Available online at www.sciencedirect.com
SCIENCE DIRECT®
Bioorganic & Medicinal Chemistry 13 (2005) 6388–6393
Bioorganic & Medicinal Chemistry

Xanthocillins as thrombopoietin mimetic small molecules

Ryuichi Sakai,[a,*] Takanori Nakamura,[b] Taito Nishino,[b] Masao Yamamoto,[b] Atsushi Miyamura,[b] Hisae Miyamoto,[c] Norihisa Ishiwata,[b] Norio Komatsu,[d] Hisao Kamiya[a] and Nobutomo Tsuruzoe[b]

[a]*Kitasato University, School of Fisheries Sciences, Sanriku-cho, Ofunato, Iwate 022-0101, Japan*
[b]*Biological Research Laboratories, Nissan Chemical Industries, Ltd, Shiraoka, Minamisaitama, Saitama 349-0294, Japan*
[c]*Chemical Research Laboratories, Nissan Chemical Industries, Ltd, Tsuboicho, Funabashi, Chiba 274-8507, Japan*
[d]*Department of Hematology, University of Yamanashi, Tamaho, Yamanashi 409-3898, Japan*

Received 14 May 2005; revised 26 June 2005; accepted 27 June 2005
Available online 22 August 2005

Abstract—Four xanthocillins (**1**–**4**), including a new compound **4**, were isolated from cultured marine fungus *Basipetospora* sp. as thrombopoietin (TPO) mimics. Compounds **1**–**4** promoted the proliferation of a TPO-sensitive human leukemia cell line, UT-7/TPO, and UT-7/EPO-mpl, genetically engineered to express c-Mpl, a receptor for TPO in dose-dependent manners. However, the proliferation of UT-7/EPO, a parental cell line of UT-7/EPO-mpl that was devoid of TPO receptor, was not affected by them. Thrombopoietic action of compound **1** was nearly as potent as that of TPO, inducing cell proliferation at a concentration ranging from 1 to 100 nM. Compound **1** also induced the phosphorylation of several proteins, including Janus kinase 2 (Jak2), signal transducers, and activators of transcription-3 (STAT3) and STAT5 in the UT-7/EPO-mpl cell line, but not in the UT-7/EPO cell line. These data indicated that xanthocillins are putative agonists for c-Mpl, as their cellular actions were analogous to those of TPO.

1. Introduction

Thrombopoietin (TPO) is a glycoprotein consisting of 332 amino acids that regulate platelet production by stimulating proliferation and differentiation of hematopoietic stem cells, megakaryocytic progenitor cells, and megakaryocytes. This regulation of the cell development is mediated through a receptor, c-Mpl, a protein encoded by proto-oncogene *c-mpl*.[1–4] Upon binding to c-Mpl, TPO activates a Janus kinase 2 (Jak2)–signal transducers and activators of transcription (STAT) signaling pathway to drive cell proliferation and differentiation. Therefore, recombinant human (rh) TPO can be an attractive treatment for thrombocytopenia. In fact, rhTPO has been tested as a treatment for patients with thrombocytopenia, including those who are receiving nonmyeloablative chemotherapy.[5] However, difficulties associated with a protein-based drug, such as limited administration methods and development of an antibody against TPO, must be considered for future drug development. Thus, small nonpeptide molecules that mimic the activity of TPO with bioavailability, preferentially through oral administration, would be of considerable medical importance.[6] Several small peptides of 14–16 amino acid residues and their dimmers have been reported as potent agonists of c-Mpl,[7] with in vivo efficacy in mice.[8] Recently, nonpeptide synthetic compounds such as a benzodiazepine derivative,[9] hydrazinonaphthalenes and azonaphthalenes,[10] and a substituted thiazole[11] have been reported to possess TPO-like activity. To date, however, none of these compounds has been developed as a thrombopoietic drug.

In search of TPO mimetic molecules with unique structure and possibly with an interesting mechanism of action from natural sources, we screened fungal strains isolated from benthic marine organisms using a UT-7/TPO cell proliferation assay, since marine microorganisms including fungi have been proven to be rich sources of bioactive secondary metabolites.[12] In the present study we report the isolation of xanthocillins (**1**–**4**) as TPO mimetics from marine fungus *Basipetospora* sp. and discuss that xanthocillins are a novel class of c-Mpl agonist.

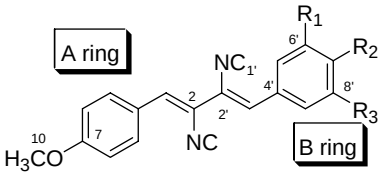
Keywords: Thrombopoietin; UT-7; c-Mpl; Xanthocillin.
* Corresponding author. Tel.: +81 192 44 1922; fax: +81 192 44 3932; e-mail: r.sakai@kitasato-u.ac.jp

0968-0896/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmc.2005.06.062

2. Results

2.1. Isolation and structure determination of xanthocillins

The lipophilic extract of cultured *Basipetospora* sp. was separated with a silica gel column to give fractions that exhibited the activity to induce cell proliferation of the UT7/TPO cells. HPLC purifications of active fractions afforded compounds **1**–**4** (Fig. 1).



compound 1:R_1 = H, R_2 = OH, R_3 = H
compound 2:R_1 = H, R_2 = OCH_3 (C-10'), R_3 = H
compound 3:R_1 = H, R_2 = OCH_3(C-10'), R_3 =OCH_3(C-11')
compound 4:R_1 = R_2 = R_3 = OCH_3

Figure 1. Structures of xanthocillins (**1**–**4**).

Spectral data for compounds **1**–**4** were very similar to each other, suggesting that they were related compounds. Structures of these compounds can be assigned readily to derivatives of xanthocillin X on the basis of its characteristic IR absorption for an isonitrile group around 2110–2120 cm^{-1}, UV absorption at 360 nm, and the molecular formula deduced from the high-resolution mass spectral data. Structures of each compound were assigned on the basis of molecular formulae and two-dimensional correlation nuclear magnetic resonance (NMR) spectra, by which the chemical shift for all the protons and carbons for compounds **1**–**4** could be assigned. The data for compounds **1**–**3** corresponded well to those of xanthocillin X monomethyl ether (XME), xanthocillin X dimethyl ether (XDE), and xanthocillin X trimethyl ether (XTE), respectively.[13] Compound **4**, 6′,8′-dimethoxy XDE, has never been reported previously. The molecular formula of **4** deduced by mass spectral data along with the NMR data indicated that **4** possesses three methoxyl groups in the B ring, although the A ring and the substituted diene are the same as those of other xanthocillins. The symmetric nature of the B ring and the correlation observed between H-5′ and C-3′ in heteronuclear multiple bond correlation spectrum (HMBC) NMR spectra confirmed the substitution pattern in the B ring. Xanthocillin X was first isolated as an anti-microbial agent.[14,15] Methylethers of xanthocillin X, compound **1**–**3**, were later isolated from *Aspergillus* sp. as anti-viral principles.[16]

2.2. The effect of compounds 1–4 on the proliferation of the various UT-7 cells

We first tested the effects of compound **1** on the cell proliferation of UT-7 sublines.[17,18] Compound **1**, at concentrations between 0.3 and 1000 ng/mL, was applied to UT-7/TPO, UT-7/EPO-mpl, and UT-7/EPO cell lines (Fig. 2). Both EPO and TPO were used as controls.

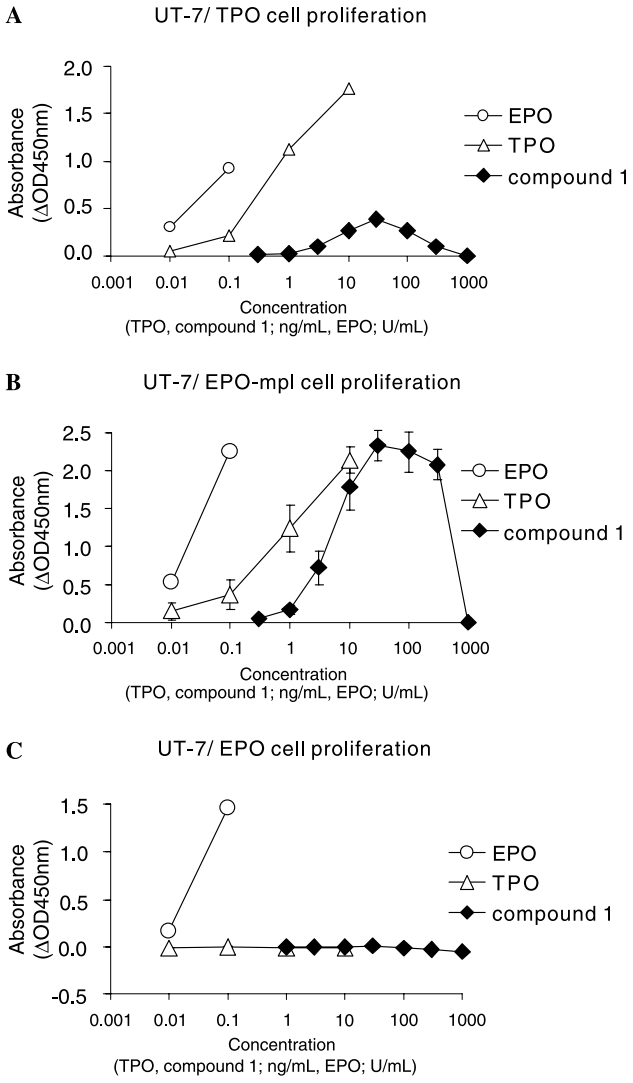


Figure 2. Proliferative activity of compound **1** on UT-7/TPO, UT-7/EPO-mpl, and UT-7/EPO cells: (A) UT-7/TPO cells, (B) UT-7/EPO-mpl cells, and (C) UT-7/EPO cells were cultured in IMDM containing 10% FBS in the presence of EPO, TPO, or compound **1** (0.3–1000 ng/mL) for 4 days. The data represents the average of two independent experiments (panels A and C), or the mean ± SE of three independent experiments (panel B).

Compound **1** induced proliferation of UT-7/TPO and UT-7/EPO-mpl in a dose-dependent manner, but not UT-7/EPO cells. The maximum response achieved by compound **1** in the UT-7/EPO-mpl cells was comparable to that induced by TPO, however that in UT-7/TPO cells was about 4 times less than that induced by TPO. Reason for this discrete response of two cell lines to compound **1** is not clear since both cell lines are equally responsive to TPO as well as EPO.

We next compared the effects of all xanthocillins isolated in the present study on the proliferations of UT-7/EPO-mpl and UT-7/EPO cells (Fig. 3). In the UT-7/EPO-mpl cells, both compounds **1** and **3** induced cell proliferation with nearly optimal responses, which were comparable to that of TPO. The efficacy of **2**, however, reached only about 78% of that of TPO, probably because of its

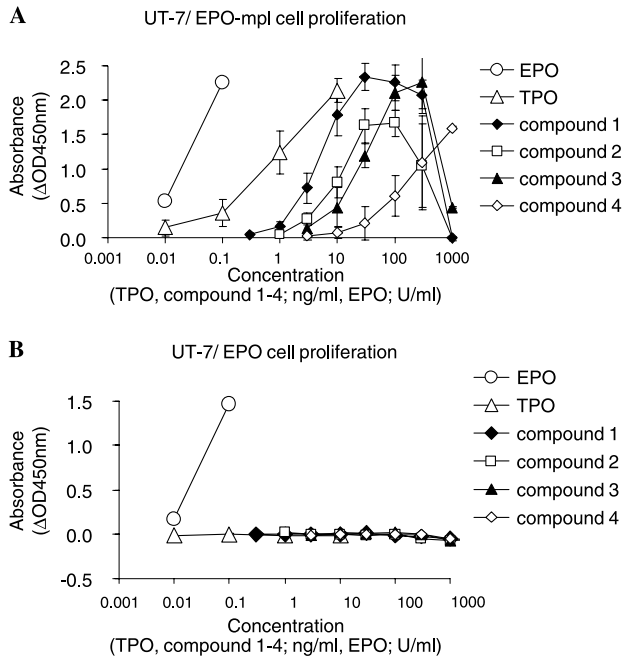


Figure 3. Proliferative activity of compounds **1**–**4** on UT-7/EPO-mpl and UT-7/EPO cells: (A) UT-7/EPO-mpl cells and (B) UT-7/EPO cells were cultured in IMDM containing 10% FBS in the presence of EPO, TPO, or compounds **1**–**4** for 4 days. The data represent the mean ± SE of three independent experiments (panel A) or the average of two independent experiments (panel B).

cytotoxicity at higher concentrations. Of note, in the UT-7/TPO cells, compounds **2**–**4** induced proliferation of the cells with maximum response of about 12–28% (data not shown). This tendency was similar to that observed for compound **1** as described above.

The efficacy and maximum response achieved by these compounds relative to TPO are summarized in Table 1. Compared to other nonpeptide thrombopoietic compounds, the activity of **1** was as potent as that of SB394725, one of the most potent small molecule thrombopoietic agents reported so far that induce cell proliferation through c-Mpl activation at about 40 nM.[19] Among the xanthocillins tested, methoxyl substitution in the B ring attenuated the potency.

Xanthocillins are known to display various biological activities. For example, **1**–**3** were cytotoxic against HeLa cells in concentrations ranging from 3.2 to 10 μg/mL.[20] Compounds **2** and **3** inhibited the growth of yeast *Candida albicans* at IC_{50} values of 12.1 and 8.2 μg/mL, respectively. Anti-viral activity of compounds **1**–**3** was also reported: **1**–**3** inhibited plaque formation of Newcastle disease virus (NDV) between concentrations of 2 and 30 μg/mL without cytotoxicity. Interestingly, we found that the effective concentration range of xanthocillins to induce cell proliferation for c-Mpl-expressed UT-7 cells was significantly lower than that of the biological activities described above, suggesting that the mechanism of TPO-like action of xanthocillins is different from the above 'inhibitory' actions to various cells and microorganisms. Interestingly, compounds **1** and **2** were reported to inhibit the biosynthesis of prostaglandin E2 and F2α at IC_{50} values of 0.2 and 30 μM, respectively, although the mechanism of this activity is not known.[13]

Table 1. UT-7/EPO-mpl cell proliferation induced by compound**1**–**4**[a]

	1	**2**	**3**	**4**
EC_{50} (ng/mL)	4.0 (2.7–6.8)[c]	14 (8.1–25)[c]	23 (14–37)[c]	210 (120–400)[c]
EC_{50} (nM)	13	44	66	560
E_{max} (%)[b]	109.1	77.5	105.7	74.4

[a] Data shown in this table are mean triplicate at each data point.
[b] TPO (10 ng/mL) = 100 %.
[c] 95% confidence interval.

2.3. Effects of xanthocillins on c-Mpl-mediated signal transduction pathways

Since the above results suggested that xanthocillins act as agonists of c-Mpl, we further examined the cellular mechanism for selective induction of UT-7/EPO-mpl cell proliferation by xanthocillins. Thus, we examined a TPO-receptor-mediated signaling pathway. Each UT-7/EPO-mpl or UT-7/EPO cell was treated with compound **1**. Harvested cells were lysed, and the cell lysates were immunoprecipitated with an anti-phosphotyrosine antibody and visualized by Western blotting with each antibody against Jak2, STAT3, and STAT5A. In the UT-7/EPO-mpl cells, compound **1** induced phosphorylation of all the above proteins in the same manner as TPO (Fig. 4).

On the other hand, neither compound **1** nor TPO induced phosphorylation of such proteins in the UT-7/EPO cells (data not shown). These data were consistent with the results from the cell proliferation assay in which xanthocillin selectively induced c-Mpl-expressed UT-7 cells.

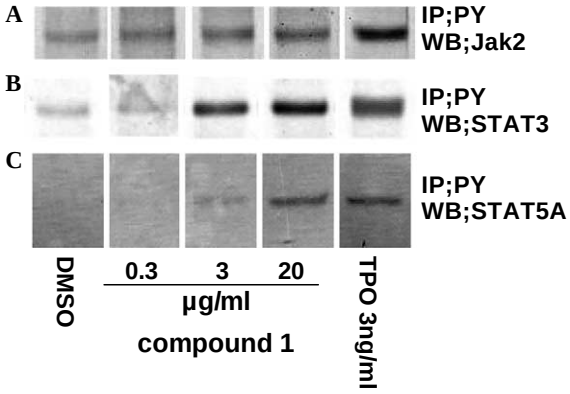


Figure 4. Signal transducing activity of compound **1** on UT-7/EPO-mpl cells. UT-7/EPO-mpl cells were treated with TPO or compound **1** (0.3–20 μg/mL) at 37 °C for 5 min. Cell lysates were immunoprecipitated with antiphosphotyrosine antibody and visualized by Western blotting with each antibody against Jak2 (panel A), STAT3 (panel B), and STAT5A (panel C).

3. Discussion

In summary, data presented in this study demonstrated that mycotoxin xanthocillins are putative ligands of c-Mpl, a TPO receptor, with dose-dependent thrombopoietic action. The xanthocillins are the first example of natural product with this activity. In the UT-7 cells, they acted as TPO mimics only when c-Mpl was expressed. The fact that xanthocillins activate a downstream signaling pathway of c-Mpl strongly suggests that these compounds are agonists of the TPO receptor. It should be noted, however that the actions of xanthocillins and TPO are comparable, but may not be the same, because response of UT-7/TPO was considerably lower than that of UT-7/EPO-mpl cell lines upon application of xanthocillins; while both of which responded similarly to TPO. This result might merely be a reflection of higher susceptibility of UT-7/TPO cells to cytotoxicity of xanthocillins, however, it is also possible that some unknown complexity underlies in TPO-receptor-mediated signaling resulted in the different responses of two cell lines to xanthocillins.

Although several nonpeptide small TPO mimetic compounds are known and the pharmacophore of some compounds has been uncovered,[10,21] the mechanisms of these drugs to activate TPO receptor are rather elusive. It is less likely that they are able to facilitate receptor dimerization as in the case of TPO itself or possibly of small-peptide TPO mimics. Thus, discoveries of nonpeptidyl TPO mimics with diverse structural feature are beneficial in the investigation of the molecular mechanisms of these compounds for the activation of TPO receptor. Because the structure of xanthocillins is significantly different from that of other nonpeptidyl thrombopoietic drugs, and the potency of xanthocillins to activate the receptor is considerably high, it is intriguing to study further to reveal the unique mechanism of receptor activation by these molecules, which would be of help in designing novel thrombopoietic drugs.

4. Experimental

4.1. General

The NMR data were recorded at 500 MHz for ^{1}H and 125 MHz for ^{13}C using methanol-d_4 as solvent. Chemical shifts for ^{1}H were reported in ppm relative to residual proton in the methanol-d_4 at δ 3.29. Mass spectra were measured in EI mode. Exact masses were determined using polyfluorokerocene (PFK) as an internal standard. An HPLC was performed using C-18 reversed-phase column (20×250 mm at a flow rate of 1 mL/min).

4.2. Biological material

The marine fungus isolate whose acetone extract induced the growth of UT-7/TPO cells was identified on the basis of its morphological and biochemical characteristics to be *Basipetospora* sp. strain 1142. The strain was deposited in international patent organism depository (IPOD) with registry number of FERM P-18940.

4.3. Isolation of xanthocillins

The fungus was grown on 100 mL of a medium consisting of 20 g of starch, 5 g of glucose, 2 g of yeast extract, 0.1 g of defatted soybean, and 0.1 g of $Mg_2P_2O_7$ in 1 L of distilled water. After incubation at 25 °C for 4 days on a rotary shaker at 140 rpm, the seed culture was transferred to 900 mL of the same medium and incubated for 11 days under the same conditions. The cell pellet from 13 L of broth was extracted with acetone and partitioned between hexane and water. The aqueous layer was re-extracted with ethyl acetate. The culture broth was passed through a DIAION HP20 (Mitsubishi Chemical) column, and the absorbed material was recovered by eluting with acetone. Both of these extracts were combined to give a crude extract. The crude extract was applied on a silica gel column and eluted with $CHCl_3$–CH_3OH 100:0–5. One of the fractions that induced UT-7/TPO cell proliferation was subjected to HPLC (acetonitrile–water 75:25, 15 mL/min) to give XME (**1**) as yellow needles. IR (KBr) ν 3300, 2929, 2117, 1604, 1511, 1259, 1177, 1031, 816 cm^{-1}; ^{1}H NMR (CD_3OD) δ 7.79 (2H, d, $J = 9$ Hz, H-5, 9), 7.72 (2H, d, $J = 9$ Hz, H-5′, 9′), 7.02 (4H, m, H-3, 3′, 6, 8), 6.87 (2H, d, $J = 9$ Hz, H-6′, 8′), 3.85 (3H, s, H-10); ^{13}C NMR (CD_3OD) δ 174.9, 174.7 (C-1, 1′), 162.7 (C-7), 161.6 (C-7′), 133.1 (C-5′), 132.7 (C-5), 129.0 (C-3′), 128.3 (C-3), 126.2 (C-4), 124.9 (C-4′), 117.5, 116.5 (C-2, 2′), 116.9 (C-6′, 8′), 115.5 (C-6, 8), 55.9 (C-10): HREIMS *m*/*z* 302.1233 (calcd for $C_{19}H_{14}N_2O_2$: 302.1055). The other active fraction was separated by HPLC (acetonitrile–water 64:36, 15 mL/min) to afford compound **2**–**4**. Compound **2**: yellow needles; IR (KBr) ν 2918, 2118, 1601, 1509, 1264, 1180, 1024, 876 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 7.78 (4H, d, $J = 9$ Hz, H-5, 5′, 9, 9′), 7.02 (2H, s, H-3, 3′), 6.98 (4H, d, $J = 9$ Hz, H-6, 6′, 8, 8′), 3.87 (6H, s, H-10, 10′); ^{13}C NMR ($CDCl_3$) δ 173.3 (C-1), 161.2 (C-7), 131.8 (C-5), 127.6 (C-3), 124.9 (C-4), 116.3 (C-2), 114.6 (C-6), 55.5 (C-10, 10′): HREIMS *m*/*z* 316.1233 (calcd for $C_{20}H_{16}N_2O_2$: 316.1212).

Compound **3**: yellow solid; IR (KBr) ν 2935, 2838, 2112, 1601, 1512, 1260, 1179, 1145, 1024 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 7.80 (2H, d, $J = 9$ Hz, H-5), 7.49 (1H, d, $J = 2$ Hz, H-5′), 7.36 (1H, dd, $J = 2$, 9 Hz, H-9′), 7.03 (1H, s, H3 or H3′), 7.02 (1H, s, H3 or H3′), 6.99 (2H, d, $J = 9$ Hz, H-6, H-8), 6.94 (1H, d, $J = 9$ Hz, H-8′), 3.96 (3H, s, H-11′), 3.95 (3H, s, H-10′), 3.88 (3H, s, H-10); ^{13}C NMR ($CDCl_3$) δ 173.5, 173.4 (C-1, 1′), 161.3 (C-7), 151.0 (C-7′), 149.1 (C-6′), 131.8 (C-5), 127.8 (C-3′), 127.6 (C-3), 125.1 (C-4′), 124.9 (C-4), 124.6 (C-9′), 116.3, 116.2 (C-2, 2′), 114.6 (C-6), 111.9 (C-5′), 111.2 (C-8′), 56.1 (C10′, 11′), 55.5 (C-10): HREIMS *m*/*z* 346.1330 (calcd for $C_{21}H_{18}N_2O_3$: 346.1317).

Compound **4**: yellow solid; IR (KBr) ν 2935, 2838, 2111, 1599, 1509, 1258, 1128 cm^{-1}; ^{1}HNMR ($CDCl_3$) δ 7.81 (2H, d, $J = 9$ Hz, H-5), 7.09 (1H, s, H-5′), 7.06 (1H, s, H-3), 7.02 (1H, s, H-3′), 6.99 (2H, d, $J = 9$ Hz, H-6), 3.93 (9H, s, H-10′, 11′, 12′), 3.88 (3H, s, H-10); ^{13}C NMR ($CDCl_3$) δ 174.0, 173.5 (C-1, 1′), 161.4 (C-7), 153.3 (C-6′), 140.1 (C-7′), 132.0 (C-5), 128.3 (C-3′), 127.8 (C-3), 127.5 (C-4′), 124.7 (C-4), 117.5 (C-2′),

116.0 (C-2), 114.6 (C-6), 107.3 (C-5′), 61.1 (C-10′), 56.3 (C-11′), 55.5 (C-10): HREIMS *m/z* 376.1390 (calcd for $C_{22}H_{20}N_2O_4$: 376.1423).

4.4. Cells and cell culture

The UT-7 cell line was established from bone marrow (BM) cells obtained from a patient with acute megakaryocytic leukemia.[22] UT-7/EPO and UT-7/TPO were established by continuous maintenance of the UT-7 cell line in the presence of EPO and TPO, respectively.[17,23] UT-7/EPO-mpl was prepared by introducing a vector that induces expression of human TPO receptor gene (*c-mpl*) into human leukemia cell line UT-7/EPO under the control of a cytomegalovirus immediate-early promoter following the method of Takatoku et al.[18] These cell lines were subcultured in Iscove's modified Dulbecco's medium (IMDM; Gibco) containing 10% fetal bovine serum (FBS; Trace Scientific) using a CO_2 incubator (5% CO_2, 37 °C).

4.5. Cell proliferation assay

The subcultured cells described above were washed twice with phosphate-buffered saline (PBS) and suspended in IMDM containing 10% FBS at a cell density of 6×10^4 cells/mL. The cell suspension was transferred to a 96-well tissue culture plate in 100-μL aliquots. Then either EPO (Epogen®, Chugai Pharmaceutical), TPO (PeproTech EC), or compounds **1**–**4** dissolved in DMSO was diluted 83-fold with IMDM containing 10% FBS and added to the aforementioned cell suspension in 20-μL aliquot. The suspension was incubated in a CO_2 incubator (5% CO_2, 37 °C) for 4 days. Cell proliferation was assayed using WST-8 reagent (Kishida Chemical) according to instructions by the manufacturer. A 10-μL aliquot of 5 mM WST-8 reagent was added to each well of the tissue culture plate, and the plate was incubated at 37 °C for 4 h. The formazan pigment generated was detected by measuring the absorbance at 450 nm with a 96-well microplate reader. The concentration of each compound producing half maximal growth (EC_{50}) and the maximal growth rate achieved by the same compound (E_{max}) to the growth of UT-7/EPO-mpl cells in the presence of 10 ng/mL TPO were determined.

4.6. Immunoprecipitation and Western blotting

The signal transducing activity of the compound to TPO receptor was assayed by SDS–PAGE and Western blotting. Briefly, UT-7/EPO-mpl cells and UT-7/EPO cells were washed 3 times with PBS and suspended in IMDM containing 10% FBS at a cell density of 9×10^5 cells/mL. The cell suspension was incubated in a CO_2 incubator (5% CO_2, 37 °C) for 18 h. To 2 mL of this cell suspension (7×10^6 cells/mL), either TPO (final concentration, 3 ng/mL) or a DMSO solution **1** (final concentration, 0.3–20 μg/mL) was added. After the mixture was incubated at 37 °C for 5 min, the cells were lysed in 1.4 mL of TNE buffer [20 mM Tris–HCl buffer (pH 7.4) containing 150 mM NaCl, 1 mM EDTA, 1% Triton X-100, 1 mM phenylmethanesulfonyl fluoride (PMSF), 1 mM Na_3VO_4, and 1/400-diluted Protease Inhibitor Cocktail (Sigma)]. The cell lysate was centrifuged to collect the supernatant for immunoprecipitation with an antibody against phosphotyrosine (PY20, Transduction Laboratories) and protein G Sepharose (Pharmacia). The immunoprecipitated protein fraction was collected and denatured in a sample buffer for separation by SDS–PAGE (7.5%). The separated proteins were transferred onto polyvinyliden difluoride (PVDF) membrane (0.2 mm pore size) at 100 V for 1 h and incubated with antibodies against proteins involved in signal transduction [anti-Jak2 (Upstate Biotechnology), anti-STAT3 (Santa Cruz Biotechnology), and anti-STAT5A (Upstate Biotechnology)] followed by an alkaline phosphatase-labeled second antibody. The antigen–antibody complex formed on the PVDF membrane was visualized with 150 μg/mL nitro blue tetrazolium chloride (NBT) and 300 μg/mL (5-bromo-4-chloro-3-indolyl-phosphate) (BCIP).

Acknowledgments

We thank Mr. Atsushi Morihara for assistance in isolation work. We are also grateful to Mr. Tetsuya Tomie, Ms. Sumiko Aikawa, Mr. Ryo Matsumoto, and Ms. Kana Ikeda for providing technical advice, assistance in the culturing of fungi, and conducting the cell proliferation assay.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2005.06.062.

References and notes

1. Kaushansky, K.; Drachman, J. G. *Oncogene* **2002**, *21*, 3359–3367.
2. Souyri, M.; Vigon, I.; Penciolelli, J. F.; Heard, J. M.; Tambourin, P.; Wendling, F. *Cell* **1990**, *63*, 1137–1147.
3. Skoda, R. C.; Seldin, D. C.; Chiang, M. K.; Peichel, C. L.; Vogt, T. F.; Leder, P. *Embo J.* **1993**, *12*, 2645–2653.
4. Vigon, I.; Mornon, J. P.; Cocault, L.; Mitjavila, M. T.; Tambourin, P.; Gisselbrecht, S.; Souyri, M. *Proc. Natl. Acad. Sci. U.S.A.* **1992**, *89*, 5640–5644.
5. Nash, R. A.; Kurzrock, R.; DiPersio, J.; Vose, J.; Linker, C.; Maharaj, D.; Nademanee, A. P.; Negrin, R.; Nimer, S.; Shulman, H.; Ashby, M.; Jones, D.; Appelbaum, F. R.; Champlin, R. *Biol. Blood Marrow Transplant* **2000**, *6*, 25–34.
6. Duffy, K. J.; Darcy, M. G.; Delorme, E.; Dillon, S. B.; Eppley, D. F.; Erickson-Miller, C.; Giampa, L.; Hopson, C. B.; Huang, Y.; Keenan, R. M.; Lamb, P.; Leong, L.; Liu, N.; Miller, S. G.; Price, A. T.; Rosen, J.; Shah, R.; Shaw, T. N.; Smith, H.; Stark, K. C.; Tian, S. S.; Tyree, C.; Wiggall, K. J.; Zhang, L.; Luengo, J. I. *J. Med. Chem.* **2001**, *44*, 3730–3745.
7. Cwirla, S. E.; Balasubramanian, P.; Duffin, D. J.; Wagstrom, C. R.; Gates, C. M.; Singer, S. C.; Davis, A. M.; Tansik, R. L.; Mattheakis, L. C.; Boytos, C. M.; Schatz, P. J.; Baccanari, D. P.; Wrighton, N. C.; Barrett, R. W.; Dower, W. J. *Science* **1997**, *276*, 1696–1699.

8. Dower, W. J.; Cwirla, S. E.; Balasubramanian, P.; Schatz, P. J.; Baccanari, D. P.; Barrett, R. W. *Stem Cells* **1998**, *16* (Suppl. 2), 21–29.
9. Kimura, T.; Kaburaki, H.; Tsujino, T.; Ikeda, Y.; Kato, H.; Watanabe, Y. *FEBS Lett.* **1998**, *428*, 250–254.
10. Duffy, K. J.; Price, A. T.; Delorme, E.; Dillon, S. B.; Duquenne, C.; Erickson-Miller, C.; Giampa, L.; Huang, Y.; Keenan, R. M.; Lamb, P.; Liu, N.; Miller, S. G.; Rosen, J.; Shaw, A. N.; Smith, H.; Wiggall, K. J.; Zhang, L.; Luengo, J. I. *J. Med. Chem.* **2002**, *45*, 3576–3578.
11. Inagaki, K.; Oda, T.; Naka, Y.; Shinkai, H.; Komatsu, N.; Iwamura, H. *Blood* **2004**, *104*, 58–64.
12. Namikoshi, M.; Kobayashi, H.; Yoshimoto, T.; Meguro, S.; Akano, K. *Chem. Pharm. Bull. (Tokyo)* **2000**, *48*, 1452–1457.
13. Kitahara, N.; Endo, A. *J. Antibiot. (Tokyo)* **1981**, *34*, 1556–1561.
14. Hagedorn, I.; Tonjes, H. *Pharmazie* **1957**, *12*, 567–580.
15. Hagedorn, I.; Tonjes, H. *Pharmazie* **1956**, *11*, 409–410.
16. Takatsuki, A.; Tamura, G.; Arima, K. *J. Antibiot. (Tokyo)* **1968**, *21*, 676–680.
17. Komatsu, N.; Kunitama, M.; Yamada, M.; Hagiwara, T.; Kato, T.; Miyazaki, H.; Eguchi, M.; Yamamoto, M.; Miura, Y. *Blood* **1996**, *87*, 4552–4560.
18. Takatoku, M.; Kametaka, M.; Shimizu, R.; Miura, Y.; Komatsu, N. *J. Biol. Chem.* **1997**, *272*, 7259–7263.
19. Erickson-Miller, C. L.; Delorme, E.; Tian, S. S.; Hopson, C. B.; Stark, K.; Giampa, L.; Valoret, E. I.; Duffy, K. J.; Luengo, J. L.; Rosen, J.; Miller, S. G.; Dillon, S. B.; Lamb, P. *Exp. Hematol.* **2005**, *33*, 85–93.
20. Takahashi, C.; Sekita, S.; Yoshihira, K.; Natori, S. *Chem. Pharm. Bull. (Tokyo)* **1976**, *24*, 2317–2321.
21. Duffy, K. J.; Shaw, A. N.; Delorme, E.; Dillon, S. B.; Erickson-Miller, C.; Giampa, L.; Huang, Y.; Keenan, R. M.; Lamb, P.; Liu, N.; Miller, S. G.; Price, A. T.; Rosen, J.; Smith, H.; Wiggall, K. J.; Zhang, L.; Luengo, J. I. *J. Med. Chem.* **2002**, *45*, 3573–3575.
22. Komatsu, N.; Nakauchi, H.; Miwa, A.; Ishihara, T.; Eguchi, M.; Moroi, M.; Okada, M.; Sato, Y.; Wada, H.; Yawata, Y., et al. *Cancer Res.* **1991**, *51*, 341–348.
23. Komatsu, N.; Yamamoto, M.; Fujita, H.; Miwa, A.; Hatake, K.; Endo, T.; Okano, H.; Katsube, T.; Fukumaki, Y.; Sassa, S., et al. *Blood* **1993**, *82*, 456–464.