Bioorganic & Medicinal Chemistry Letters 22 (2012) 5093–5097

Contents lists available at SciVerse ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

The potential role of anibamine, a natural product CCR5 antagonist, and its analogues as leads toward development of anti-ovarian cancer agents

Yan Zhang [a,b,*], Christopher K. Arnatt [a], Feng Zhang [a], Jiannan Wang [a], Kendra M. Haney [a], Xianjun Fang [b,c]

[a] Department of Medicinal Chemistry, Virginia Commonwealth University, Richmond, VA 23298, USA
[b] Massey Cancer Center, Virginia Commonwealth University, Richmond, VA 23298, USA
[c] Department of Biochemistry, Virginia Commonwealth University, Richmond, VA 23298, USA

ARTICLE INFO

Article history:
Received 12 April 2012
Revised 22 May 2012
Accepted 29 May 2012
Available online 16 June 2012

Keywords:
Ovarian cancer
Chemokines
CCR5
Anibamine

ABSTRACT

Chemokines and their receptors play important roles in the development of primary tumors and their metastases. Particularly CC chemokine receptor 5 (CCR5) and its ligand CC chemokine ligand 5 (CCL5/RANTES) seem to be critical in proliferation and invasion of ovarian cancer, the leading cause of death from gynecological malignancies in the United States. Anibamine, the first natural product CCR5 antagonist, and its analogues were examined for their effects on proliferation of the OVCAR-3 ovarian cancer cells in order to validate their candidacy as leads to develop novel anti-ovarian cancer agents. Acting as CCR5 antagonists, anibamine and its analogues significantly suppressed CCL5-induced intracellular Ca^{2+} flux. The compounds also inhibited the proliferation of OVCAR-3 at micromolar to submicromolar range. Moreover, anibamine and several analogues did not show significant cytotoxicity in NIH 3T3 cells at concentrations up to 20 μM. Based on these results, anibamine and one of its synthetic analogues were defined as potential leads to develop novel agents against ovarian cancer.

Published by Elsevier Ltd.

Ovarian cancer is the second most common (affecting about every one out of seventy women) and the deadliest (1% of all women die of it) gynecological cancer in the United States, and it is also the fifth leading cause of cancer related deaths in women.[1,2] Ovarian cancer is called a 'silent killer' because symptoms normally won't be evident until the disease has advanced and the chance of cure or remission becomes very slim.[3] Therefore, identification of factors and pathways responsible for the ovarian cancer development and progression is of critical importance and such effort may lead to development of novel therapeutic agents.

Tumor cell growth can be directly regulated, among others, by chemokines, a family of small proteins inducing directed cell migration (chemotaxis), via specific G-protein coupled receptors.[4–10] Initially, chemokines are considered to be pro-inflammatory which can be induced during an immune response as regulating leukocyte recruitment at sites of inflammation. During the last decade, it has become increasingly clear that chemokines also have the capacity to mediate several other functions and therefore are more than simple trafficking controllers. Recently, a number of reports demonstrated that a complex network of chemokines and their receptors influence the development of primary tumors and their metastasis.[11–15] For example, the expression of chemokines was detected in several types of human and murine tumors.[16–22] In some cases, chemokines were found to be autocrine factors produced by tumor cells that are essential to tumor cell proliferation or survival.[23–26] In view of their chemotactic properties, it has been suggested that chemokines may mediate the recruitment of tumor-associated leukocytes to tumor sites,[27–34] a process postulated to accelerate the progression of several malignant diseases.

The C–C chemokine receptor CCR5 is a G-protein coupled receptor. The ligands of this receptor include monocyte chemoattractant protein 2 (MCP-2), macrophage inflammatory protein 1 alpha (MIP-1 alpha), macrophage inflammatory protein 1 beta (MIP-1 beta), and RANTES (Regulated on activation normal T expressed and secreted protein), which is also known as CCL5. Along with other chemokine receptors, CCR5 has been characterized for its potential role in cancer development,[35] particularly, in ovarian oncogenesis. For instance, analysis of the levels of the chemokine in plasma of patients at different stages of the disease revealed an association between CCL5 and ovarian carcinoma progression while it appeared that CCL5 protein levels were higher in ovarian cancer patients than in patients diagnosed with benign ovarian cysts, and elevated in stages III–IV of ovarian cancer compared to stages I–II.[36,37] This suggested that intrinsic CCL5 levels could be useful for detection of malignant ovarian tumors. Another study showed that interleukin-12 (IL-12) induced the regression of xenografts of the OV-HM ovarian carcinoma cells, which was mediated by the chemokine receptor CCR5, while the CCR5 antagonist TAK-779 in tumor-bearing mice prevented IL-12 induced T-cell migration.[38] This certainly suggested a therapeutic opportunity of

* Corresponding author.
E-mail address: yzhang2@vcu.edu (Y. Zhang).

0960-894X/$ - see front matter Published by Elsevier Ltd.
http://dx.doi.org/10.1016/j.bmcl.2012.05.127

using CCR5 antagonist in ovarian cancer. Meanwhile ovarian tumors have been observed to over-express CCR5 and inhibition of CCL11 (one of the CCR5 endogenous agonists) signaling by the combination of neutralizing antibodies against CCL11 and its receptors significantly increased the ovarian carcinoma cell sensitivity to cisplatin.[39] Overall, these findings suggested that development of appropriate chemokine receptor CCR5 antagonists could provide a novel strategy to treat ovarian cancer.

Anibamine, a unique pyridine quaternary alkaloid recently isolated from *Aniba panurensis*, was found to bind to CCR5 with an IC_{50} of 1 μM in competition with ^{125}I-gp120, an HIV viral envelop protein.[40] As the first natural product CCR5 antagonist, anibamine provides a structural skeleton that is remarkably different from all previously identified lead CCR5 antagonists (Fig. 1) derived from extensive high throughput screening studies. The chemokine receptor CCR5 has mainly been employed in AIDS therapies since it was first cloned more than a decade ago.[41–44] To date only one drug, maraviroc, has been approved by the FDA in 2007 while concerns remained that maraviroc could be associated with increased risks of liver damage, lymphoma, infections and heart attack.[45] Apparently, there is an urgent need to explore new chemical structures and templates with a wider range of structural features to develop novel CCR5 antagonists. Compared to those high-throughput screening hits, lead compounds derived from natural products, often contain more diverse skeletons with wider ranges of shape, chemical features, and specific biological activities.[46,47] Thus, natural products are desirable and useful resources for drug discovery and development.

Recent studies showed that anibamine inhibited proliferation of prostate cancer cell lines at micromolar to submicromolar concentrations and prevented adhesion and invasion of the highly metastatic M12 prostate cancer cell line.[48] Meanwhile the total synthesis of anibamine has been reported a couple of years ago[49] while an updated synthetic route was made available very recently.[50] A homology modeling based docking study of anibamine and other known CCR5 antagonists further verified the possible binding mode of anibamine in the CCR5.[51] The development of these synthetic pathways and modeling studies provided the opportunity to prepare diversified anibamine derivatives in order to investigate the structure–activity relationship of anibamine as a CCR5 antagonist and potential anti-cancer agent.

Characterization of anibamine as the lead for anti-ovarian cancer agent. To validate anibamine's function as a CCR5 antagonist, its effect on the intracellular calcium mobilization stimulated by CCL5 (RANTES) in MOLT4/CCR5 cells was first examined by following the reported procedure[50] since the inhibitory effects of CCR5 antagonists in chemokine-induced calcium ion mobilization have been demonstrated to correlate well with their affinity in radioligand competition binding assays.[52,53] Anibamine showed moderate inhibition of calcium flux at an IC_{50} of 5.4 μM, which was in line with its reported binding affinity to the CCR5 receptor.[40]

Anibamine was then evaluated for its ability to inhibit growth of the OVCAR-3 ovarian cancer cells through a cell proliferation assay. In the initial experiments, CCL5 was not added in the protocol in the consideration of avoiding possible non-specific stimulatory effect of CCL5 on other chemokine receptors. It turned out that anibamine effectively inhibited the proliferation of OVCAR3 cells at an IC_{50} around 1 μM, which again was in line with its binding affinity to the CCR5 receptor and the inhibitory effect on the CCL5 stimulated calcium flux (Table 1).

Since it has been reported that CCR5 and CCL5 were expressed on OVCAR3 cells,[39] it would be interesting to see if CCL5 could act as a cancer cell growth stimulator. Therefore, different concentrations of CCL5 were applied to the OVCAR3 cells and its effect on cell proliferation was determined as illustrated in Figure 3. Interestingly, at the lower concentrations (0.3 nM–3 nM), CCL5 significantly promoted the growth of OVCAR3 cells by at least 50% (P <0.01) while higher concentrations of CCL5 tested (10 nM and 30 nM) showed some but not very significant effect (Fig. 2). Such observation was in coincidence with the reported stimulatory pattern of CCL5 on several prostate cancer cells.[22]

To examine whether the inhibitory effect of anibamine on the OVCAR3 cells observed previously was in correlation to its antagonist activity on the CCL5/CCR5 system, its inhibitory effect on the proliferation of OVCAR-3 cells was further tested in the presence of CCL5 (1 nM). Under such condition, anibamine showed relatively stronger inhibitory effect on the OVCAR3 cell proliferation (IC_{50} at 0.9 μM) than the condition without CCL5 stimulation, which implied that the inhibitory effect of anibamine on ovarian cancer cell growth may be mediated through its antagonist activity on the CCR5 receptor (Table 1).

Afterwards, the basal cytotoxicity assay was performed using the NIH 3T3 cells under similar proliferation conditions (WST-1 as the proliferation agent) to determine whether the inhibitory effect of anibamine on cancer cells was due to its basal cytotoxicity. It turned out that much higher concentration of anibamine (IC_{50} = 23.5 μM) was required to inhibit the growth of 3T3 cells, suggesting a very promising selectivity of anibamine in inhibiting the growth of cancer cells.

Application of 'deconstruction-reconstruction-elaboration' method to study the structure–activity relationship of anibamine as anti-ovarian cancer lead compound. The initial structural modification of anibamine was conducted by following the 'deconstruction-reconstruction-elaboration' method. This method has been successfully

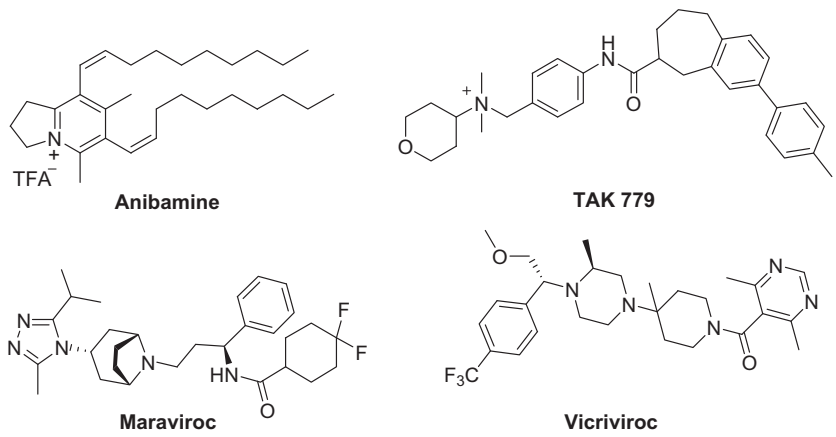

Figure 1. Anibamine and some known CCR5 antagonists.

Table 1
Biological characterization of anibamine and its analogs

Compound	CCR5 antagonism MOLT-4/CCR5 IC_{50} (μM)	Anti-proliferation OVCAR3 IC_{50} (μM)		Basal cytotoxicity NIH 3T3 TC_{50} (μM)
		w/o CCL5	w/CCL5	
1, Aanibamine	5.4 ± 0.9	1.1 ± 0.3	0.86 ± 0.1	23.5 ± 2.4
2	9.2 ± 0.4	1.3 ± 0.2	0.9 ± 0.1	6.9 ± 1.6
3	16.3 ± 4.2	0.4 ± 0.1	1.0 ± 0.1	7.8 ± 1.5
4	>100	ND[a]	ND	ND
5	>100	ND	ND	ND
6	6.5 ± 1.8	0.8 ± 0.3	0.7 ± 0.2	4.4 ± 0.6
7	7.8 ± 1.4	0.6 ± 0.1	0.3 ± 0.1	5.1 ± 2.4
8	10.1 ± 3.9	0.9 ± 0.1	0.5 ± 0.1	3.3 ± 0.3
9	9.2 ± 0.6	0.9 ± 0.1	0.6 ± 0.1	4.0 ± 0.3
10	10.0 ± 0.4	1.7 ± 0.4	0.3 ± 0.1	29.1 ± 0.6
11	8.4 ± 0.9	1.2 ± 0.3	0.7 ± 0.1	10.1 ± 1.1
12	4.6 ± 1.6	2.4 ± 1.2	3.4 ± 2.0	17.5 ± 4.6
13	48.1 ± 21.7	3.6 ± 0.9	0.4 ± 0.2	4.8 ± 0.6
14	15.2 ± 7.9	1.7 ± 0.6	0.4 ± 0.1	3.8 ± 0.5
15	37.6 ± 5.4	2.1 ± 0.4	0.8 ± 0.1	11.7 ± 1.5

[a] ND, not determined.

applied to improve the pharmacological activities of both synthetic agents and natural products.[54–57] In our case (Fig. 3), each structural component of the lead compound, anibamine, was 'removed' one at a time in order to test the influence of that component on its CCR5 antagonism and anti-ovarian cancer activity. Once the essential structural components were defined, they were retained in the core structure. Further modification was then done on the core structure to improve its activities.

Total fourteen analogues of anibamine were prepared following the synthetic route of anibamine with appropriate modifications (See the Supplementary data for details).[49,50] Similar to the characterization of anibamine, all of its analogues were first evaluated in a CCL5-induced Ca^{2+} mobilization experiment to see if they act as antagonists to the CCR5 receptor and inhibit the calcium ion flux induced by CCL5. These analogues were then tested in the cell proliferation assay against OVCAR3 cells in the absence or presence of CCL5. Similarly, 3T3 cells were adopted to characterize the basal cytotoxicity of these analogues.

Anibamine deconstruction analogues and their activity studies. Two side chain deconstruction analogues of anibamine, compound **2** and **3**, were prepared to study the necessity of the side chains for the binding to the CCR5 receptor and potential anti-ovarian cancer activity (Table 1). The fact that both of them showed significantly decreased inhibition to the receptor function of calcium flux compared to the parent anibamine indicated that both side chains should be retained to facilitate the binding to the receptor complementarily.

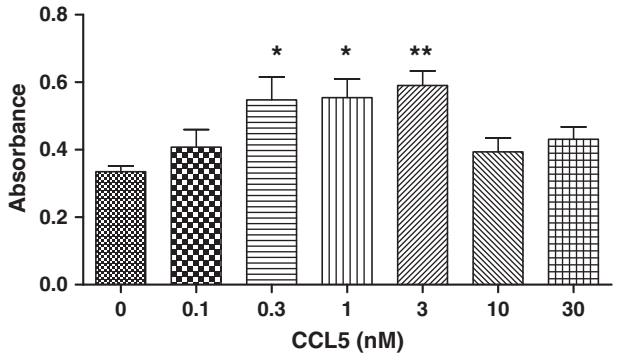

Figure 2. CCL5 stimulation of OVCAR3 cell proliferation. The statistical analysis was conducted by ANOVA.

On the other hand, compound **2** and **3** showed similar or somewhat higher activity than the parent natural product in the anti proliferation assay in OVCAR3 cells. Considering their relatively lower affinity to the receptor CCR5 as indicated by the calcium mobilization inhibition activity, such improved anti-proliferative effect on OVCAR-3 cells may be conferred in part by interaction with other off-target proteins. This was further supported by their significantly higher cytotoxicity in the 3T3 cells compared with the parent lead compound.

To test if the core ring system would be essential, compound **4** and **5** were prepared (Fig. 3). In compound **4**, a methyl group was retained at position 2 to minimize the steric hindrance alteration due to the removal of the fused ring part. The second molecule, compound **5,** was designed to retain the same number of the carbon atoms from the aliphatic core ring system in order to minimize the change of hydrophobicity. To facilitate the synthesis, a methoxyl group was applied to cap the end of the new side chain. The fact that these two core ring deconstruction analogues carried no significant antagonism to the receptor CCR5 (calcium flux inhibition IC_{50} higher than 100 μM, Table 1) demonstrated that the positively charged nitrogenous center was critical for binding to the receptor, which was consistent with the observation from the molecular modeling studies reported previously.[48] Therefore this ring system needs to be retained for the future operation.

Anibamine elaboration analogues activity study. Four analogues of anibamine were prepared to evaluate the possible influence of double bond configurations to the recognition of the receptor and potential anti cancer activity (Fig. 3). Among these four compounds, **6** through **9**, it seemed that in general, the double bond configurations (either trans, cis, or saturated) on the two side chains did not influence the functional inhibition to the receptor significantly based on the calcium mobilization assay results. They also showed either similar or slightly higher anti-proliferation activity as indicated by their IC_{50} values compared with the parent anibamine. In the presence of CCL5 stimulation, all of them showed significantly higher anti-proliferation activity against the OVCAR3 cells, indicating that they inhibited OVCAR3 cells through targeting the CCR5 receptor. On the other hand, their higher basal cytotoxicity compared with the parent natural product was somehow discouraging (Table 1).

To further evaluate the core ring size influence on the receptor CCR5 affinity as indicated by their functional inhibition to calcium flux as well as the potential anti-ovarian cancer activity, six core ring size modified analogues were prepared and studied (Fig. 3,

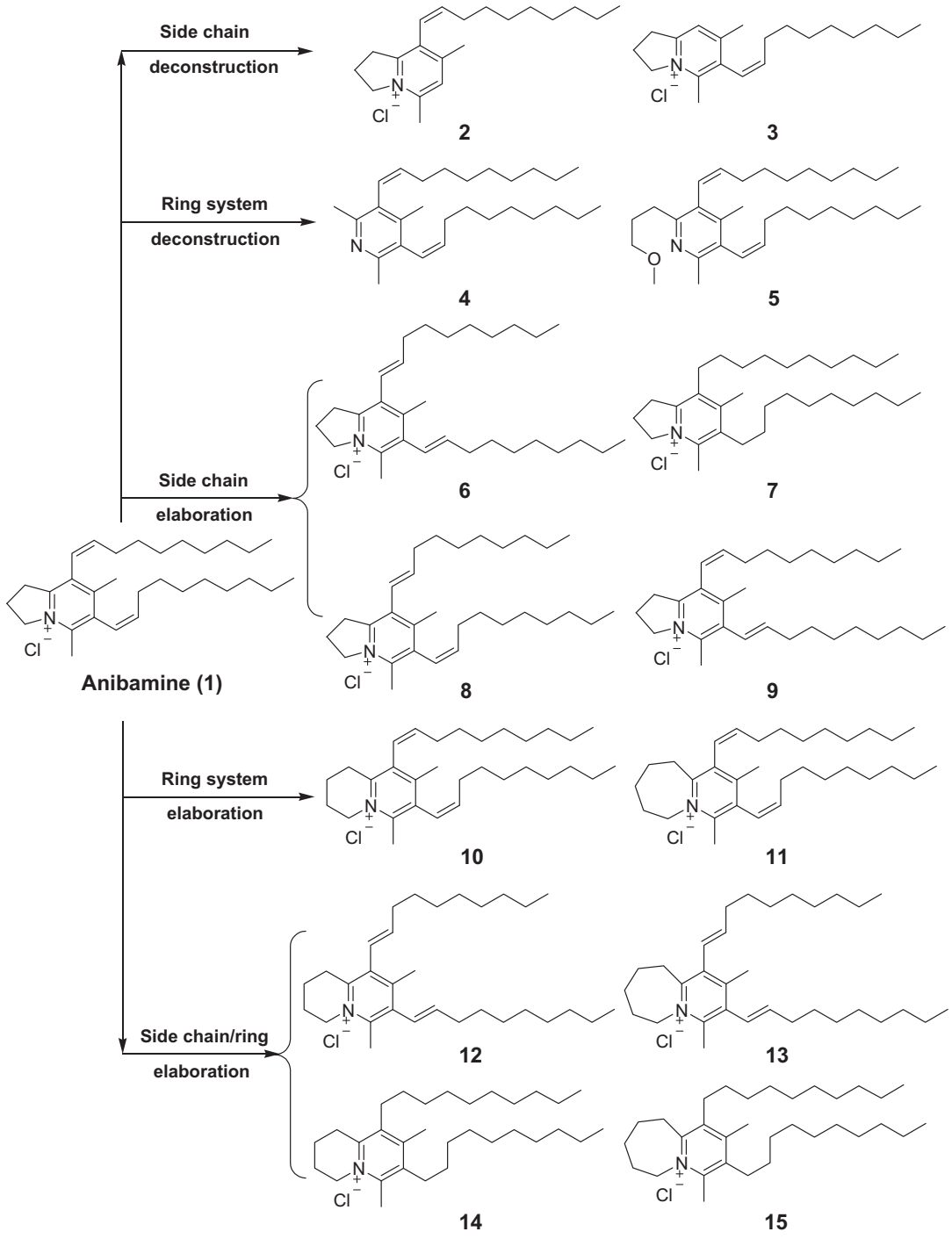

Figure 3. The application of 'deconstruction-reconstruction-elaboration' concept to design the analogues of the lead compound, anibamine.

and Table 1). In general, it seemed that six-member ring analogues of anibamine (compound **10**, **12** and **14**) showed relatively higher receptor affinity than the seven-member ring analogues (compound **11**, **13**, and **15**). By comparing their anti-proliferative activity against OVCAR3 cells, it seemed that they all showed very similar profile. Given the fact that the relatively lower receptor affinity and higher basal cytotoxicity pattern, the seven-member ring analogues probably interacted with some off-target protein(s).

A closer look at compound **10** and **12**, both of which showed relatively lower basal cytotoxicity profile with reasonable anti-proliferative activity against OVCAR3 cells, provided some insight into the potential role of the ring size combined with the side chain configuration. Compound **10**, which showed the lowest basal cytotoxicity among all the analogues, also carried a similar anti-proliferative effect compared with the natural product lead, anibamine. More importantly, the presence of CCL5 dramatically increased its anti-proliferative activity, indicating its specific antagonistic interaction with the CCR5. For compound **12**, no significant difference was observed in its anti-proliferative activity with or without the stimulation of CCL5. Therefore compound **10** was considered our next generation lead compound based on its biological activity profile.

In summary, the natural product CCR5 antagonist, anibamine, was characterized as a lead to develop novel anti-ovarian cancer

agent. Its biological profile seemed to be encouraging. It exhibited significant anti-proliferative activity against the OVCAR3 cells with much lower basal cytotoxic effect in the nontransformed NIH 3T3 cells. The application of 'deconstruction-reconstruction-elaboration' concept on anibamine structure–activity relationship characterization revealed that the core ring system was critical for the binding affinity to the receptor CCR5 while both side chains seemed to be important for the binding affinity to the receptor and selective inhibition of the chemokine CCL5 correlated cancer cell proliferation. The ring size modification of the core ring system provided us a new lead, compound **10**, with reasonable receptor binding affinity and promising anti-ovarian cancer proliferative activity while no significant basal cytotoxicity was observed at a concentration up to 29 μM. The identification of anibamine together with compound **10** showed promise in development of novel chemokine receptor CCR5 antagonist for therapeutic intervention of ovarian cancer.

Acknowledgments

We are grateful to the funding support from US Army Prostate Cancer Research Program PC073739. The content is solely the responsibility of the authors and does not necessarily represent the official views US Army Prostate Cancer Research Program. We thank NIH AIDS Research and Reference Reagent Program for providing the MOLT-4/CCR5 cell line.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2012.05.127.

References and notes

1. Gynecologic Neoplasms. In *Merck Manual of Diagnosis and Therapy*; Porter, R. S., Kaplan, J. L., Eds.; Pharmaceutical company Merck Co., 2011.
2. Ovarian Cancer, 2011, National Cancer Institute, http://www.cancer.gov/ Mar.25 2011.
3. Ovarian Cancer Symptoms Consensus Statement, 2007, http://web.archive.org/web/20070814110910/http://www.sgo.org/publications/OvarianCancerSymptoms.pdf Mar. 30 2011.
4. Rossi, D.; Zlotnik, A. *Annu. Rev. Immunol.* **2000**, *18*, 217.
5. Baggiolini, M. *J. Intern. Med.* **2001**, *250*, 91.
6. Gerard, C.; Rollins, B. J. *Nat. Immunol.* **2001**, *2*, 108.
7. Proudfoot, A. E. *Nat. Rev. Immunol.* **2002**, *2*, 106.
8. Fernandez, E. J.; Lolis, E. *Annu. Rev. Pharmacol. Toxicol.* **2002**, *42*, 469.
9. Zlotnik, A.; Yoshie, O. *Immunity* **2000**, *12*, 121.
10. Bacon, K.; Baggiolini, M.; Broxmeyer, H.; Horuk, R.; Lindley, I.; Mantovani, A.; Maysushima, K.; Murphy, P.; Nomiyama, H.; Oppenheim, J.; Rot, A.; Schall, T.; Tsang, M.; Thorpe, R.; Van Damme, J.; Wadhwa, M.; Yoshie, O.; Zlotnik, A.; Zoon, K. *J. Interferon Cytokine Res.* **2002**, *22*, 1067.
11. Frederick, M. J.; Clayman, G. L. *Expert Rev. Mol. Med.* **2001**, *3*, 1.
12. Allavena, P.; Marchesi, F.; Mantovani, A. *Curr. Cancer Ther. Rev.* **2005**, *1*, 81.
13. Kakinuma, T.; Hwang, S. T. *J. Leukoc. Biol.* **2006**, *79*, 639.
14. Zlotnik, A. *Int. J. Cancer* **2006**, *119*, 2026.
15. Raman, D.; Baugher, P. J.; Thu, Y. M.; Richmond, A. *Cancer Lett.* **2007**, *256*, 137.
16. Nasu, K.; Matsui, N.; Narahara, H.; Tanaka, Y.; Takai, N.; Miyakawa, I.; Higuchi, Y. M. *Arch. Pathol. Lab. Med.* **1998**, *122*, 836.
17. Melani, C.; Pupa, S. M.; Stoppacciaro, A.; Menard, S.; Colnaghi, M. I.; Parmiani, G.; Colombo, M. P. *Int. J. Cancer* **1995**, *62*, 572.
18. Selvan, R. S.; Butterfield, J. H.; Krangel, M. S. *J. Biol. Chem.* **1994**, *269*, 13893.
19. Phillips, R. J.; Burdick, M. D.; Lutz, M.; Belperio, J. A.; Keane, M. P.; Strieter, R. M. *Am. J. Respir. Crit. Care Med.* **2003**, *167*, 1676.
20. Chen, G. S.; Yu, H. S.; Lan, C. C.; Chow, K. C.; Lin, T. Y.; Kok, L. F. *Br. J. Dermatol.* **2006**, *154*, 910.
21. Luker, K. E.; Luker, G. D. *Cancer Lett.* **2006**, *238*, 30.
22. Vaday, G. G.; Peehl, D. M.; Kadam, P. A.; Lawrence, D. M. *Prostate* **2006**, *66*, 124.
23. Schadendorf, D.; Moller, A.; Algermissen, B.; Worm, M.; Sticherling, M.; Czarnetzki, B. M. *J. Immunol.* **1993**, *151*, 2667.
24. Miyamoto, M.; Shimizu, Y.; Okada, K.; Kashii, Y.; Higuchi, K.; Watanabe, A. *Cancer Immunol. Immunother.* **1998**, *47*, 47.
25. Lu, Y.; Cai, Z.; Galson, D. L. *Prostate* **2006**, *66*, 1311.
26. Palchevskiy, V.; Hashemi, N.; Weigt, S. S.; Xue, Y. Y.; Derhovanessian, A.; Keane, M. P.; Strieter, R. M.; Fishbein, M. C.; Deng, J. C.; Lynch, J. P.; Elashoff, R.; Belperio, J. A. *Fibrogenesis Tissue Repair* **2011**, *4*, 10.
27. Mantovani, A.; Bottazzi, B.; Colotta, F.; Sozzani, S.; Ruco, L. *Immunol. Today* **1992**, *13*, 265.
28. Scotton, C. J.; Wilson, J. L.; Milliken, D.; Stamp, G.; Balkwill, F. R. *Cancer Res.* **2001**, *61*, 4961.
29. Ding, Y.; Shimada, Y.; Maeda, M.; Kawabe, A.; Kaganoi, J.; Komoto, I. *Clin. Cancer Res.* **2003**, *9*, 3406.
30. Schimanski, C. C.; Schwald, S.; Simiantonaki, N.; Jayasinghe, C.; Gonner, U.; Wilsberg, V.; Junginger, T.; Berger, M. R.; Galle, P. R.; Moehler, M. *Clin. Cancer Res.* **2005**, *11*, 1743.
31. Schimanski, C. C.; Bahre, R.; Gockel, I.; Muller, A.; Frerichs, K.; Horner, V. *Br. J. Cancer* **2006**, *95*, 210.
32. Repeke, C. E.; Ferreira, S. B., Jr.; Claudino, M.; Silveira, E. M.; de Assis, G. F.; Avila-Campos, M. J.; Silva, J. S.; Garlet, G. P. *Bone* **2010**, *46*, 1122.
33. Opdenakker, G.; Van Damme, J. *Immunol. Today* **1992**, *13*, 463.
34. Opdenakker, G.; Van Damme, J. *Cytokine* **1992**, *4*, 251.
35. Soria, G.; Ben-Baruch, B. *Cancer Drug Discov. Dev.* **2009**, 109.
36. Milliken, D.; Scotton, C.; Raju, S.; Balkwill, F.; Wilson, J. *Clin. Cancer Res.* **2002**, *8*, 1108.
37. Tsukishiroa, S.; Suzumori, N.; Nishikawa, H.; Arakawa, A.; Suzumoria, K. *Gynecol. Oncol.* **2006**, *102*, 542.
38. Uekusa, Y.; Yu, W. G.; Mukai, T.; Gao, P.; Yamaguchi, N.; Murai, M.; Matsushima, K.; Obika, S.; Imanishi, T.; Higashibata, Y.; Nomura, S.; Kitamura, Y.; Fujiwara, H.; Hamaoka, T. *Cancer Res.* **2002**, *62*, 3751.
39. Levina, V.; Nolen, B. M.; Marrangoni, A. M.; Cheng, P.; Marks, J. R.; Szczepanski, M. J.; Szajnik, M. E.; Gorelik, E.; Lokshin, A. E. *Clin. Cancer Res.* **2009**, *15*, 2647.
40. Jayasuriya, H.; Herath, K. B.; Ondeyka, J. G.; Polishook, J. D.; Bills, G. F.; Dombrowski, A. W.; Springer, M. S.; Siciliano, S.; Malkowitz, L.; Sanchez, M.; Guan, Z.; Tiwari, S.; Stevenson, D. W.; Borris, R. P.; Singh, S. B. *J. Nat. Prod.* **2004**, *67*, 1036.
41. Littman, D. R. *Cell* **1998**, *93*, 677.
42. Chinen, J.; Shearer, W. T. *J. Allergy Clin. Immunol.* **2003**, *110*, 189.
43. Kedzierska, K.; Crowe, S. M.; Turville, S.; Cunningham, A. L. *Rev. Med. Virol.* **2003**, *13*, 39.
44. Svicher, V.; Balestra, E.; Cento, V.; Sarmati, L.; Dori, L.; Vandenbroucke, I.; D'Arrigo, R.; Buonomini, A. R.; Marck, H. V.; Surdo, M.; Saccomandi, P.; Mostmans, W.; Aerssens, J.; Aquaro, S.; Stuyver, L. J.; Andreoni, M.; Ceccherini-Silberstein, F.; Perno, C. F. *Antiviral Res.* **2011**, *90*, 42.
45. Wall Street Journal. 2007, April 21.
46. Verdine, G. L. *Nature* **1996**, *384*, 1.
47. Feher, M.; Schmidit, J. M. *J. Chem. Inf. Comput. Sci.* **2003**, *43*, 218.
48. Zhang, X.; Haney, K. M.; Richardson, A. C.; Wilson, E.; Gewirtz, D. A.; Ware, J. L.; Zehner, Z. E.; Zhang, Y. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 4627.
49. Li, G.; Watson, K.; Buckheit, R. W.; Zhang, Y. *Org. Lett.* **2007**, *9*, 2043.
50. Zhang, F.; Zaidi, S.; Haney, K. M.; Kellogg, G. E.; Zhang, Y. *J. Org. Chem.* **2011**, *76*, 7945.
51. Li, G.; Haney, K. M.; Kellogg, G. E.; Zhang, Y. *J. Chem. Inf. Model.* **2009**, *49*, 120.
52. Thoma, G.; Nuninger, F.; Schaefer, M.; Akyel, K. G.; Albert, R.; Beerli, C.; Bruns, C.; Francotte, E.; Luyten, M.; MacKenzie, D.; Oberer, L.; Streiff, M. B.; Wagner, T.; Walter, H.; Weckbecker, G.; Zerwes, H. G. *J. Med. Chem.* **1939**, *2004*, 47.
53. Kaighn, M. E.; Narayan, S.; Ohnuki, Y.; Lechner, J. F.; Jones, L. W. *Invest. Urol.* **1979**, *17*, 16.
54. Erlanson, D. A.; McDowell, R. S.; O'Brien, Y. *J. Med. Chem.* **2004**, *47*, 3463.
55. Glennon, R. A. *Mini Rev. Med. Chem.* **2005**, *5*, 927.
56. Ghosh, S.; Elder, A.; Guo, J.; Mani, U.; Patane, M.; Carson, K.; Ye, Q.; Bennett, R.; Chi, S.; Jenkins, T.; Guan, B.; Kolbeck, R.; Smith, S.; Zhang, C.; LaRosa, G.; Jaffee, B.; Yang, H.; Eddy, P.; Lu, C.; Uttamsingh, V.; Horlick, R.; Harriman, G.; Flynn, D. *J. Med. Chem.* **2006**, *49*, 2669.
57. Parker, K. A.; Wang, P. *Org. Lett.* **2007**, *9*, 4793.