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Synthesis of 5-substituted benzyl-2,4-diamino pyrimidine derivatives as c-Fms kinase inhibitors

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Abstract

A serials of novel 5-substituted benzyl-2,4-diamino pyrimidine derivatives have been synthesized and evaluated as inhibitors of c-Fms kinase by the standard MTT method. The results showed that compound 15,5-[3-methoxy-4-(pyridine-3-yl)benzyl]-2,4-diamino pyrimidine, had an IC_{50} of 1.45 μmol/L in inhibiting the proliferation of M-CSF-dependent myeloid leukemia cells in mice (NFS-60), which was similar with GW2580, a selective inhibitor of c-Fms kinase.

Keywords: C-Fms kinase inhibitors; Synthesis; 2,4-Diamino pyrimidine

The macrophage colony-stimulating factor-1 receptor (c-Fms, also known as M-CSFR, CSF-1R, or Fms) is encoded by the c-fms proto-oncogene and is the exclusive receptor for its ligand, colony-stimulating factor-1 (CSF-1 or macrophage colony-stimulating factor, M-CSF), which regulates proliferation, differentiation and survival of cells of the mononuclear phagocyte lineage [1,2]. Recent studies have demonstrated a direct correlation between tumor-associated macrophage numbers and tumor progression [3,4] and between synovial macrophage numbers and disease severity in rheumatoid arthritis [5]. Hence the inhibition of c-Fms appears to be of therapeutic value in treating diseases such as rheumatoid arthritis as well as certain cancers where macrophages are pathogenic. This hypothesis is also well-supported by the biological studies conducted with CSF-1 deficient mice [6,7].

A recent publication described the identification of 5-substituted benzyl-2,4-diamino pyrimidine compound GW2580 as c-Fms kinase specific inhibitors. GW2580 can completely inhibit human c-Fms kinase *in vitro*, whereas it was inactive against 26 other kinases. It is a useful probe for the elucidation of the regulation mechanism of c-Fms kinase in normal and pathological processes [8]. But the structure-activity relationships (SAR) of this chemotype have not been reported. Herein we designed a serial compounds based on the structure of GW2580, the analysis of c-Fms kinase binding site and the rule of bioisosterism. The generic structures of designed compounds are in Scheme 1 as compound **5**. The aromatic ring A of compound **5** was retained for keeping the hydrogen bonds between the ligand and the binding site. The aromatic ring B was substituted by methoxyl group and bromine in different positions to investigate the SAR of ring B. The aromatic ring C has hydrophobic interaction with the receptor, so the methyl group

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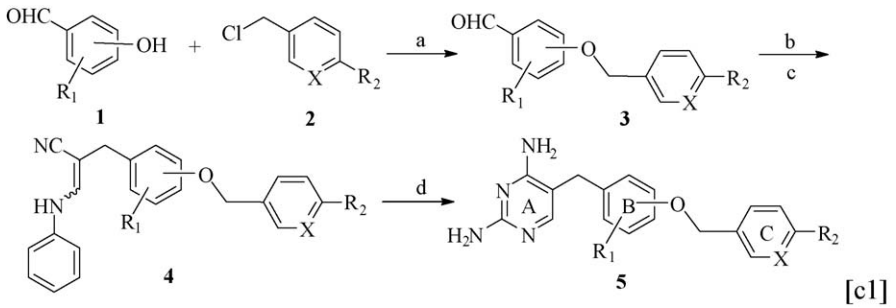


Scheme 1. Reagents and conditions: (a) K_2CO_3, KI, DMF, 90 °C, 82%; (b) 3-morpholinopropionitrile, $NaOCH_3$, DMSO, 75 °C; (c) aniline hydrochloride, isopropanol, reflux, 45–55%; (d) guanidine hydrochloride, $NaOCH_3$, ethanol, reflux, 90%.

was introduced to replace methoxyl group to enhance the hydrophobic effect. Heteroaromatic rings were also introduced to increase the structural diversity of ring C. Thus two categories of compounds, 5-substituted benzyl-2,4-diaminepyrimidine and 5-substituted benzyl-2-mercapto-4-aminopyrimidine were designed and synthesized. Their structures were confirmed by ^{1}H NMR and FAB-MS.

The general synthetic route of all designed compounds was illustrated in Scheme 1. Substituted benzaldehyde **3** was synthesized by alkylation of the corresponding substituted hydroxybenzaldehyde **1** in the presence of K_2CO_3/KI in DMF solution at 90 °C. Condensation of **3** with 3-morpholinopropionitrile in anhydrous DMSO containing a catalytic amount of $NaOCH_3$ led to a red oil, which on sequential refluxing with aniline hydrochloride in isopropanol solution afforded 3-anilinoacrylonitrile **4**. Condensation of **4** with guanidine hydrochloride in the presence of $NaOCH_3$ in ethanol solution afforded 5-substituted benzyl-2,4-diamino pyrimidine **5** [9].

Compounds **6–15** were synthesized according to the above method. The structure and analysis data of the compounds shown in Table 1. Melting point was detected by YRT-3 Melting point apparatus made by Tianjing

Table 1
The structure of compounds **6–15**.

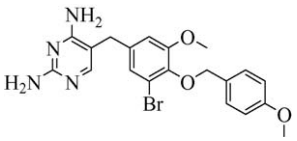
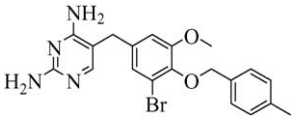
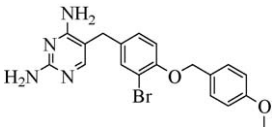
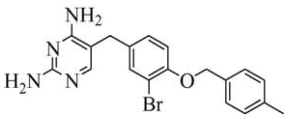
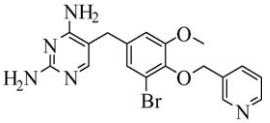
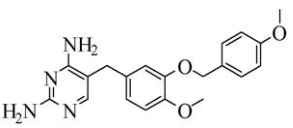
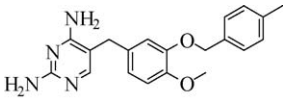
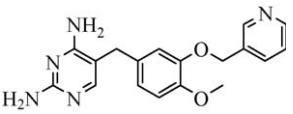
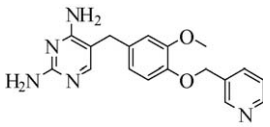
ID	Structure	mp (°C)	Yield (%)	FAB-MS (*m/z*)	^{1}H NMR (400 MHz, DMSO-d_6, δ, J Hz)
6		173.8–175.8	14.5	447.0[a]	3.56 (s, 2H), 3.76 (s, 3H), 3.83 (s, 3H), 4.84 (s, 2H), 5.74 (s, 2H), 6.15 (s, 2H), 6.92–6.94 (d, 2H, J = 8.7 Hz), 6.97 (d, J = 1.7 Hz, 1H), 7.01 (d, 1H, J = 1.7 Hz), 7.34–7.40 (d, 2H, J = 8.7 Hz), 7.57 (s,1H)
7		177.4–179.5	13.0	431.0[a]	2.31 (s, 3H), 3.65 (s, 2H), 3.82 (s, 3H), 4.87 (s, 2H), 5.72 (s, 2H), 6.12 (s, 2H), 6.97 (d, 1H, J = 1.7 Hz), 7.00 (d, 1H, J = 1.7 Hz), 7.17–7.19 (d, J = 8.0 Hz, 1H), 7.35–7.37 (d, 2H, J = 8.0 Hz), 7.57(s, 1H)
8		193 dec.	13.2	417.0[a]	3.53 (s, 2H), 3.75 (s, 3H), 5.07 (s, 2H), 5.74 (s, 2H), 6.12 (s, 2H), 6.94–6.96 (m, 2H), 7.11 (m, 2H), 7.37–7.45 (m, 3H), 7.53 (s, 1H)
9		177.9–179.6	14.5	399.0	2.30 (s, 3H), 3.53 (s, 2H), 5.11 (s, 2H), 5.73 (s, 2H), 6.11 (s, 2H), 7.09 (d, 2H, J = 8.4 Hz), 7.14–7.16 (dd 1H, J_1 = 8.4 Hz, J_2 = 2.0 Hz), 7.33–7.35 (d, 2H, J = 8.4 Hz), 7.42–7.43 (d, 2H, J = 2.0 Hz), 7.53 (s, 1H)
10		187.6–189.3	13.7	418.0[a]	3.56 (s, 2H), 3.82 (s, 3H), 4.98 (s, 2H), 5.76 (s, 2H), 6.16 (s, 2H), 6.98 (d, 1H J = 1.7 Hz), 7.03 (d, 1H, J = 1.7Hz), 7.41–7.44 (dd, 1H, J_1 = 7.8 Hz, J_2 = 4.9 Hz), 7.58 (s, 1H), 7.88–7.90 (d, 2H, J = 7.8 Hz), 8.54 (dd, 1H, J_1 = 4.9 Hz, J_2 = 1.7 Hz), 8.65 (d, 1H, J = 1.7 Hz)

Table 1 (*Continued*)

ID	Structure	mp (°C)	Yield (%)	FAB-MS (*m*/*z*)	^{1}H NMR (400 MHz, DMSO-d_6, δ, J Hz)
11		219.3–221.3	13.6	386.0	3.55 (s, 2H), 4.21 (s, 2H), 5.74 (s, 2H), 6.13 (s, 2H), 7.18 (m, 3H), 7.45 (m, 2H), 7.53 (s, 1H), 7.86–7.88 (m, 2H), 8.54 (dd, 1H, J_1 = 4.8 Hz, J_2 = 1.7 Hz), 8.68–8.68 (d, 1H, J = 1.7 Hz)
12		185.1–187.8	9.3	367.1[b]	3.52 (s, 2H), 3.71 (s, 3H), 3.76 (s, 3H), 4.93 (s, 2H), 6.14 (br s, 2H), 6.53 (br s, 2H), 6.73–6.76 (dd, 1H, J_1 = 8.4 Hz, J_2 = 2.0 Hz), 6.86–6.88 (d, 1H, J = 8.4 Hz), 6.92–6.94 (d, 2H, J = 8.7 Hz), 6.97 (d, 1H, J = 2.0 Hz), 7.33–7.36 (d, 2H, J = 8.7 Hz), 7.45 (s, 1H)
13		172.8–174.8	11.5	351.1[b]	2.30 (s, 3H), 3.49 (s, 2H), 3.71 (s, 3H), 4.96 (s, 2H), 5.69 (s, 2H), 6.05 (s, 2H), 6.72–6.74 (dd, 1H, J_1 = 8.1 Hz, J_2 = 1.7 Hz), 6.85–6.87 (d, 1H, J = 8.1 Hz), 6.95–6.96 (d, 1H, J = 1.7 Hz), 7.17–7.19 (d, 2H, J = 7.8 Hz), 7.30–7.32 (d, 2H, J = 7.8 Hz), 7.46 (s, 1H)
14		184.5–186.5	9.6	338.1[b]	3.51 (s, 2H), 3.72 (s, 3H), 5.07 (s, 2H), 5.69 (s, 2H), 6.05 (s, 2H), 6.76–6.78 (d, 1H, J = 8.0 Hz), 6.88–6.90 (d, 1H, J = 8.0 Hz), 6.994 (s, 1H), 7.40–7.45 (m, 2H), 7.47 (s, 1H), 7.84–7.86 (d, 1H, J = 7.8 Hz), 8.54–8.55 (d, 1H, J = 4.6 Hz), 8.650 (s, 1H)
15		181.0–183.0	12.6	338.1[b]	3.52 (s, 2H), 3.73 (s, 3H), 5.07 (s, 2H), 5.66 (br s, 2H), 6.03 (br s, 2H), 6.70 (dd, 1H, J_1 = 8.1 Hz, J_2 = 2.0 Hz), 6.89 (d, 1H, J = 2.0 Hz), 6.94–6.96 (d, 1H, J = 8.1 Hz), 7.40–7.43 (dd, 1H, J_1 = 7.6 Hz, J_2 = 4.8 Hz), 7.47 (s, 1H), 7.83–7.85 (d, 1H, J = 7.6 Hz), 8.52 (dd, 1H, J_1 = 4.8 Hz, J_2 = 1.6 Hz), 8.63–8.64 (d, 1H, J = 1.6 Hz)

[a] $[M + 2]^+$.
[b] $[M + 1]^+$.

China. MS spectra were recorded on a Micromass Zabspec spectrophotometer. NMR spectra were recorded on a JEOL JNM-ECA-400 (400 MHZ) spectrophotometer. The inhibitory c-Fms kinase activities of compounds were evaluated by inhibiting M-CSF-dependent M-NFS-60 tumor cell proliferation effect [8]. The test cells also included a normal cell (HLF). The 50% inhibitory concentration (IC_{50}) mean values and selective index are summarized in Table 2.

Table 2
Inhibition of cell proliferation effect by GW2580, compound **6**–**15**.

Compound ID	HLF[a]	M-NFS-60[a]	SI[b]
6	32.96	2.87	11.48
7	15.64	3.68	4.25
8	14.06	6.56	2.14
9	9.24	16.56	0.56
10	40.19	2.57	15.64
11	35.17	1.13	31.12
12	172.33	3.29	52.38
13	58.91	5.20	11.33
14	176.70	5.49	32.17
15	≈300	1.45	206.70
GW2580	158.00	0.69	228.98

[a] The test concentration ranges were from 0.1 to 300 μmol/L. The given data are mean values of three parallel experiments.
[b] SI is the mean of the ratio of IC_{50} of Inhibition of HLF and IC_{50} of Inhibition of M-NFS-60.

As shown in Table 2, compound **15**, 5-[3-methoxy-4-(pyridine-3-yl) benzyl]-2,4-diamino pyrimidine, had an IC_{50} of 1.45 μmol/L in inhibiting the proliferation of M-CSF-dependent myeloid leukemia cells in mice (NFS-60) and SI of 206.70, both are similar with GW2580. These results are of great significance for the design of news compounds with higher activity and drug likeness. Further studies on the mechanism of action of these compounds are in progress.

Acknowledgments

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