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The natural product CCR5 antagonist anibamine and its analogs as anti-prostate cancer agents

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ABSTRACT

Prostate cancer is a leading cause of death among males in the United States. As the chemokine receptor CCR5 is over-expressed in more aggressive forms of prostate cancer, and is also a critical receptor in inflammation, chemokine receptor CCR5 antagonists could potentially act as anti-prostate cancer agents. Anibamine, a natural product CCR5 antagonist, provides a unique molecular scaffold for the generation of novel analogs with possible anti-prostate cancer activity. A series of analogs of anibamine were designed, synthesized and tested against several prostate cancer cell lines. The analogs all acted as CCR5 antagonists at micromolar range affinity to the receptor while their anti-proliferative activity varied depending on the cell line type and their chemical structural properties. Further basal cytotoxicity characterization on these compounds indicated some of them may be suitable for in vivo studies.

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The CC chemokine receptor CCR5 belongs to the G-protein-coupled seven-transmembrane receptor superfamily, and its endogenous ligands include the chemokines CCL3 (MIP-1α), CCL4 (MIP-1β), and CCL5 (RANTES).[1,2] CCR5 has been identified as an essential co-receptor for HIV virus entry to host cells[3–6] and has therefore become an attractive target for anti-HIV therapeutics development.[7–11] A number of small molecule CCR5 antagonists identified through high-throughput screening efforts have shown potent activities in blocking chemokine function and HIV entry, such as Maraviroc,[12] TAK-779,[13] and Vicriviroc[14] (Fig. 1).

Prostate cancer is a leading cause of death in American males and there is no cure for this disease once it becomes androgen-independent and/or metastatic.[15] Historically, chronic inflammation has been believed to be at the root of several cancers, including prostate cancer[16] and increased expression of the members of the chemokine network (an important component in inflammation response) by tumor cells has been found to correlate with the progression of many types of cancers.[17–20] For example, the chemokine CCL5 was shown to induce breast cancer cell migration, mediated by the chemokine receptor CCR5. It was then shown that the expression of CCR5 and CCL5 correlated with breast cancer disease progression while a CCR5 antagonist inhibited breast tumor growth in the presence of CCL5.[21,22] Because of the possible link between prostate cancer and chronic inflammation, several genes and proteins of the inflammatory network were evaluated in prostatic tissues of various disease states.[23] It was found that in some cases prostate cancer tissues express CCL5 and CCR5 mRNA.[23] This discovery prompted the investigation of the effect of a CCR5 antagonist, TAK-779, which significantly reduced the growth and invasiveness of prostate cancer cells in the presence of CCL5.[24] Therefore, development of an appropriate chemokine receptor CCR5 antagonist may provide a novel prostate cancer therapy.

Anibamine, a unique pyridine quaternary alkaloid recently isolated from *Aniba panurensis,* has been found to effectively bind to the chemokine receptor CCR5 with an IC_{50} at 1 μM in competition with ^{125}I–gp120, a HIV viral envelop protein that has high binding affinity to CCR5.[25] 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). Our recent studies[26] demonstrated that anibamine produced significant inhibition of prostate cancer cell proliferation at micromolar to submicromolar concentrations as well as suppressing adhesion and invasion of the highly metastatic M12 prostate cancer cell line. Preliminary in vivo studies indicated that anibamine also inhibits prostate tumor growth in mice.[26] These findings indicate that anibamine may serve as a new lead compound for the development of prostate cancer therapeutic agents.

Compared with other known CCR5 antagonists, anibamine's moderate binding affinity to CCR5 is likely due to its long aliphatic side chains. It is believed that rational structural modification of

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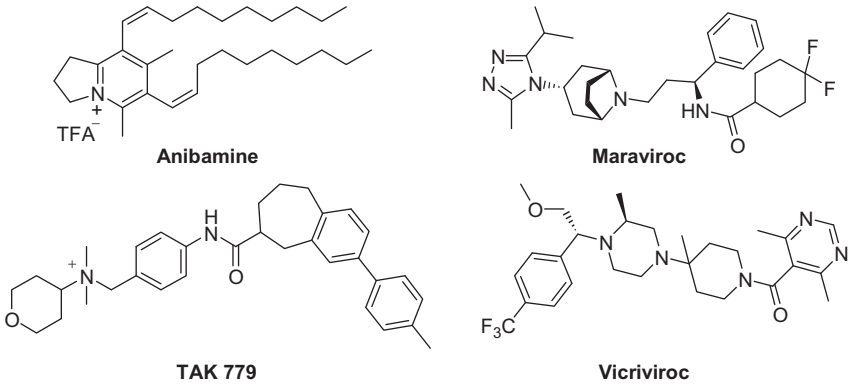
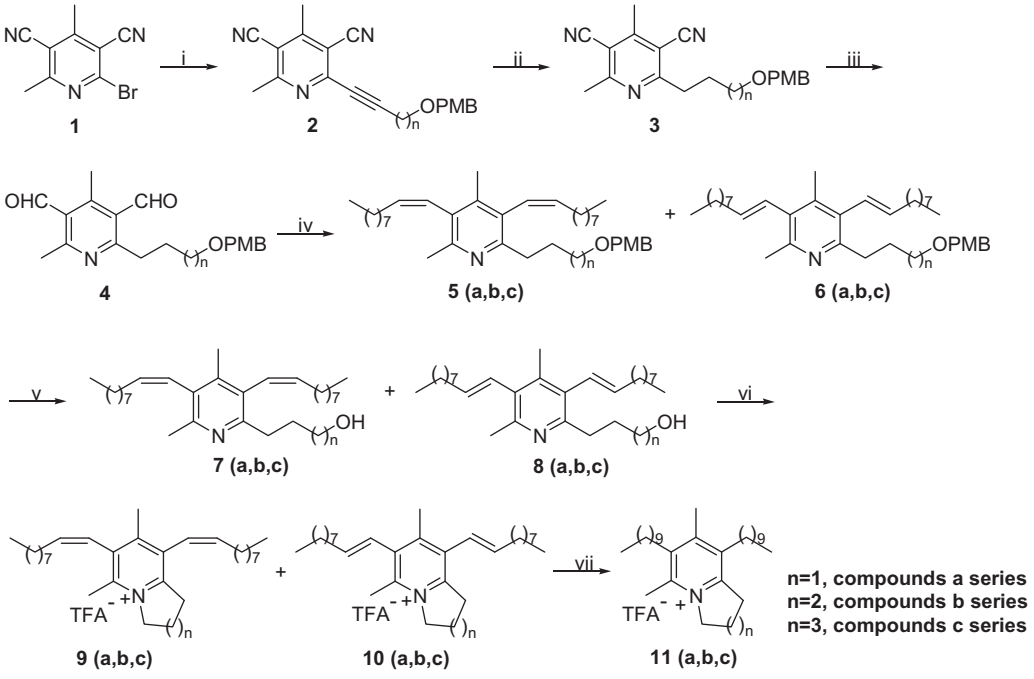


Figure 1. Anibamine and some known CCR5 antagonists.

this natural product based on its novel structural skeleton could lead to the development of new lead compounds that would act as CCR5 antagonists and also express some unique pharmacological profiles that would be useful for anti-cancer activity. Recently, the total synthesis of anibamine was reported by our group.[27] Modification of this synthetic pathway provides opportunities for generating anibamine analogs whose structure–activity relationship could be investigated as CCR5 antagonists. Here we report the initial structural modification of anibamine and the biological screening of these analogs against prostate tumor cell lines.

Primary structural modifications of anibamine were focused on the size of the fused ring embodied pyridine quaternary ion and the configuration of the double bond in each side chain, two major structure features of the parent natural product. The central fused ring system of anibamine largely determines the binding mode of the whole molecule to the receptor[28] as the central positively charged nitrogen atom is believed to be essential for the binding of anibamine to CCR5. Altering the size of the fused ring might influence the binding affinity of the whole molecule to the receptor and the anti-prostate cancer activity of the products. Isomerization and saturation of the double bond were carried out to test the influence on the anti-prostate cancer activity since, that is, another important structural feature of anibamine. The ring-size-modified anibamine analogs were prepared following previously reported procedures (Scheme 1).[27] The palladium-catalyzed Sonogashira reaction between the bromide **1** and the appropriate PMB protected alcohol afforded the intermediate **2**. The hydrogenation of compound **2** using palladium on carbon as a catalyst at room temperature gave the side chain saturated intermediate **3** in quantitative yield. The aldehyde **4** was prepared by DIBAL-H reduction of **3** in toluene. Adopting LHMDS as the base, the Wittig reaction products **5** and **6** were obtained as major products. Without further separation of the isomers, the PMB group of compounds **5** and **6** was removed under acidic conditions to give compounds **7** and **8**, respectively. The ring-closure reaction was achieved by treating compounds **7** and **8** with methanesulfonylchloride and triethylamine at room temperature except for the seven-membered ring derivatives which required heating up to 50 °C. The crude products **9** and **10** were purified by preparative HPLC using the previously reported condition.[27] The saturated products **11** were obtained by catalytic hydrogenation at room temperature. All the final compounds were obtained with reasonable yields and characterized



Scheme 1. Reagents: (i) $HC{\equiv}C(CH_2)_nOPMB$ (n = 1, 2, and 3), CuI, $PdCl_2(PPh_3)_2$, TEA; (ii) Pd/C; (iii) DIBAL-H, toluene; (iv) $CH_3(CH_2)_7CH{=}PPh_3$, LHMDS; (v) 1 N HCl, EtOH; (vi) (a) MsCl, TEA; (b) preparative HPLC; (vii) Pd/C.

with NMR, IR, MS, and HPLC (see Supplementary data). It should be noted that two other minor products (isomers with double bond configuration as (11*E*, 22*Z*) and (11*Z*, 22*E*), respectively) were obtained as a mixture. The stereo-selective syntheses of these two isomers have been pursued and will be reported in due course.

Inhibitory concentrations of CCR5 antagonists determined by inhibition of chemokine induced calcium ion mobilization has been demonstrated to correlate well with radioligand binding inhibitory concentrations.[29] Anibamine and its eight analogs along with the established CCR5 antagonist TAK-779 (as a control), were evaluated for their ability to inhibit CCL5 stimulated calcium mobilization in MOLT-4 cells. This CCR5-expressing T-lymphoblastoid cell line[30] seemed to be the most sensitive one in our calcium mobilization assay among all the cell lines we explored. The results are shown in Table 1. The fact that the IC_{50} values of TAK-779 (7.9 ± 2.5 nM) and anibamine (**9a**, 5.43 ± 0.91 μM) correlated with their CCL5 inhibitory binding affinities reported in literature (TAK-779, 1.4 nM,[13b] and anibamine, 1.0 μM[25]) provides a validation of this experimental approach. For five- and six-membered ring analogs of anibamine (**9b**, **9c**, **10a**, **10b**, and **10c**), their side chain double bond configuration did not appear to influence binding affinity as significantly as for the seven-membered ring analogs (**11a**, **11b**, and **11c**). In addition, it appeared that a seven-membered ring was unfavorable for binding affinity. All of the analogs were also tested for their capacity to stimulate calcium release in the absence of CCL5, but none acted as agonists (data not shown). Not surprisingly, most of the new ligands did not show much improved receptor affinity in comparison to the parent compound anibamine, likely due to the fact that at this stage the structural modifications did not significantly alter the shape and hydrophobicity of the molecule, and therefore no observable change to the binding mode to the receptor either.

As indicated previously, the expression of CCL5 and CCR5 has been observed in various prostate cancer cell lines, including M12, DU145 and PC-3.[26,29,31] Therefore, the anti-proliferative activity of anibamine and its analogs was evaluated against these prostate cancer cell lines. The first CCR5 antagonist, TAK-779, has been reported to inhibit proliferation and invasion of cancer cells pre-treated with CCL5 (10 ng/mL).[24] Under such CCL5 stimulation condition, TAK-779, at 500 nM, showed significant proliferation inhibition effect (40–50%) for DU145 and LNCaP cell lines. A modified protocol without the CCL5 stimulation condition was adopted here for anibamine and its analogs in an effort to avoid the potential non-specific stimulation effect of CCL5 interacting with other chemokine receptors expressed in the cancer cells that it can bind to[20] and to better simulate a clinically applicable scenario for drug development purpose. Results of these assays were summarized in Table 2. Each compound showed dose-dependent anti-proliferative activity in all three cell lines (data not shown). Across the board, for a given cell line, the analogs showed similar anti-proliferation activity as indicated by their ED_{50} values, which is at least partially in correlation to their comparable binding affinity to the receptor. In general, the PC-3 cells appeared to be more sensitive to either the M-12 or the DU-145 cells. This could be related to the different receptor expression levels for each prostate cancer cell line.[23,24,26] Among all the analogs, compound **10b** appeared to be the most potent agent against all three cancer cell lines. For example, it showed about 10 times improvement in ED_{50} over the original lead (anibamine) against M12 and PC-3 prostate cancer cell lines. Therefore this compound was defined as the new lead.

Interestingly, in our assay TAK-779 showed lower anti-proliferative activity (ED_{50} values, M-12, 20.40 ± 1.10 μM; DU-145, 57.27 ± 9.47 μM; and PC-3, 37.85 ± 0.99 μM, respectively) than reported by others.[24] This may be because the current studies were performed in the absence of CCL5 stimulation. TAK-779 entered clinical trial based on its anti-HIV activity as a result of optimization of a series of compounds by following their binding affinity to the CCR5 receptor as well as their anti-HIV activities.[13] Its mechanism of action against prostate cancer cell is not clear and the involvement of the CCL5/CCR5 axis in prostate cancer development is not yet defined. Studies to optimize lead compounds based on their binding affinity to CCR5 as well as their anti-cancer activities may help elucidate the role of CCL5/CCR5 pathway in prostate cancer progression. In this context, the fact that anibamine and its analogs showed somewhat higher potency than TAK-779 in the anti-proliferation assays suggests the possibility that these new ligands may also be affecting other target sites. Because of the structure similarity of these ligands and their limited 'drug-like' property based on Lipinski's Rule of 5,[32] more extensive structural modification would be needed to further understand their structure–activity relationship.

Table 1
Inhibitory effects on CCL5 induced Ca^{2+} mobilization in MOLT-4/CCR5 cell

Compounds	IC_{50} ± SE[a] (μM)
9a (Anibamine)	5.43 ± 0.91
10a	6.53 ± 1.79
11a	7.80 ± 1.38
9b	10.01 ± 0.38
10b	4.60 ± 1.60
11b	15.24 ± 7.87
9c	8.40 ± 0.94
10c	48.09 ± 21.72
11c	37.61 ± 5.40

[a] Values shown were mean ± S.E. mean from at least three separate experiments performed in triplicate. The IC_{50} values (concentration to inhibit 50% of calcium release induced by CCL5 compared with the control) were calculated using Prism.

Table 2
Inhibition of proliferation of prostate cancer cell lines

Compounds	ED_{50} ± SE[a] (μM)		
	M12	DU-145	PC-3
9a (Anibamine)	3.26 ± 0.35	1.02 ± 0.17	1.13 ± 0.01
10a	2.18 ± 0.34	0.84 ± 0.02	0.64 ± 0.06
11a	1.92 ± 0.44	1.31 ± 0.15	0.64 ± 0.08
9b	3.24 ± 0.46	0.36 ± 0.10	0.28 ± 0.07
10b	0.43 ± 0.13	0.46 ± 0.07	0.19 ± 0.04
11b	1.93 ± 0.47	0.97 ± 0.01	0.55 ± 0.19
9c	2.57 ± 0.07	0.76 ± 0.12	0.31 ± 0.05
10c	4.20 ± 2.99	0.60 ± 0.15	0.24 ± 0.08
11c	2.67 ± 0.03	1.41 ± 0.18	0.43 ± 0.19

[a] Values shown were mean ± S.E. mean from at least three separate experiments performed in triplicate. The ED_{50} (concentration to inhibit 50% of cell proliferation compared with the control) values were calculated using Prism.

Table 3
Cytotoxicity results of anibamine and its analogs

Compounds	HC_{50} ± SE[a] (μM) Sheep red blood cells	TC_{50} ± SE[a] (μM) NR/3T3	TC_{50} ± SE[a] (μM) WST-1/3T3
9a (Anibamine)	>100	22.65 ± 7.38	23.47 ± 2.36
10a	>100	2.52 ± 0.75	4.35 ± 0.57
11a	77.1 ± 6.7	2.79 ± 1.11	5.08 ± 2.40
9b	>100	19.00 ± 1.65	29.08 ± 0.60
10b	58.1 ± 12.5	6.81 ± 1.77	17.53 ± 4.56
11b	96.2 ± 6.5	1.98 ± 0.47	3.76 ± 0.47
9c	63.0 ± 11.4	7.66 ± 0.91	10.09 ± 1.21
10c	>100	0.83 ± 0.35	4.76 ± 0.58
11c	>100	1.86 ± 0.18	11.66 ± 1.48

[a] Values shown were mean ± S.E. mean from at least three separate experiments performed in triplicate. The HC_{50} (concentration to induce 50% of hemolysis compared with the control) values and TC_{50} (concentration to induce 50% of cell death compared with the control) values were calculated using Prism.

Table 4
Therapeutic index (TI) for **9a** (anibamine), **9b**, and **10b**

Compound	3T3 versus M12		3T3 versus DU145		3T3 versus PC3	
	NR	WST-1	NR	WST-1	NR	WST-1
9a (Anibamine)	6.95	7.20	22.21	23.01	20.04	20.77
9b	5.86	8.98	52.78	80.78	67.86	103.86
10b	15.84	40.77	14.80	38.11	35.84	92.26

To further verify whether the inhibitory effect of our lead compound, anibamine, against prostate cancer cell line proliferation was due to its inhibition of CCL5 binding on the chemokine receptor CCR5, we retested its anti-proliferation activity against the CCL5 (30 nM) treated M12 cell line. Compound **9**, anibamine, showed higher potency under this condition, as indicated by its ED_{50} value at 0.84 ± 0.08 μM. Such result at least partially supported our hypothesis that our lead compound may inhibit the proliferation of prostate cancer cell M12 through its inhibition of CCL5/CCR5 axial function.

Due to the reported hemolysis by anibamine,[32] all the compounds were further evaluated for their hemolytic toxicity at concentrations up to 100 μM. The results are summarized in Table 3. None of the analogs exhibited significant hemolytic activity under 50 μM, and the HC_{50} value was generally from one to two orders of magnitude higher than their IC_{50} concentrations for the prostate cancer cell lines.

To further characterize their basal cytotoxicity and to assess whether their anti-proliferative activity might be associated with non-selective cytotoxicity, anibamine and its analogs were tested by using a Neutral Red and WST-1 protocol in NIH3T3 cell.[33] The data presented in Table 3 indicate that most of these compound required significantly higher concentrations to demonstrate cytotoxicity against 3T3 cells than the prostate cancer cell lines. The therapeutic index values for compound **9b** and **10b** (Table 4) validated their candidacy for our future animal model study.

In summary, as the CCL5/CCR5 axis seems to be important in prostate cancer progression, chemokine receptor CCR5 antagonists are likely to have anti-prostate cancer activity. The natural product CCR5 antagonist anibamine represents a novel structural skeleton and has been applied as a lead to design novel CCR5 antagonists as anti-prostate cancer agents. A series of anibamine analogs were designed and synthesized based on this hypothesis. In the MOLT-4/CCR5 Ca^{2+} mobilization assay against the endogenous CCR5 agonist CCL5, these compounds seemed to act consistently as CCR5 antagonists. In anti-proliferative activity screening against prostate cancer cell lines, all the analogs were active against three different cancer cell lines with compound **10b** demonstrating the most promising activities. Studies of selectivity using red blood cells and NIH3T3 cells indicated that significantly higher concentrations of the analogs were required before toxicity was evident in these normal cells models. A more comprehensive modification of anibamine's structural skeleton is ongoing in order to characterize its structure–activity relationship thoroughly with the goal of developing more potent anti-prostate cancer agents. Such efforts may also facilitate the clarification of the role of CCL5/CCR5 axis in prostate cancer progression.

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Supplementary data

Supplementary data (chemical synthesis procedures and compounds analysis data) associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2011.07.058.

References and notes

1. Opperman, M. *Cell Signal.* **2004**, *16*, 1201.
2. Fernandez, E. J.; Lolis, E. *Annu. Rev. Pharmacol. Toxicol.* **2002**, *42*, 469.
3. Samson, M.; Libert, F.; Doranz, B. J.; Rucker, J.; Liesnard, C.; Farber, C. M.; Saragosti, S.; Lapouméroulie, C.; Cognaux, J.; Forceille, C.; Muyldermans, G.; Verhofstede, C.; Burtonboy, G.; Georges, M.; Imai, T.; Rana, S.; Yi, Y.; Smyth, R. J.; Collman, R. J.; Doms, R. W.; Vassart, G.; Parmentier, M. *Nature* **1996**, *382*, 722.
4. Littman, D. R. *Cell* **1998**, *93*, 677.
5. Chinen, J.; Shearer, W. T. *J. Allergy Clin. Immunol.* **2002**, *110*, 189.
6. Kedzierska, K.; Crowe, S. M.; Turville, S.; Cunningham, A. L. *Rev. Med. Virol.* **2003**, *13*, 39.
7. Howard, O. M.; Oppenheim, J. J.; Wang, J. M. *J. Clin. Immunol.* **1999**, *19*, 280.
8. Reeves, J. D.; Piefer, A. J. *Drugs* **2005**, *65*, 1747.
9. Este, J. A. *Curr. Med. Chem.* **2003**, *10*, 1617.
10. Schwarz, M. K.; Wells, T. N. C. *Nat. Rev. Drug Disc.* **2002**, *1*, 347.
11. Lusso, P. *EMBO J.* **2006**, *25*, 447.
12. (a) Dorr, P.; Westby, M.; Dobbs, S.; Griffin, P.; Irvine, B.; Macartney, M.; Mori, J.; Rickett, G.; Smith-Burchnell, C.; Napier, C.; Webster, R.; Armour, D.; Price, D.; Stammen, B.; Wood, A.; Perros, M. *Antimicrob. Agents Chemother.* **2005**, *49*, 4721; (b) Wood, A.; Armour, D. *Prog. Med. Chem.* **2005**, *43*, 239.
13. (a) Baba, M.; Nishimura, O.; Kanzaki, N.; Okamoto, M.; Sawada, H.; Iizawa, Y.; Shiraishi, M.; Aramaki, Y.; Okonogi, K.; Ogawa, Y.; Meguro, K.; Fujino, M. *Proc. Natl. Acad. Sci. U.S.A.* **1999**, *96*, 5698; (b) Maeda, K.; Nakata, H.; Koh, Y.; Miyakawa, T.; Ogata, H.; Takaoka, Y.; Shibayama, S.; Sagawa, K.; Fukushima, D.; Moravek, J.; Koyanagi, Y.; Mitsuya, H. *J. Virol.* **2004**, *78*, 8654.
14. (a) Tagat, J. R.; McCombie, S. W.; Nazareno, D.; Labroli, M. A.; Xiao, Y.; Steensma, R. W.; Strizki, J. M.; Baroudy, B. M.; Cox, K.; Lachowicz, J.; Varty, G.; Watkins, R. *J. Med. Chem.* **2004**, 2405; (b) Strizki, J. M.; Tremblay, C.; Xu, S.; Wojcik, L.; Wagner, N.; Gonsiorek, W.; Hipkin, R. W.; Chou, C. C.; Pugliese-Sivo, C.; Xiao, Y.; Tagat, J. R.; Cox, K.; Priestley, T.; Sorota, S.; Huang, W.; Hirsch, M.; Reyes, G. R.; Baroudy, B. M. *Antimicrob. Agents Chemother.* **2005**, *49*, 4911; (c) Palani, A.; Tagat, J. R. *J. Med. Chem.* **2006**, *49*, 2851.
15. (a) *US Cancer Statistics*: 2002 Incidence and Mortality, the most comprehensive federal report available on state-specific cancer rates. The Department of Health and Human Services, November 2005.; (b) Miller, B. A.; Ries, L. A. G.; Hankey, B. F. Eds.; 1993, Seer Cancer Statistics Review, NIH Publ., No. 93-2789, 1973.; (c) http://www.cancer.gov/cancertopics/treatment/prostate.
16. (a) Balkwill, F.; Mantavani, A. *Lancet* **2001**, *357*, 539; (b) Nelson, W. G.; DeMarzo, A.; Isaacs, W. B. *N. Engl. J. Med.* **2003**, *349*, 366.
17. Mantovani, A.; Bottazzi, B.; Colotta, F.; Sozzani, S.; Ruco, L. *Immunol. Today* **1992**, *13*, 265.
18. Opdenakker, G.; Van Damme, J. *Immunol. Today* **1992**, *13*, 463.
19. Opdenakker, G.; Van Damme, J. *Cytokine* **1992**, *4*, 251.
20. Frederick, M. J.; Gary, L. *Rev. Mol. Med.* **2001**, 1.
21. Robinson, S. C.; Scott, K. A.; Wilson, J. L.; Thompson, R. G.; Proudfoot, A. E. I.; Balkwill, F. *Cancer Res.* **2003**, *63*, 8360.
22. Coussens, L. M.; Werb, Z. *Nature* **2002**, *420*, 860.
23. Koenig, J. E.; Senge, T.; Allhoff, E. p.; Koenig, W. *Prostate* **2004**, *58*, 121.
24. Vaday, G. G.; Peehl, D. M.; Kadam, P. A.; Lawrence, D. M. *Prostate* **2006**, *66*, 124.
25. 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. Q.; Tiwari, S.; Stevenson, D. W.; Borris, R. P.; Singh, S. B. *J. Nat. Prod.* **2004**, *67*, 1036.
26. 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.
27. Li, G.; Watson, K.; Buckheit, R. W.; Zhang, Y. *Org. Lett.* **2007**, *9*, 2043.
28. Li, G.; Haney, K. M.; Kellogg, G. E.; Zhang, Y. *J. Chem. Inf. Model.* **2009**, *49*, 120.
29. (a) 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.* **2004**, *47*, 1939; (b) Kaighn, M. E.; Narayan, S.; Ohnuki, Y.; Lechner, J. F.; Jones, L. W. *Invest. Urol.* **1979**, *17*, 16.
30. Baba, M.; Miyake, H.; Okamoto, M.; Iizawa, Y.; Okonogi, K. *AIDS Res. Hum. Retroviruses* **2000**, *16*, 935.
31. Webber, M. M.; Bello, D.; Quadar, S. *Prostate* **1997**, *30*, 58.
32. Klausmeyer, P.; Chmurny, G. N.; McCloud, T. G.; Tucker, K. D.; Shoemaker, R. H. *J. Nat. Prod.* **2004**, *67*, 1732.
33. (a) Borenfreund, E.; Puerner, J. *Toxicol. Lett.* **1985**, *24*, 119; (b) Triglia, D. *In Vitro Cell. Dev. Biol.* **1991**, *27A*, 239; (c) Test method protocol for the BALB/c 3T3 Neutral Red Uptake Cytotoxicity Test, a test for basal cytotoxicity for an in vitro validation study, Phase III, November 4, 2003. Prepared by The National Toxicology Program (NTP) Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM).