 closest results for each mass)
Elements Used:
C: 0-30  H: 0-20  N: 0-5  O: 0-2
C26H21N2O
1511119033 19 (0.352) Cm (17:20)

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2-((4-Bromophenyl)amino)-6-phenyl-4-(thiophen-2-yl)nicotinonitrile
Elemental Composition Report

Single Mass Analysis
Tolerance = 10.0 PPM / DBE: min = -1.5, max = 100.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 2

Monoisotopic Mass, Even Electron Ions
127 formula(s) evaluated with 1 results within limits (up to 10 closest results for each mass)
Elements Used:
C: 0.27 H: 0.30 N: 0.50 O: 0.20 S: 0.10 Br: 0.10

C283/MALO/073 B
151016013:21 (0.339) C0m (21:22:5.11x0.500)

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<td>C22 H15 N3 S Br</td>
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6-(4-Chlorophenyl)-2-((2,3-dimethylphenyl)amino)-4-phenylnicotinonitrile
Elemental Composition Report

Single Mass Analysis
Tolerance = 5.0 PPM / DBE: min = -1.5, max = 100.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 2

Monoisotopic Mass, Even Electron Ion
54 formula(e) evaluated with 1 results within limits (up to 10 closest results for each mass)

Elements Used:
C: 0-27  H: 0-30  N: 0-5  Cl: 0-1
C283/MALDI073 G
151016014 24 (0.440) Cm (23.26)

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4-(4-Bromophenyl)-6-phenyl-2-(o-tolylamino)nicotinonitrile
Elemental Composition Report

Single Mass Analysis
Tolerance = 5.0 PPM / DBE: min = -1.5, max = 100.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 2

Monoisotopic Mass, Even Electron Ions
68 formula(e) evaluated with 1 results within limits (up to 10 closest results for each mass)
Elements Used:
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C263/MALO1/075 A
15119067 21 (0.400) Cm (21.23)

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Minimum: 5.0  Maximum: 5.0  Tolerance: 1.5

4-(Furan-2-yl)-6-phenyl-2-(o-tolylamino)nicotinonitrile
Elemental Composition Report

Single Mass Analysis
Tolerance = 20.0 PPM / DBE: min = -1.5, max = 100.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 2

Monoisotopic Mass, Even Electron Ions
42 formula(e) evaluated with 1 results within limits (all results up to 1000) for each mass

Elements Used:
C: 0-30  H: 0-23  N: 0-6  O: 0-2

![Chemical Structure]

C138/MALD01003
160122004 25 (0.516) Cm (27:28-49:52=0.500)

<table>
<thead>
<tr>
<th>Mass</th>
<th>Calc. Mass</th>
<th>mDa</th>
<th>PPM</th>
<th>DBE</th>
<th>i-FIT</th>
<th>Formula</th>
</tr>
</thead>
<tbody>
<tr>
<td>352.1458</td>
<td>352.1450</td>
<td>0.8</td>
<td>2.3</td>
<td>16.5</td>
<td>21.8</td>
<td>C23 H18 N3 O</td>
</tr>
</tbody>
</table>
6-(4-Cyanophenyl)-2-((2,3-dimethylphenyl)amino)-4-phenylnicotinonitrile
**Elemental Composition Report**

**Single Mass Analysis**
Tolerance = 5.0 PPM / DBE: min = -1.5, max = 100.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 2

Monoisotopic Mass, Even Electron Ions
44 formula(e) evaluated with 1 results within limits (all results (up to 1000) for each mass)

Elements Used:
C: 0-30  H: 0-25  N: 0-6  S: 0-2

C138H110N5O8S
180122084 43 (0.79%) Cm (43.0-69.7;3x0.500)

<table>
<thead>
<tr>
<th>Mass</th>
<th>calc. mass</th>
<th>mDa</th>
<th>PPM</th>
<th>DBE</th>
<th>i-FIT</th>
<th>Formula</th>
</tr>
</thead>
<tbody>
<tr>
<td>401.1749</td>
<td>401.1766</td>
<td>-1.7</td>
<td>-4.2</td>
<td>19.5</td>
<td>0.3</td>
<td>C27 H21 N4</td>
</tr>
</tbody>
</table>
2-((2,3-Dimethylphenyl)amino)-6-phenyl-4-propynicotinonitrile
Elemental Composition Report

Single Mass Analysis
Tolerance = 5.0 PPM / DBE: min = -1.5, max = 100.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 2

Monoisotopic Mass, Even Electron ions
10 formula(s) evaluated with 1 results within limits (all results up to 1000) for each mass
Elements Used:
C: 0.26  H: 0.25  N: 0.5

C138H40C10N10(A)
1603240588 (1.617) Cm (87.93 101:104x0.500)

<table>
<thead>
<tr>
<th>Mass</th>
<th>Calc. Mass</th>
<th>mDa</th>
<th>FPM</th>
<th>DBE</th>
<th>i-FIT</th>
<th>Formula</th>
</tr>
</thead>
<tbody>
<tr>
<td>342.1972</td>
<td>342.1970</td>
<td>0.2</td>
<td>0.6</td>
<td>13.5</td>
<td>17.1</td>
<td>C23 H24 N8</td>
</tr>
</tbody>
</table>

Minimum: 5.0  Maximum: 100.0
<table>
<thead>
<tr>
<th>User Spectra</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fragmentor Voltage</td>
</tr>
<tr>
<td>0</td>
</tr>
<tr>
<td>+ Scan (0.27 min) 160630003.d Subtract (1)</td>
</tr>
<tr>
<td>200.10</td>
</tr>
<tr>
<td>Counts (%) vs. Mass-to-Charge (m/z)</td>
</tr>
<tr>
<td>0</td>
</tr>
<tr>
<td>0</td>
</tr>
</tbody>
</table>

Sample Name: C138/HA01/033 A
Sample Type: Sample
Instrument Name: Instrument 1
Acq Method: ESI.m
DA Method: CACHB.m
Position: Vial 65
User Name: Success
$^1$HNMR spectra of compound 6 (Table 1) in CDCl$_3$
Mass Analysis Report

Data Filename: 160517003.d
Sample Name: C2E3/MALO1/056 Int
Sample Type: Sample
Instrument Name: Instrument 1
Acc Method: ESIm
DA Method: CACH8.m

User Spectra

Fragmentor Voltage: 10
Collision Energy: 0
Ionization Mode: ESI

+ Scan (0.27 min) 160517003.d Subtract (1)
292.10 M+NH4+
212.10
$^1$HNMR spectra of compound 7 in CDCl$_3$
$^1$HNMR spectra of compound 8 in CDCl$_3$
IR spectra of recovered and fresh FeF₃ catalyst