Published in final edited form as:

Heterocycl Comm. 2014 May ; 20(3): 149–153. doi:10.1515/hc-2014-0041.

Synthesis of pyridine derivatives as potential antagonists of chemokine receptor type 4

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Abstract

A series of pyridine derivatives were synthesized as potential inhibitors of chemokine receptor type 4. This chemokine receptor has been linked to various disease pathways including HIV-1 proliferation, autoimmune disorders, inflammatory diseases, and cancer metastasis. The compounds were tested for activity using an affinity binding assay and an assay that tests the ability to inhibit cell invasion. Two hit compounds (**2b** and **2j**) have been identified for further evaluation that inhibit cell invasion by at least 50% and have an effective concentration of less than 100 nM in the binding affinity assay. The structures of the synthesized compounds were confirmed by spectral data.

Keywords

anti-cancer; anti-inflammatory; CXC chemokine receptor type 4 (CXCR4); pyridine derivatives

Pyridine derivatives have been explored for their potential as pharmaceutical agents [1, 2]. In this paper, we report the synthesis of a series of pyridine compounds as potential CXC chemokine receptor type 4 (CXCR4) antagonists. Chemokines and their receptors are involved in the pathogenesis of cancer, HIV, autoimmune and inflammatory diseases [3, 4]. One such chemokine receptor is CXCR4. When CXCR4 interacts with its natural ligand (CXCL12), it triggers a number of signaling pathways that result in physiological responses, such as chemotaxis, cell survival and proliferation, and gene transcription [5–11]. These pathways are involved in the progression of autoimmune diseases and cancer metastasis. As

a result, CXCR4 has been a therapeutic target for these conditions. The development of small molecule CXCR4 inhibitors may be an effective treatment for many CXCR4-related conditions [12, 13].

Several classes of CXCR4 small-molecule antagonists have shown anti-RA activity [14, 15]. Others have shown anti-cancer activity by inhibiting cancer growth and metastasis [16–19]. AMD3100 (Figure 1) has been one of the most successful CXCR4 antagonists [15, 20, 21]. AMD3100 is approved by the FDA as a stem cell mobilizer in patients with leukemia. However, because of toxicity issues, CXCR4 is only for limited use. Currently, there are no other small-molecule antagonists of CXCR4 approved by the FDA. Therefore, drugs with improved toxicity profiles will be required for long-term clinical use.

Other derivatives of AMD3100, such as WZ811 (Figure 1), have been synthesized and are active at nanomolar concentration as a CXCR4 antagonist [22, 23]. However, in preclinical testing, WZ811 failed to exhibit any *in vivo* efficacy due to poor bioavailability. Thus far, the central aromatic ring of WZ811 and other derivatives has remained free from modification and other aromatic cores have not been explored. To this end, we have synthesized a new class of compounds containing a central pyridine ring (Figure 1) with the potential to improve potency and bioavailability. To determine their effectiveness as potential CXCR4 inhibitors, these compounds have been screened using two preliminary assays.

Synthesis of pyridine derivatives **2a–l** was accomplished by reductive amination of pyridine-2,6-dicarbaldehyde (**1**) in the presence of an amine in methanol with zinc chloride as a catalyst and sodium cyanoborohydride as the reducing agent (Scheme 1). The ^1H NMR, ^{13}C NMR, and HRMS analyses are reported for all final compounds.

The synthesized analogs were subjected to two preliminary screening assays. The compounds were initially screened using a binding affinity assay [16, 24]. This assay involves competition of a potent CXCR4 peptidic antagonist, TN140, with the synthesized compounds. In this assay, MDA-MB-231 (breast cancer cells) are preincubated with analogs at concentrations of 1, 10, 100, and 1000 nM, and then incubated with biotinylated TN140 that has been conjugated to the red fluorescent dye rhodamine. The binding efficiency of the new analogs to the CXCL12 binding domain of CXCR4 can be determined. The effective concentration (EC) is defined as the lowest concentration at which a significant reduction in the rhodamine fluorescent color (red) is observed as compared to the control reflecting the competitive displacement by the peptide, TN140 (Figure 2). This assay is a semi-quantitative, preliminary measure of the level of activity and should not be confused with EC_{50} .

The compounds were also screened using the Matrigel invasion assay [25]. This assay was employed as a secondary functional assay to test whether the compounds can block CXCR4/CXCL12-mediated chemotaxis and invasion at 100 nM. The compound and cells are added on the upper chamber of a vessel and ligand (CXCL12) is added in the lower chamber as a chemoattractant. A Matrigel membrane separates the upper and lower chambers. For potent

compounds, very few cells will move through the Matrigel membrane; that is, invasion of cells is inhibited.

Eight of the 12 synthesized compounds exhibit moderate to good activity in the binding affinity assay (< 100 nM) (Table 1). Derivatives **2b** (3-fluorophenyl) and **2j** (4-ethylphenyl) show consistently good potency in both assays. Compound **2b** has an EC of 10 nM and inhibits invasion by 50% and compound **2j** has an EC value of 1 nM and inhibits invasion by 64%. For comparison, WZ811 (Figure 1) displays the EC value of 10 nM in the binding affinity assay and inhibits invasion by 90%. Interestingly, the 2-pyridyl derivative **2l** is structurally similar to WZ811 (also a 2-pyridyl derivative) but has low activity in both assays.

These 2,6-disubstituted pyridine derivatives represent a new generation of CXCR4 antagonists that warrants further exploration. Our goal is to continue the synthesis of additional analogs to improve the potency and biopharmaceutical properties of this class of compounds. Additional analogs with pyrazine and thiophene moieties are being synthesized. A comprehensive structure-activity relationship (SAR) study of these compounds will be conducted.

Experimental details

Chemistry

Melting points were recorded using a Stuart SMP40 apparatus and are uncorrected. The ^1H NMR (400 MHz) and ^{13}C NMR (100 MHz) spectra were recorded on a Bruker Ac 400 FT NMR spectrometer in CDCl_3 . Mass spectra were recorded on a JEOL spectrometer. The syntheses were carried out under nitrogen in dry glassware.

General procedure for the synthesis of pyridine analogs 2a–l

To a solution of pyridine-2,6-dicarbaldehyde (50 mg, 0.37 mmol) in methanol (4 mL) was added an aniline (0.81 mmol) and ZnCl_2 (100 mg, 0.74 mmol). The solution was stirred for 2 h at room temperature, then treated with NaBH_3CN (46.5 mg, 0.81 mmol). The solution was then stirred overnight. The crude product was purified by column chromatography.

2,6-Bis(anilinomethyl)pyridine (2a)—This product was obtained in 37% yield as an off-white semi-solid; ^1H NMR: δ 4.38 (s, 4H), 6.59 (d, $J = 7.8$ Hz, 4H), 6.65 (t, $J = 7.2$ Hz, 2H), 7.06–7.15 (m, 6 H), 7.49 (t, $J = 7.8$ Hz, 1H); ^{13}C NMR: δ 158.1, 148.0, 137.3, 129.3, 119.9, 117.7, 113.1, 49.3. HRMS. Calcd for $\text{C}_{19}\text{H}_{20}\text{N}_3$ ($[\text{M} + \text{H}]^+$): m/z 290.1657. Found: m/z 290.1657.

2,6-Bis(3-fluoroanilinomethyl)pyridine (2b)—This product was obtained in 62% yield as a brown semi-solid; ^1H NMR: δ 4.34 (s, 4H), 6.21–6.40 (m, 6H), 6.95–7.06 (m, 2H), 7.10 (d, $J = 7.8$ Hz, 2H), 7.51 (t, $J = 7.8$ Hz, 1H); ^{13}C NMR: δ 165.4, 162.9, 157.5, 149.8, 149.7, 137.4, 130.3, 120.1, 109.0, 104.2, 104.0, 99.9, 99.6, 48.9. HRMS. Calcd for $\text{C}_{19}\text{H}_{18}\text{N}_3\text{F}_2$ ($[\text{M} + \text{H}]^+$): m/z 326.1469. Found: m/z 326.1462.

2,6-Bis(4-fluoroanilinomethyl)pyridine (2c)—This product was obtained in 34% yield as a brown semi-solid; ^1H NMR: δ 4.33 (s, 4H), 6.51 (dd, J = 8.7 and 4.2 Hz, 4H), 6.81 (t, J = 8.7 Hz, 4H), 7.12 (d, J = 7.6 Hz, 2 H), 7.52 (t, J = 7.6 Hz, 1H); ^{13}C NMR: δ 158.0, 157.2, 154.8, 137.4, 137.2, 120.0, 115.9, 115.7, 115.6, 115.5, 114.3, 113.5, 113.4, 49.9. HRMS. Calcd for $\text{C}_{19}\text{H}_{18}\text{N}_3\text{F}_2$ ($[\text{M} + \text{H}]^+$): m/z 326.1469. Found: m/z 326.1462.

2,6-Bis(4-methoxyanilinomethyl)pyridine (2d)—This product was obtained in 30% yield as a brown solid; mp 63–65°C; ^1H NMR: δ 3.66 (s, 6 H), 4.33 (s, 4 H), 6.55 (d, J = 8.8 Hz, 4 H), 6.70 (d, J = 8.8 Hz, 4 H), 7.12 (d, J = 7.6 Hz, 2 H), 7.49 (t, J = 7.6 Hz, 1 H); ^{13}C NMR: δ 158.4, 152.2, 142.2, 137.2, 119.9, 114.9, 114.4, 55.8, 50.2. HRMS. Calcd for $\text{C}_{21}\text{H}_{24}\text{N}_3\text{O}_2$ ($[\text{M} + \text{H}]^+$): m/z 350.1869. Found: m/z 350.1865.

2,6-Bis(3-chloroanilinomethyl)pyridine (2e)—This product was obtained in 14% yield as a yellow semi-solid; ^1H NMR: δ 4.36 (s, 4 H), 6.46 (dd, J = 8.0 and 1.0 Hz, 2 H), 6.58 (s, 2 H), 6.61 (d, J = 7.7 Hz, 2 H), 7.01 (t, J = 8.0 Hz, 2 H), 7.12 (d, J = 7.7 Hz, 2 H), 7.54 (t, J = 7.7 Hz, 1 H); ^{13}C NMR: δ 157.4, 149.0, 137.5, 135.1, 130.3, 120.1, 117.5, 112.7, 111.4, 48.9. HRMS. Calcd for $\text{C}_{19}\text{H}_{18}\text{N}_3\text{Cl}_2$ ($[\text{M} + \text{H}]^+$): m/z 358.0872. Found: m/z 358.0864.

2,6-Bis(4-chloroanilinomethyl)pyridine (2f)—This product was obtained in 15% yield as a yellow solid; mp 116–118°C; ^1H NMR: δ 4.36 (br s, 4H), 6.51 (d, J = 8.3 Hz, 4H), 7.06 (d, J = 8.3 Hz, 4H), 7.12 (d, J = 7.6 Hz, 2H), 7.54 (t, J = 7.6 Hz, 1H); ^{13}C NMR: δ 157.6, 146.4, 137.4, 129.1, 122.3, 120.0, 114.2, 49.2. HRMS. Calcd for $\text{C}_{19}\text{H}_{18}\text{N}_3\text{Cl}_2$ ($[\text{M} + \text{H}]^+$): m/z 358.0872. Found: m/z 358.0864.

2,6-Bis[(3-trifluoromethyl)anilinomethyl]pyridine (2g)—This product was obtained in 18% yield as a light brown semi-solid; ^1H NMR: δ 4.40 (br s, 4H), 6.71 (d, J = 7.7 Hz, 2H), 6.80 (br s, 2H), 6.88 (d, J = 7.7 Hz, 2H), 7.13 (d, J = 7.6 Hz, 2H), 7.17 (d, J = 7.6 Hz, 2H), 7.55 (t, J = 7.7 Hz, 1H); ^{13}C NMR: δ 157.3, 148.0, 137.5, 129.7, 125.7, 123.0, 120.2, 116.0, 114.1, 109.3, 48.8. HRMS. Calcd for $\text{C}_{21}\text{H}_{17}\text{F}_6\text{N}_3$ ($[\text{M} + \text{H}]^+$): m/z 426.1405. Found: m/z 426.1403.

2,6-Bis(3-nitroanilinomethyl)pyridine (2h)—This product was obtained in 34% yield as a yellow-orange solid; mp 147–149°C; ^1H NMR: δ 4.54 (d, J = 5.0 Hz, 4H), 6.96 (d, J = 8.1 Hz, 2H), 7.24 (br s, 2H), 7.31 (t, J = 8.1 Hz, 2H), 7.49 (br s, 2 H), 7.56 (d, J = 8.1 Hz, 2H), 7.65–7.71 (m, 1H); ^{13}C NMR: δ 156.8, 148.6, 137.7, 129.8, 120.4, 119.2, 112.4, 106.6, 48.7. HRMS. Calcd for $\text{C}_{19}\text{H}_{17}\text{N}_5\text{O}_4$ ($[\text{M} + \text{H}]^+$): m/z 380.1359. Found: m/z 380.1358.

2,6-Bis(4-nitroanilinomethyl)pyridine (2i)—This product was obtained in 10% yield as a yellow semi-solid; ^1H NMR: δ 4.51 (d, J = 5.1 Hz, 4H), 6.56 (d, J = 9.1 Hz, 4H), 7.17 (d, J = 7.8 Hz, 2H), 7.64 (t, J = 7.8 Hz, 1H), 8.05 (d, J = 9.1 Hz, 2H); ^{13}C NMR: δ 156.1, 152.7, 137.9, 126.4, 120.5, 111.6, 48.9. HRMS. Calcd for $\text{C}_{19}\text{H}_{18}\text{N}_5\text{O}_4$ ($[\text{M} + \text{H}]^+$): m/z 380.1359. Found: m/z 380.1359.

2,6-Bis(4-ethylanilinomethyl)pyridine (2j)—This product was obtained in 11% yield as an orange oil; ^1H NMR: δ 1.18 (t, J = 7.7 Hz, 6H), 2.50–2.57 (m, 4H), 4.41 (s, 4H), 6.59

(d, $J = 8.3$ Hz, 4H), 6.97 (d, $J = 8.3$ Hz, 2H), 7.01 (d, $J = 8.3$ Hz, 4H), 7.53 (t, $J = 7.7$ Hz, 1H); ^{13}C NMR: δ 158.4, 146.0, 137.3, 133.6, 128.7, 119.9, 115.3, 113.3, 49.7, 28.0, 16.02. HRMS. m/z $[\text{M} + \text{H}]^+$ Calcd for $\text{C}_{23}\text{H}_{28}\text{N}_3$ ($[\text{M} + \text{H}]^+$): m/z 346.2283. Found: m/z 346.2291.

2,6-Bis(2-methoxyanilinomethyl)pyridine (2k)—This product was obtained in 51% yield as a brown solid; mp 112–114°C; ^1H NMR: δ 3.81 (s, 6 H), 4.43 (s, 4 H), 6.49 (d, $J = 7.7$ Hz, 2H), 6.58–6.64 (m, 2H), 6.70–6.79 (m, 4H), 7.14 (d, $J = 7.7$ Hz, 2H), 7.48 (t, $J = 7.7$ Hz, 1H); ^{13}C NMR: δ 158.7, 147.0, 137.9, 137.3, 121.3, 119.6, 116.7, 110.3, 109.5, 55.5, 49.4. HRMS. Calcd for $\text{C}_{21}\text{H}_{23}\text{N}_3\text{O}_2$ ($[\text{M} + \text{H}]^+$): m/z 350.1869. Found: m/z 350.1862.

2,6-Bis(pyridin-2-aminomethyl)pyridine (2l)—This product obtained in 14% yield as a white solid; mp 152–153°C (lit mp 153–155°C) [26]; ^1H NMR: δ 4.66 (d, $J = 5.1$ Hz, 4H), 6.47 (d, $J = 7.7$ Hz, 2H), 6.61 (d, $J = 5.1$ Hz, 2H), 7.21 (d, $J = 7.7$ Hz, 2H), 7.42 (t, $J = 7.7$ Hz, 2H), 7.60 (t, $J = 7.7$ Hz, 1H), 7.7 (d, $J = 5.1$ Hz, 2H); ^{13}C NMR: δ 159.0, 156.2, 149.4, 148.6, 137.6, 129.8, 120.3, 119.0, 106.7, 48.7. HRMS. Calcd for $\text{C}_{17}\text{H}_{17}\text{N}_5$ ($[\text{M} + \text{H}]^+$): m/z 292.1562, Found: m/z 292.1566.

Biology

Initial screening of anti-CXCR4 small molecules based on a binding affinity assay

Binding affinity and cell invasion assays are basic assay tools that apply to the initial screening. MDA-MB-231 cells cultured in an eight-well slide chamber were preincubated with the test compounds at concentrations of 1, 10, 100, and 1000 nM. The cells were fixed with 4% formaldehyde and incubated with 50 nM biotinylated compounds **2** followed by rhodamine staining.

Matrigel cell invasion assay

Matrigel invasion chambers from BD Biocoat Cellware (San Jose, CA, USA) were used for invasion assays. MDA-MB-231 cells were cultured on a layer of Matrigel in the upper chamber with testing compounds at the concentration of 100 nM, while 200 ng/mL CXCL12 was added to the lower chamber as a chemoattractant. Detailed procedures for the binding affinity and invasion assays have been described previously [19].

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Figure 1.
Rationale for design of a new class of pyridine analogs.

Figure 2.

Results from the binding assay for two selected analogs. Compound **2b** shows EC of 10 nM and compound **2k** shows EC of 1000 nM (PC, positive control; NC, negative control).

2a: R = Ph

2b: R = 3-fluorophenyl

2c: R = 4-fluorophenyl

2d: R = 4-methoxyphenyl

2e: R = 3-chlorophenyl

2f: R = 4-chlorophenyl

2g: R = 3-trifluoromethyl

2h: R = 3-nitrophenyl

2i: R = 4-nitrophenyl

2j: R = 4-ethylphenyl

2k: R = 2-methoxyphenyl

2l: R = 2-pyridyl

Scheme 1.

Reagents and conditions: (a) RNH₂, ZnCl₂, NaBH₃CN, dry MeOH, rt, 12 h.

Table 1

Preliminary results of the binding affinity assay and the Matrigel invasion assay for compounds **2a–l**, compared with the results of a potent CXCR4 antagonist, WZ811 [22].

Compound	Binding affinity assay, EC (nM) ^a	Matrigel invasion assay (% inhibition of invasion) ^b
WZ811	10	90
2a	100	0
2b	10	50
2c	100	4.5
2d	100	4.5
2e	100	34
2f	100	20
2g	1000	15
2h	100	40
2i	> 1000	9
2j	1	64
2k	1000	0
2l	> 1000	5

^aCompounds were tested at 1, 10, 100, and 1000 nM only.

^bCompounds were tested for inhibition of invasion at 100 nM.