Available online at www.sciencedirect.com
SCIENCE DIRECT
Bioorganic & Medicinal Chemistry Letters 16 (2006) 1905–1908
Bioorganic & Medicinal Chemistry Letters

Synthesis of miltirone analogues as inhibitors of Cdc25 phosphatases

Weigang Huang,[b] Jingya Li,[b] Wei Zhang,[b] Yueyang Zhou,[b] Chuanming Xie,[b] Yu Luo,[b] Yunfei Li,[b] Jingli Wang,[a] Jia Li[b,*] and Wei Lu[a,*]

[a]*Department of Chemistry, East China Normal University, Shanghai 200062, China*
[b]*The National Center for Drugs Screening, Shanghai Institute of Materia Medica, SIBS, Graduate School of Chinese Academy of Sciences, Chinese Academy of Sciences, Shanghai 201203, China*

Received 31 October 2005; revised 3 December 2005; accepted 24 December 2005
Available online 24 January 2006

Abstract—Miltirone analogues were synthesized and evaluated for inhibitory activity against Cdc25 and PTP1B. Most of the compounds demonstrated potent Cdc25 inhibitory activity, and several exhibited higher selectivity for Cdc25 than for PTP1B. In a cytotoxic assay, most of the compounds displayed cytotoxicity against the tumor cell lines A549 and HCT-116, producing IC_{50} values in the micromolar range.

The loss of cell cycle control leading to deregulated cell proliferation is one of the hallmarks of cancer. The Cdc25 phosphatases function as key regulators of the cell cycle during normal eukaryotic cell division and as mediators of the checkpoint response in cells with DNA damage.[1] Three Cdc25 homologues exist in humans: Cdc25A, Cdc25B, and Cdc25C.[1–4] Overexpression of Cdc25A and B occurs in various forms of cancer and is strongly associated with tumor aggressiveness and poor prognosis,[5–8] making Cdc25 an attractive drug target for cancer therapy. Over the last few years, some small molecule Cdc25 inhibitors have been described.[9–34]

In our program of extensive screening for inhibitors of Cdc25, we have found that some tanshinones demonstrate inhibitory activity for Cdc25[35] and cytotoxicity against a number of cultured human tumor cell lines.[36] We chose miltirone, one of these tanshinones,[37] as the pharmacophore from which we synthesized 19 analogues and evaluated their antitumor activity as Cdc25 phosphatase inhibitors.

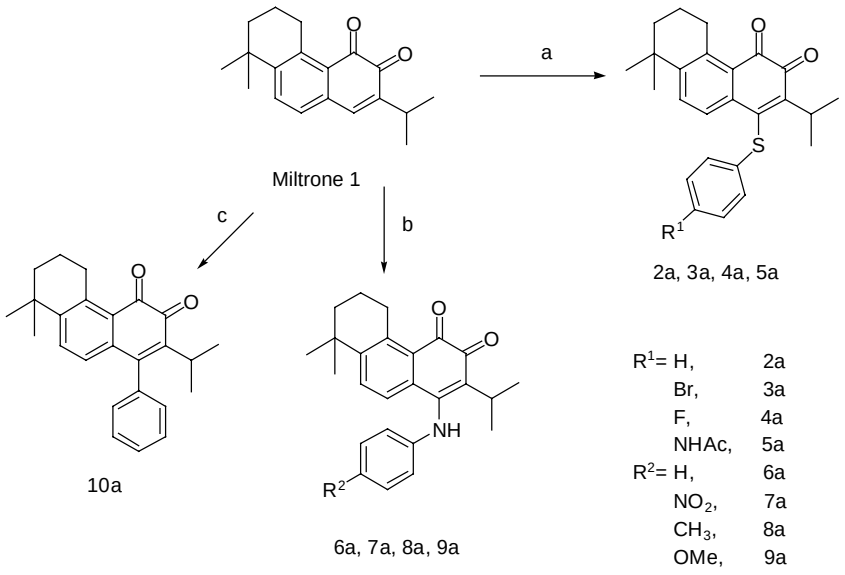
As shown in Scheme 1, the N-substituted series analogues, **6a**–**9a** were prepared by addition of the corresponding aniline to the solution of miltirone in methanol. Treatment of miltirone with the corresponding thiophenol in $CHCl_3$/MeOH produced sulfides **2a**–**5a** in 50–80% yield. Compound **10a** was obtained by the reaction of miltirone with benzene in acetic acid, with $Pd(OAc)_2$ as the catalyst under reflux conditions.

In our previous study, some tanshinones without the A ring displayed more potent antitumor activities.[35] Here, we prepared a series of analogues without the A ring. We started the synthesis of the important intermediate **16**, also a miltirone analogue, from compound **11** (Scheme 2), which we prepared as reported previously.[38] Reduction of **11** with $NaBH_4$ produced the alcohol **12**, which was directly treated with 20% H_2SO_4 to give **13**, a colorless oil, in 77% yield (from **11**). Dehydrogenation of **13** with DDQ in toluene afforded 2-isopropyl-3-methoxy naphthalene **14** in 100% yield. Treatment of **14** with boron tribromide and oxidation with Dess–Martin periodinane gave **16**, a red solid. Compounds **2b**–**10b** were prepared according to the procedure described above for compounds **2a**–**10a**.

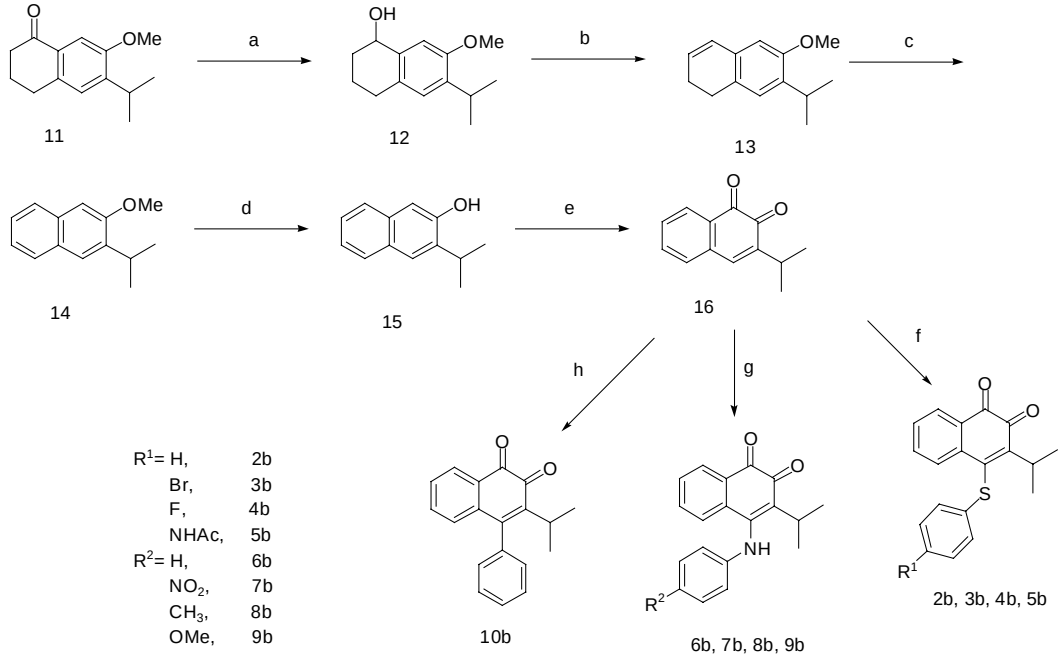
Miltirone and its analogues were evaluated for their antitumor activities[39,40] and the results are summarized in Table 1. Miltirone demonstrated no activity for Cdc25A and Cdc25B. All of its analogues, except **7a**, were potent inhibitors of Cdc25 phosphatases in the micromolar range. We speculated that substitution of the 1-position of miltirone or the lack of the A ring

Keywords: Miltirone; Cdc25 phosphatases; Cancer.
* Corresponding authors. Tel./fax: +86 21 62602475 (W.L.); fax: +86 21 50801552 (J.L.); e-mail addresses: jli@mail.shcnc.ac.cn; wlu@chem.ecnu.edu.cn

0960-894X/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2005.12.080



Scheme 1. Reagents: (a) different thiophenol, MeOH/$CHCl_3$, 50–80%; (b) different aniline, MeOH, 50–60%; (c) benzene, $Pd(OAc)_2$, HOAc, 35%.



Scheme 2. Reagents: (a) $NaBH_4$, MeOH; (b) aq H_2SO_4, reflux, 76.77% from **11**; (c) DDQ, toluene; (d) BBr_3, CH_2Cl_2; (e) Dess–Martin periodinane, CH_2Cl_2, 42% two steps from **14**; (f) different thiophenol, MeOH/$CHCl_3$, 50–80%; (g) different aniline, MeOH, 50–60%; (h) benzene, $Pd(OAc)_2$, HOAc, 75%.

increases the inhibitory activity for Cdc25. However, contrary to our expectation, compounds **2b**–**10b**, which lack the A ring, did not display more potent Cdc25 inhibitory activity than **2a**–**10a**, suggesting that the A ring is not necessary for the Cdc25 inhibitory activity. To investigate the relationship between the electronic effect on the aromatic ring and antitumor activities, we introduced different substituents. However, we observed no obvious relationship. To study the specificity for Cdc25, we also tested the inhibition of PTP1B, another protein-tyrosine phosphatase. Compounds **16**, **6a**, **6b**, **7b**, **8a**, **8b**, **9a**, **9b,** and **10b**, which inhibit Cdc25 phosphatases in the micromolar range, demonstrated no activity for PTP1B, indicating the high selectivity of these compounds. We note that the rest of these compounds inhibited PTP1B, producing IC_{50} values of 4.11–13.66 μM.

We evaluated the cytotoxicity of all compounds using the A549 and HCT-116 cell lines. Miltirone was cytotoxic against the A549 and HCT-116 cell lines, producing IC_{50} values of 24.59 ± 0.42 and 12.39 ± 0.75 μM, respectively, which are consistent with previous results.[36] Compounds **4b** and **10b** displayed similar or inferior cytotoxicity to that produced by miltirone. The rest of the compounds displayed more potent cytotoxicity. Our findings suggest that the A ring is also unnecessary for their cytotoxicity.

Table 1. Inhibitory activity for Cdc25A, Cdc25B, and PTP1B phosphotases and cytotoxicity against A549 and HCT-116 cell lines

Compounds	Cdc25A $IC_{50} \pm SD$[a] (μM)	Cdc25B $IC_{50} \pm SD$ (μM)	PTP1B $IC_{50} \pm SD$ (μM)	A549 $IC_{50} \pm SD$ (μM)	HCT-116 $IC_{50} \pm SD$ (μM)
Miltirone 5	NA[b]	NA	NA	24.59 ± 0.42	12.39 ± 0.75
16	11.18 ± 1.38	24.10 ± 1.37	NA	4.14 ± 0.18	4.00 ± 0.03
2a	3.97 ± 0.63	5.07 ± 0.84	10.40 ± 2.086	26.64 ± 5.34	5.75 ± 0.03
2b	5.86 ± 1.79	10.86 ± 1.95	13.66 ± 0.50	7.59 ± 0.11	8.03 ± 0.07
3a	20.31 ± 6.41	3.78 ± 1.38	7.19 ± 0.75	5.07 ± 0.04	4.94 ± 0.07
3b	5.43 ± 1.11	3.63 ± 0.70	7.57 ± 1.35	5.93 ± 0.18	5.85 ± 0.15
4a	22.06 ± 9.03	2.58 ± 0.66	4.55 ± 0.14	1.25 ± 0.54	5.71 ± 0.13
4b	12.75 ± 1.32	7.55 ± 2.14	7.22 ± 1.13	20.42 ± 0.97	20.78 ± 0.55
5a	5.96 ± 0.73	4.85 ± 1.88	9.05 ± 1.01	15.43 ± 0.87	5.18 ± 0.04
5b	21.87 ± 0.28	13.82 ± 1.27	7.63 ± 0.54	7.36 ± 0.86	6.32 ± 0.08
6a	8.81 ± 0.38	3.99 ± 0.08	27.16 ± 4.67	19.06 ± 0.51	6.25 ± 0.01
6b	10.88 ± 0.96	8.71 ± 0.79	NA	25.01 ± 0.56	8.22 ± 0.15
7a	NA	NA	NA	5.34 ± 0.05	7.10 ± 1.24
7b	6.87 ± 1.11	5.33 ± 0.33	19.08 ± 5.33	6.59 ± 0.28	2.39 ± 0.03
8a	27.58 ± 6.12	7.54 ± 2.43	NA	4.25 ± 2.45	24.94 ± 1.10
8b	7.06 ± 0.29	4.53 ± 0.24	19.59 ± 3.45	8.20 ± 3.19	6.16 ± 2.03
9a	11.82 ± 1.69	4.09 ± 0.59	NA	9.19 ± 4.53	5.51 ± 0.09
9b	4.90 ± 0.23	4.63 ± 0.13	NA	87.01 ± 20.80	4.04 ± 1.23
10a	3.96 ± 0.63	3.16 ± 0.22	4.11 ± 0.40	6.24 ± 0.17	6.28 ± 0.12
10b	7.83 ± 0.59	3.23 ± 0.31	NA	24.71 ± 0.46	29.60 ± 4.36

[a] The IC_{50} values are means of three distinct experiments.
[b] NA: not active, $IC_{50} > 20$ μg/mL.

In conclusion, we synthesized analogues of miltirone and evaluated their antitumor activities as Cdc25 inhibitors. As expected for Cdc25 inhibitors, most of these compounds were potent Cdc25 inhibitors and exhibited cytotoxic activity against A549 and HCT-116 cell lines in the micromolar range. Some compounds exhibited higher selectivity for Cdc25 than for PTP1B. We conclude that these miltirone analogues might be attractive lead molecules for developing drugs against the Cdc25 phosphatase.

Acknowledgment

Financial supports from the National Natural Science Foundation of China (30572232) and Shanghai Science and Technology Mission (054319902) are gratefully acknowledged.

References and notes

1. Nilsson, I.; Hoffmann, I. *Prog. Cell Cycle Res.* **2000**, *4*, 107.
2. Galaktionov, K.; Beach, D. *Cell* **1991**, *67*, 1181.
3. Sadhu, K.; Reed, S. I.; Richardson, H.; Russell, P. *Prog. Natl. Acad. Sci. U.S.A.* **1990**, *87*, 5139.
4. Nagata, A.; Igarashi, M.; Jinno, S.; Suto, K.; Okayama, H. *New Biol.* **1991**, *3*, 959.
5. Sasaki, H.; Yukiue, H.; Kobayashi, Y.; Tanahashi, M.; Moriyama, S.; Nakashima, Y.; Fukai, I.; Kiriyama, M.; Yamakawa, Y.; Fujii, Y. *Cancer Lett.* **2001**, *173*, 187.
6. Cangi, M. G.; Gukor, B.; Soung, P.; Signoretti, S.; Moreira, G., Jr.; Ranashinge, M.; Cady, B.; Pagano, M.; Loda, M. *J. Clin. Invest.* **2000**, *106*, 753.
7. Takemasa, I.; Yamamoto, H.; Sekimoto, M.; Ohue, M.; Noura, S.; Miyake, Y.; Matsumoto, T.; Aihara, T.; Tomita, N.; Tamaki, Y.; Sakita, I.; Kikkawa, N.; Matsuura, N.; Shiozaki, H.; Monden, M. *Cancer Res.* **2000**, *60*, 3043.
8. Hernandez, S.; Bessa, X.; Bea, S.; Hernmandez, L.; Nadal, A.; Mallofre, C.; Muntane, J.; Castells, A.; Fernandez, P. L.; Cardesa, A.; Campo, E. *Lab. Invest.* **2001**, *81*, 465.
9. Pestell, K. E.; Ducruet, A. P.; Wipf, P.; Lazo, J. S. *Oncogene* **2000**, 6607, and the references reviewed in this paper.
10. Lazo, J. S.; Aslan, D. C.; Pestell, K. E.; Cooley, K.; Ducruet, A. P.; Joo, B.; Vogt, A.; Wipf, P. *J. Med. Chem.* **2001**, *44*, 4042.
11. Peng, H.; Otterness, D. M.; Abraham, R. T.; Zalkow, L. H. *Tetrahedron* **2001**, *57*, 1891.
12. Loukaci, A.; Saout, I. L.; Samadi, M.; Leclerc, S.; Damiens, E.; Meijer, L.; Debitus, C.; Guyot, M. *Bioorg. Med. Chem.* **2001**, *9*, 3049.
13. Sodeoka, M.; Sampe, R.; Kojima, S.; Baba, Y.; Usui, T.; Ueda, K.; Osada, H. *J. Med. Chem* **2001**, *44*, 3216.
14. Aligiannis, N.; Mitaku, S.; Mitorocotsa, D.; Leclerc, S. *Planta Med.* **2001**, *67*, 468.
15. Lazo, J. S.; Nemoto, K.; Pestell, K. E.; Cooley, K.; Southwick, E. C.; Mitchell, D. A.; Furey, W.; Gussio, R.; Zaharevitz, D. W.; Joo, B.; Wipf, P. *Mol. Parmacol.* **2002**, *61*, 720.
16. Chen, C.; Liu, Y. Z.; Shia, K. S.; Teseng, H. Y. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 2729.
17. Wipf, P.; Hopkins, C. R.; Phillips, E. O.; Lazo, J. S. *Tetrahedron* **2002**, *58*, 6367.
18. Pu, l.; Amoscato, A. A.; Bier, M. E.; Lazo, J. S. *J. Biol. Chem.* **2002**, *277*, 46877.
19. Brohm, D.; Phillippe, N.; Metzger, S.; Bhargava, A.; Muller, O.; Lieb, F.; Waldmann, H. *J. Am. Chem. Soc.* **2002**, *124*, 13171.
20. Kar, S.; Lefterov, I. M.; Wang, W.; Lazo, J. S.; Scott, C. N.; Wilcox, C. S.; Carr, B. I. *Biochemistry* **2003**, *42*, 10490.
21. Sohn, J.; Kiburz, B.; Li, Z.; Deng, L.; Safi, A.; Pirrung, M. C.; Rudolph, J. *J. Med. Chem.* **2003**, *46*, 2580.
22. Brezak, M. C.; Quaranta, M.; Mondesert, O.; Galcera, M. O.; Lavergne, O.; Alby, F.; Cazales, M.; Baldin, V.; Thurieau, C.; Harnett, J.; Lanco, C.; Kasprzyk, P.; Prevost, G. P.; Ducommun, B. *Cancer Res.* **2004**, *64*, 3320.

23. Baurle, S.; Blume, T.; Gunther, J.; Henschel, D.; Hillig, R. C.; Husemann, M.; Mengel, A.; Parchmann, C.; Schmid, E.; Skuballa, W. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 1673.
24. Erdogan-orhan, I.; Sener, B.; Rosa, S. D.; Perez-Baz, J.; Lozach, O.; Leost, M.; Rakhilin, S.; Meijer, L. *Nat. Prod. Res.* **2004**, *18*, 1.
25. Furstner, A.; Reinecke, K.; Prinz, H.; Waldmann, H. *ChemBioChem* **2004**, *5*, 1575.
26. Brisson, M.; Nguyen, T.; Vogt, A.; Yalowich, J.; Giorgianni, A.; Tobi, D.; Bahar, I.; Stephenson, C. R. J.; Wipf, P.; Lazo, J. S. *Mol. Pharmacol.* **2004**, *66*, 824.
27. Ham, S. W.; Lee, S. *Bull. Korean Chem. Soc.* **2004**, *25*, 1755.
28. Galcera, M. O. C.; Lavergne, O.; Brezak, M. C.; Ducommun, B.; Prevost, G. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 5809.
29. Cao, S.; Foster, C.; Brisson, M.; Lazo, J. S.; Kingston, D. G. I. *Bioorg. Med. Chem.* **2005**, *13*, 999.
30. Shimbashi, A.; Tsuchiya, A.; Imoto, M.; Nishiyama, S. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 61.
31. Cao, S.; Foster, C.; Lazo, J. S.; Kingston, D. G. I. *Bioorg. Med. Chem.* **2005**, *13*, 5830.
32. Lavergne, O.; Fernandes, A. C.; Brehu, L.; Sidhu, A.; Brezak, M. C.; Prevost, G.; Ducommum, B.; Galcera, M. O. C. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 171.
33. Brun, M. P.; Braud, E.; Angotti, D.; Mondesert, O.; Quaranta, M.; Montes, M.; Miteva, M.; Gresh, N.; Ducommun, B.; Garbay, C. *Bioorg. Med. Chem. Lett.* **2005**, *13*, 4871.
34. Cao, S.; Foster, C.; Lazo, J. S.; Kingston, D. G. I. *Bioorg. Med. Chem.* **2005**, *13*, 5094.
35. Huang, W. G.; Jiang, Y. Y.; Li, Q.; Li, J.; Li, J. Y.; Lu, W.; Cai, J. C. *Tetrahedron* **2005**, *61*, 1863.
36. Ryu, S. Y.; Lee, C. O.; Choi, A. U. *Planta Med.* **1997**, *63*, 339.
37. Hayashi, T.; Kakisawa, H.; Hsu, H. Y.; Chen, Y. P. *J. Chem. Soc., Chem. Commun.* **1970**, 299.
38. Chang, H. M.; Cheng, K. P.; Choang, T. F.; Chow, H. F.; Chui, K. Y.; Hon, P. M.; Tan, F. W. L.; Yang, Y.; Zhong, Z. P. *J. Org. Chem.* **1990**, *55*, 3537.
39. Cdc25s and PTP1B assay: Cdc25s and PTP1B phosphatase catalytic domain were expressed with the glutathione *S*-transferase (GST) and purified by the GSTrap affinity chromatography as previously reported[a,b]. The typical inhibition assay for cdc25s was carried out in a 100 μL system containing 50 mM Tris–HCl, pH 8.0, 50 mM NaCl, 2 mM DTT, 2 mM EDTA, 1% glycerol, 10 μM OMFP, 2% DMSO, and 200–300 nM GST–cdc25A or 40–80 nM GST–cdc25B. For the PTP1B assay, the reaction system was 50 mM MOPs, pH 6.5, 2 mM DTT, 1 mM EDTA, 2 mM pNPP, 2% DMSO, and 30–40 nM GST–PTP1B. (a) Walchli, S.; Curchod, M. L.; Gobert, R. P.; Arkinstall, S.; Huijsduijnen, R. H. V. *J. Biol. Chem.* **2000**, *275*, 9792; (b) Tamura, K.; Southwick, E. C.; Kerns, J.; Rosi, K.; Carr, B. I.; Wilcox, C.; Lazo, J. S. *Cancer Res.* **2000**, *60*, 1317.
40. Inhibition of cell proliferation assay: growth inhibition of the tumor cells was analyzed by the MTT method. Human tumor cell lines HCT116 and A549 were trypsined and seeded with 3000 cells/well in 96-well plates, after attaching, cells were treated with different concentration compounds for subsequent 72 h incubation. Then, 40 μL MTT (5 mg/mL) was added into 200 μL medium each well and incubated for 3 h, finally the medium was removed and 500 μL dimethylsulfoxide was added to each well. The absorbance of each well at 550 nM (reference wave length is 690 nM) was determined using SpectraMAX340 microplate reader (Molecular Devices, Sunnyvale, CA).