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Inhibitors of NF-κB derived from thalidomide

Esperanza J. Carcache de-Blanco, Bulbul Pandit, Zhigen Hu, Jiandong Shi, Andrew Lewis and Pui-Kai Li*

Division of Medicinal Chemistry and Pharmacognosy, College of Pharmacy, The Ohio State University, Columbus, OH 43210, USA

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Abstract—A series of compounds originally derived from thalidomide were synthesized and evaluated. The most potent compounds in this series, 5HPP-33 and compound 20, inhibited NF-κB activation in HeLa cells. Preliminary study indicated that the mechanism of inhibition of NF-κB activation is through inhibition of its translocation from the cytoplasm to the nucleus.

Nuclear factor kappa B (NF-κB) is a group of transcription factors known to regulate immune and inflammatory responses.[1] The factors are homo and heterodimers from the Rel protein family containing five members: NF-κB1 (p50/105), NF-κB2 (p52/100), Rel A (p65), Rel B, and c-Rel. In its inactive form, the dimers are located in the cytoplasm and sequestered with the inhibitory proteins IκBs. Upon activation, the IκBs are phosphorylated by IκB kinase (IKK) and liberated from the NF-κB dimers. These dimers will enter the nucleus, where they enhance the transcription of genes encoding for immune and inflammatory response.

Recently, constitutive activation of NF-κB has been described in a number of tumors.[2–5] The activation resulted in upregulation of growth factors, cytokines, cell adhesion molecules, and antiapoptotic proteins which mediate promotion, angiogenesis, metastasis, and chemoresistance of tumor. Thus, inhibiting the activation of NF-κB is a viable strategy for both chemotherapy as well as chemoprevention.

Many classes of compounds have been reported to inhibit NF-κB activation which include natural products such as genistein,[6,7] caffeic acid[8,9] and curcumin;[10,11] glucocorticoids[12] and nonsteroidal anti-inflammatory agents;[13–16] IKK[17–21], and proteasome inhibitiors.[22,23]

In addition to the many classes of compounds mentioned above, thalidomide (Fig. 1) was also reported to be an inhibitor of NF-κB activation.[24–28] Thalidomide, a drug discovered in the mid-1950s in a search for better anti-epileptic agents, was found to possess little anti-epileptic effect, but was marketed as a safe, non-addictive sedative-hypnotic drug with good anti-emetic activity. The drug was used extensively for morning sickness during pregnancy until it was found to cause significant fetal abnormalities.[29] In the mid-1960s, thalidomide was found to be very useful in the treatment of leprosy leading to resurgence in interest in the drug and its eventual approval in 1998 by the FDA for the acute treatment of erythema nodosum leprosum[2] and recently for multiple myeloma. Today, thalidomide is in clinical trials for, among other things, HIV ulcers and wasting[30–33] Crohn's disease,[34] rheumatoid arthritis,[35,36] chronic host-versus-graft disease,[37] Behcet's vasculitis,[38] and cancer including AIDS Kaposi's sarcoma[39] and other solid tumors.[40–43] Recently, a number of novel thalidomide analogs have been reported.[44–47] These analogs can be classified into two groups: the Selective Cytokine Inhibitory Drugs (SelCIDs), which possess phosphodiesterase Type 4 (PDE4) inhibitory activities, and the immunomodulatory drugs (IMiDs). Currently, two IMiDs are in clinical trials for the treatment of cancers. Revlimid (Fig. 1) is now in phase III clinical trials for multiple myeloma and metastatic melanoma, while Actimid (Fig. 1) has entered a phase I/II trial for multiple myeloma and a phase II trial for prostate cancer.

Recently, we reported the anti-angiogenic activities of several putative metabolites of thalidomide.[48] Our ongoing search for potent anti-angiogenic thalidomide

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* Corresponding author. Tel.: +1 614 688 0253; fax: +1 614 688 8556; e-mail: li.27@osu.edu

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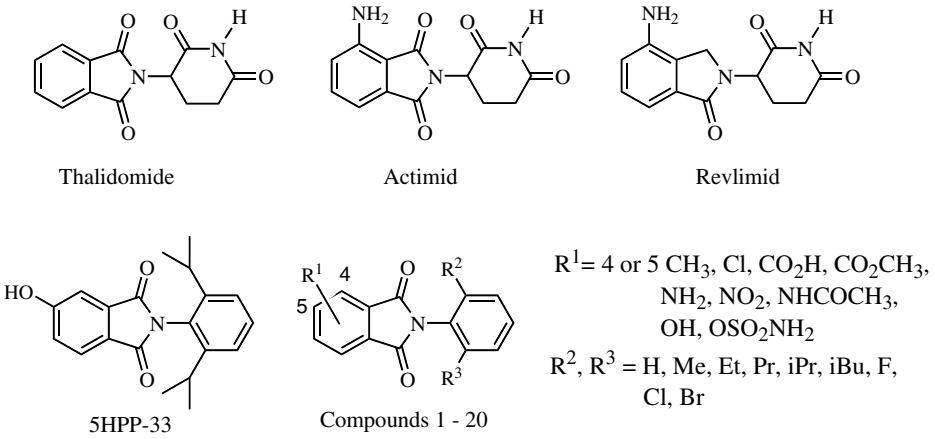


Figure 1. Structures of thalidomide, actimid, revlimid, 5HPP-33, and its analogs.

analogs revealed a compound, 5-hydroxy-(2,6-diisopropylphenyl)-1*H*-isoindole-1,3-dione (5HPP-33, Fig. 1), with a paclitaxel-like anti-microtubule mode of action.[49] In addition, 5HPP-33 has been reported to have tumor necrosis factor-α inhibitory activity in vitro.[50–52] In the evaluation of 5HPP-33 described here, we determined that 5HPP-33 inhibits the activation of NF-κB through inhibition of its translocation to the nucleus. Twenty 5HPP-33 analogs are evaluated. 5HPP-33 and one of its analogs are approximately 4-fold more potent than thalidomide as inhibitors of NF-κB activation.

Thalidomide,[53] actimid[47], and 5HPP-33[54] were synthesized according to the reported procedures. The syntheses of compounds **1**–**10** and **13**–**20** are shown in Figure 2. The syntheses of compounds **1**–**3** and **5**–**9** involved the coupling of various substituted phthalic anhydrides with substituted anilines. Compounds **4** and **10** were obtained by catalytic hydrogenation of compounds **3** and **9**, respectively. Condensation of 4-hydroxyphthalic acid with substituted anilines yielded compounds **13**–**20**. Esterification of **5** with MeOH in acidic reflux yielded compound **6**. The syntheses of compounds **11** and **12** are shown in Figure 2. In brief, compound **11** was obtained by sulfamoylation[55] of 5HPP-33. Acetylation of compound **10** yielded compound **12**.

5HPP-33 and its analogs were assayed for their inhibitory activity toward IL-1α induced NF-κB activation in HeLa cells, with (*E*)-3-(*tert*-butylphenylsulfonyl)-2-propenenitrile (BAY-11-7085), a potent IKK inhibitor,[19,20] as the positive control. Normally, NF-κB is present in the cytoplasm as homo or heterodimer with members of the IκB inhibitor family. Upon phosphorylation and degradation of IκB, the nuclear localization sequence becomes accessible and NF-κB translocates to the nucleus. Thus, translocation from the cytoplasm to the nucleus is a definitive measure of NF-κB activation. A quantitative NF-κB nuclear translocation assay was used to measure the inhibitory activities of the compounds.[56] In brief, HeLa cells in a 96-well plate were stimulated with IL-1α (5 ng/ml, 25 μl/well) with or without inhibitors and the cells were incubated for 30 min. The cells were fixed and incubated with anti-p65 antibody and then stained with Hoechst dye. The plates were then scanned with fluorescent imaging microscope (Axiovert 40 CFL, Carl Zeiss Microimaging, Inc., Thornwood, NY). The results are shown in Figure 3. Immunofluorescence staining of p65 in HeLa cells (unstimulated, PBS control) shows that p65 is mainly located in cytoplasm with minimal amount in the nucleus. Upon stimulation with IL-1α for 30 min, significant amount of the p65 is translocated into the nucleus.

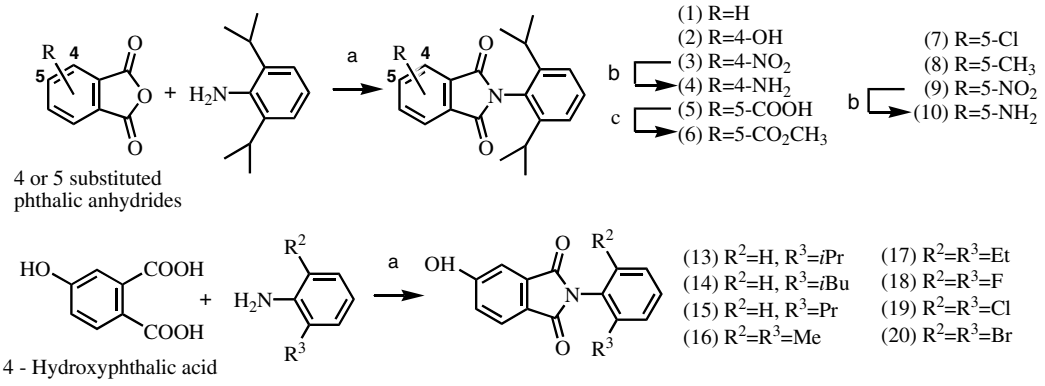


Figure 2. Syntheses of compounds **1**–**10** and **13**–**20**. Reagents and conditions: (a) AcOH, reflux, 5–7 h, 67–95% yield; (b) H_2, 10% Pd/C, acetone, reflux, 2 h, 88–90% yield; (c) MeOH, dil H_2SO_4, reflux, 12 h, 84% yield.

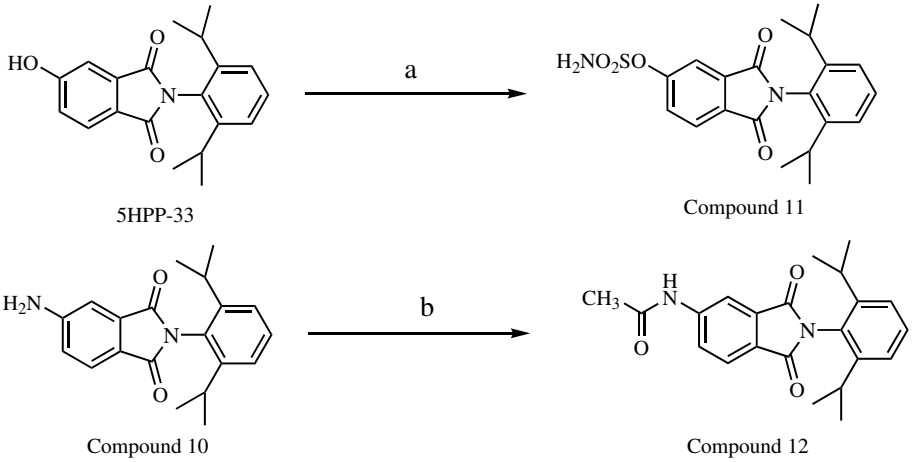
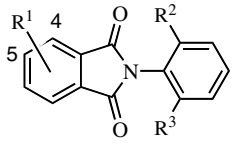


Figure 3. Syntheses of compounds **11** and **12**. Reagents and conditions: (a) $ClSO_2NH_2$/2,6-di-*tert*butyl-4-methyl pyridine/DMF/rt/24 h; (b) CH_3COCl/Et_3N/THF/rt/1 h.

Table 1. Inhibitory activity of thalidomide, actimid, BAY-11-7085, 5HPP-33, and its analogs on IL-1 induced nuclear translocation of NF-κB in HeLa cells



Compound	R^1	R^2	R^3	Inhibition of p50/p65 translocation IC_{50} (μM)
Thalidomide	—	—	—	2.04
Actimid	—	—	—	1.27
BAY-11-7085	—	—	—	0.1
5HPP-33	5-OH	*i*-Pr	*i*-Pr	0.53
1	H	*i*-Pr	*i*-Pr	>50
2	4-OH	*i*-Pr	*i*-Pr	4.95
3	4-NO_2	*i*-Pr	*i*-Pr	>50
4	4-NH_2	*i*-Pr	*i*-Pr	>50
5	5-CO_2H	*i*-Pr	*i*-Pr	>50
6	5-CO_2CH_3	*i*-Pr	*i*-Pr	>50
7	5-Cl	*i*-Pr	*i*-Pr	>50
8	5-CH_3	*i*-Pr	*i*-Pr	>50
9	5-NO_2	*i*-Pr	*i*-Pr	>50
10	5-NH_2	*i*-Pr	*i*-Pr	29.5
11	5-OSO_2NH_2	*i*-Pr	*i*-Pr	>50
12	5-$NHCOCH_3$	*i*-Pr	*i*-Pr	>50
13	5-OH	H	*i*-Pr	>50
14	5-OH	H	*t*-Bu	>50
15	5-OH	H	Pr	>50
16	5-OH	Me	Me	>50
17	5-OH	Et	Et	>50
18	5-OH	F	F	>50
19	5-OH	Cl	Cl	>50
20	5-OH	Br	Br	0.43

BAY-11-7085 (an IKK inhibitor), thalidomide, 5HPP-33, and their analogs inhibit the nuclear translocation of p65 (Fig. 3).

In the IL-1α stimulated cells, the amount of nuclear fluorescence is increased, while the cytoplasmic fluorescence markedly is decreased. The nuclear minus fluorescence is high. The inhibitory activities of the compounds (IC_{50}) are designated as the concentration of the compounds to reduce 50% of the nuclear minus cytoplasmic fluorescence in the cells. The inhibitory activities of the compounds are shown in Table 1.

Thalidomide has been reported to be an inhibitor of NF-κB activation.[24–28] In this study, we demonstrated that thalidomide inhibited the nuclear translocation of NF-κB in IL-1α activated HeLa cells with an IC_{50} of 2.04 μM (Table 1). In addition, we also demonstrated

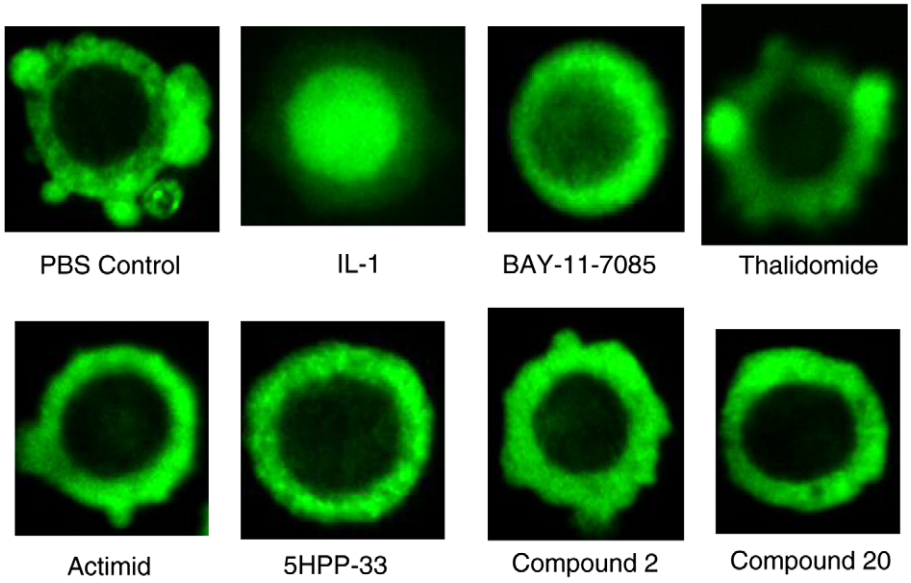


Figure 4. IL-1 induced NF-κB nuclear translocation. Representative fields of p65 fluorescence are shown in HeLa cells that are unstimulated (PBS control), IL-1 stimulated, and IL-1 stimulated in the presence of BAY-11-7085, thalidomide, actimid, 5HPP-33, and compounds **2** and **20** (please refer Table 1 for the structures of compounds **2** and **20**).

for the first time that actimid, a thalidomide analog currently in clinical trial for multiple myeloma,[57,58] also inhibited NF-κB activation with a slightly higher potency than thalidomide (IC_{50}—1.27 μM, Table 1). 5HPP-33, a thalidomide analog reported to have tumor necrosis factor-α (TNF-α) inhibitory activity, in vitro is four times more potent than thalidomide as an inhibitor of NF-κB activation (IC_{50}—0.53 μM, Table 1). Thus, we conducted a detailed structure–activity relationship study of 5HPP-33 in regard to its ability to inhibit NF-κB activation. Twenty 5HPP-33 analogs (compounds **1**–**20**) were synthesized with modifications on 2,6-diisopropyl phenyl ring or on the phthalimide ring (Table 1). The 2,6-diisopropyl groups in 5HPP-33 are necessary but not essential for activity. Replacing one of the isopropyl groups with a H (compounds **13**–**15**) or substituting the 2,6-diisopropyl groups with Me, Et, F or Cl (compounds **16**–**19**) resulted in elimination of inhibitory activity. However, 2,6-dibromo analog of 5HPP-33 (compound **20**) is as active as 5HPP-33 (IC_{50}—0.43 μM, Table 1).

The 5-OH group in 5HPP-33 is essential for activity. Substituting the 5-OH group with H (compound **1**), COOH (compound **5**), CO_2CH_3 (compound **6**), Cl (compound **7**), CH_3 (compound **8**), NO_2 (compound **9**), OSO_2NH_2 (compound **11**), and $NHCOCH_3$ (compound **12**) all resulted in the elimination of inhibitory activity. The inhibitory activity is decreased by 500-fold when the 5-OH is substituted with a 5-NH_2 group (compound **10**). Transferring the OH group of 5HPP-33 from the 5 to the 4 position (compound **2**) resulted in 10-fold reduction in inhibitory activity. Substituting the 4-OH in compound **2** with NO_2 or NH_2 all resulted in the elimination of the inhibitory activity of NF-κB (Fig. 4).

In conclusion, we have discovered a series of compounds derived from thalidomide with potent NF-κB inhibitory activity. Preliminary studies indicated that the active analogs inhibit NF-κB activation through inhibition of its translocation from the cytoplasm to the nucleus. We are currently investigating the molecular target(s) of the inhibition.

References and notes

1. Ghosh, S.; May, M. J.; Kopp, E. B. *Annu. Rev. Immunol.* **1998**, *16*, 225.
2. Bottero, V.; Busuttil, V.; Loubat, A.; Magne, N.; Fischel, J. L.; Milano, G.; Peyron, J. F. *Cancer Res.* **2001**, *61*, 7785.
3. Gilmore, T.; Gapuzan, M. E.; Kalaitzidis, D.; Starczynowski, D. *Cancer Lett.* **2002**, *181*, 1.
4. Yang, J.; Richmond, A. *Cancer Res.* **2001**, *61*, 4901.
5. Romieu-Mourez, R.; Landesman-Bollag, E.; Seldin, D. C.; Traish, A. M.; Mercurio, F.; Sonenshein, G. E. *Cancer Res.* **2001**, *61*, 3810.
6. Li, Y.; Ellis, K. L.; Ali, S.; El-Rayes, B. F.; Nedeljkovic-Kurepa, A.; Kucuk, O.; Philip, P. A.; Sarkar, F. H. *Pancreas* **2004**, *28*, e90.
7. Gong, L.; Li, Y.; Nedeljkovic-Kurepa, A.; Sarkar, F. H. *Oncogene* **2003**, *22*, 4702.
8. Natarajan, K.; Singh, S.; Burke, T. R., Jr.; Grunberger, D.; Aggarwal, B. B. *Proc. Natl. Acad Sci. U.S.A.* **1996**, *93*, 9090.
9. Watabe, M.; Hishikawa, K.; Takayanagi, A.; Shimizu, N.; Nakaki, T. *J. Biol. Chem.* **2004**, *279*, 6017.
10. Li, L.; Aggarwal, B. B.; Shishodia, S.; Abbruzzese, J.; Kurzrock, R. *Cancer* **2004**, *101*, 2351.
11. Aggarwal, S.; Takada, Y.; Singh, S.; Myers, J. N.; Aggarwal, B. B. *Int. J. Cancer* **2004**, *111*, 679.
12. Brown, K.; Gerstberger, S.; Carlson, L.; Franzoso, G.; Siebenlist, U. *Science* **1995**, *267*, 1485.
13. Yin, M. J.; Yamamoto, Y.; Gaynor, R. B. *Nature* **1998**, *396*, 77.
14. Yamamoto, Y.; Yin, M. J.; Lin, K. M.; Gaynor, R. B. *J. Biol. Chem.* **1999**, *274*, 27307.
15. Wahl, C.; Liptay, S.; Adler, G.; Schmid, R. M. *J. Clin. Invest.* **1998**, *101*, 1163.

16. Weber, C. K.; Liptay, S.; Wirth, T.; Adler, G.; Schmid, R. M. *Gastroenterology* **2000**, *119*, 1209.
17. Olsen, L. S.; Hjarnaa, P. J.; Latini, S.; Holm, P. K.; Larsson, R.; Bramm, E.; Binderup, L.; Madsen, M. W. *Int. J. Cancer* **2004**, *111*, 198.
18. Hovstadius, P.; Larsson, R.; Jonsson, E.; Skov, T.; Kissmeyer, A. M.; Krasilnikoff, K.; Bergh, J.; Karlsson, M. O.; Lonnebo, A.; Ahlgren, J. *Clin. Cancer Res.* **2002**, *8*, 2843.
19. Mabuchi, S.; Ohmichi, M.; Nishio, Y.; Hayasaka, T.; Kimura, A.; Ohta, T.; Kawagoe, J.; Takahashi, K.; Yada-Hashimoto, N.; Seino-Noda, H.; Sakata, M.; Motoyama, T.; Kurachi, H.; Testa, J. R.; Tasaka, K.; Murata, Y. *Clin. Cancer Res.* **2004**, *10*, 7645.
20. Mabuchi, S.; Ohmichi, M.; Nishio, Y.; Hayasaka, T.; Kimura, A.; Ohta, T.; Saito, M.; Kawagoe, J.; Takahashi, K.; Yada-Hashimoto, N.; Sakata, M.; Motoyama, T.; Kurachi, H.; Tasaka, K.; Murata, Y. *J. Biol. Chem.* **2004**, *279*, 23477.
21. Pierce, J. W.; Schoenleber, R.; Jesmok, G.; Best, J.; Moore, S. A.; Collins, T.; Gerritsen, M. E. *J. Biol. Chem.* **1997**, *272*, 21096.
22. Adams, J.; Palombella, V. J.; Elliott, P. J. *Invest. New Drugs* **2000**, *18*, 109.
23. Hideshima, T.; Chauhan, D.; Richardson, P.; Mitsiades, C.; Mitsiades, N.; Hayashi, T.; Munshi, N.; Dang, L.; Castro, A.; Palombella, V.; Adams, J.; Anderson, K. C. *J. Biol. Chem.* **2002**, *277*, 16639.
24. Keifer, J. A.; Guttridge, D. C.; Ashburner, B. P.; Baldwin, A. S., Jr. *J. Biol. Chem.* **2001**, *276*, 22382.
25. Yagyu, T.; Kobayashi, H.; Matsuzaki, H.; Wakahara, K.; Kondo, T.; Kurita, N.; Sekino, H.; Inagaki, K.; Suzuki, M.; Kanayama, N.; Terao, T. *J. Clin. Endocrinol. Metab.* **2005**, *90*, 3017.
26. Majumdar, S.; Lamothe, B.; Aggarwal, B. B. *J. Immunol.* **2002**, *168*, 2644.
27. Moreira, A. L.; Corral, L. G.; Ye, W.; Johnson, B.; Stirling, D.; Muller, G. W.; Freedman, V. H.; Kaplan, G. *AIDS Res. Hum. Retroviruses* **1997**, *13*, 857.
28. Moreira, A. L.; Friedlander, D. R.; Shif, B.; Kaplan, G.; Zagzag, D. *J. Neurooncol.* **1999**, *43*, 109.
29. Lenz, W.; Knapp, K. *Arch. Environ. Health* **1962**, *5*, 100.
30. Gorin, I.; Vilette, B.; Gehanno, P.; Escande, J. P. *Lancet* **1990**, *335*, 1343.
31. Reyes-Teran, G.; Sierra-Madero, J. G.; Martinez del Cerro, V.; Arroyo-Figueroa, H.; Pasquetti, A.; Calva, J. J.; Ruiz-Palacios, G. M. *Aids* **1996**, *10*, 1501.
32. Marriott, J. B.; Cookson, S.; Carlin, E.; Youle, M.; Hawkins, D. A.; Nelson, M.; Pearson, M.; Vaughan, A. N.; Gazzard, B.; Dalgleish, A. G. *AIDS Res. Hum. Retroviruses* **1997**, *13*, 1625.
33. Jacobson, J. M.; Spritzler, J.; Fox, L.; Fahey, J. L.; Jackson, J. B.; Chernoff, M.; Wohl, D. A.; Wu, A. W.; Hooton, T. M.; Sha, B. E.; Shikuma, C. M.; MacPhail, L. A.; Simpson, D. M.; Trapnell, C. B.; Basgoz, N. *J. Infect. Dis.* **1999**, *180*, 61.
34. Ehrenpreis, E. D.; Kane, S. V.; Cohen, L. B.; Cohen, R. D.; Hanauer, S. B. *Gastroenterology* **1999**, *117*, 1271.
35. Raza, A. *Microsc. Res. Tech.* **2000**, *50*, 229.
36. Keesal, N.; Wasserman, M. J.; Bookman, A.; Lapp, V.; Weber, D. A.; Keystone, E. C. *J. Rheumatol.* **1999**, *26*, 2344.
37. Koc, S.; Leisenring, W.; Flowers, M. E.; Anasetti, C.; Deeg, H. J.; Nash, R. A.; Sanders, J. E.; Witherspoon, R. P.; Appelbaum, F. R.; Storb, R.; Martin, P. J. *Blood* **2000**, *96*, 3995.
38. Yazici, H.; Fresko, I.; Hamuryudan, V.; Mat, C.; Melikoglu, M.; Ozyazgan, Y.; Tuzun, Y.; Yurdakul, S. *Adv. Exp. Med. Biol.* **1999**, *455*, 135.
39. Little, R. F.; Wyvill, K. M.; Pluda, J. M.; Welles, L.; Marshall, V.; Figg, W. D.; Newcomb, F. M.; Tosato, G.; Feigal, E.; Steinberg, S. M.; Whitby, D.; Goedert, J. J.; Yarchoan, R. *J. Clin. Oncol.* **2000**, *18*, 2593.
40. Patt, Y. Z.; Hassan, M. M.; Lozano, R. D.; Ellis, L. M.; Peterson, J. A.; Waugh, K. A. *Am. J. Clin. Oncol.* **2000**, *23*, 319.
41. Eisen, T.; Boshoff, C.; Mak, I.; Sapunar, F.; Vaughan, M. M.; Pyle, L.; Johnston, S. R.; Ahern, R.; Smith, I. E.; Gore, M. E. *Br. J. Cancer* **2000**, *82*, 812.
42. Fine, H. A.; Figg, W. D.; Jaeckle, K.; Wen, P. Y.; Kyritsis, A. P.; Loeffler, J. S.; Levin, V. A.; Black, P. M.; Kaplan, R.; Pluda, J. M.; Yung, W. K. *J. Clin. Oncol.* **2000**, *18*, 708.
43. Stebbing, J.; Benson, C.; Eisen, T.; Pyle, L.; Smalley, K.; Bridle, H.; Mak, I.; Sapunar, F.; Ahern, R.; Gore, M. E. *Br. J. Cancer* **2001**, *85*, 953.
44. Corral, L. G.; Muller, G. W.; Moreira, A. L.; Chen, Y.; Wu, M.; Stirling, D.; Kaplan, G. *Mol. Med.* **1996**, *2*, 506.
45. Muller, G. W.; Corral, L. G.; Shire, M. G.; Wang, H.; Moreira, A.; Kaplan, G.; Stirling, D. I. *J. Med. Chem.* **1996**, *39*, 3238.
46. Corral, L. G.; Haslett, P. A.; Muller, G. W.; Chen, R.; Wong, L. M.; Ocampo, C. J.; Patterson, R. T.; Stirling, D. I.; Kaplan, G. *J. Immunol.* **1999**, *163*, 380.
47. Muller, G. W.; Chen, R.; Huang, S. Y.; Corral, L. G.; Wong, L. M.; Patterson, R. T.; Chen, Y.; Kaplan, G.; Stirling, D. I. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 1625.
48. Marks, M. G.; Shi, J.; Fry, M. O.; Xiao, Z.; Trzyna, M.; Pokala, V.; Ihnat, M. A.; Li, P. K. *Biol. Pharm. Bull.* **2002**, *25*, 597.
49. Li, P. K.; Pandit, B.; Sackett, D. L.; Hu, Z.; Zink, J.; Zhi, J.; Freeman, D.; Robey, R. W.; Werbovetz, K.; Lewis, A.; Li, C. *Mol. Cancer Ther.* **2006**, *5*, 450.
50. Hashimoto, Y. *Curr. Med. Chem.* **1998**, *5*, 163.
51. Miyachi, H.; Azuma, A.; Hashimoto, Y. *Yakugaku Zasshi* **1997**, *117*, 91.
52. Hashimoto, Y. *Bioorg. Med. Chem.* **2002**, *10*, 461.
53. Shah, J. H.; Swartz, G. M.; Papathanassiu, A. E.; Treston, A. M.; Fogler, W. E.; Madsen, J. W.; Green, S. J. *J. Med. Chem.* **1999**, *42*, 3014.
54. Shibata, Y.; Sasaki, K.; Hashimoto, Y.; Iwasaki, S. *Chem. Pharm. Bull. (Tokyo)* **1996**, *44*, 156.
55. Li, P. K.; Chu, G. H.; Guo, J. P.; Peters, A.; Selcer, K. W. *Steroids* **1998**, *63*, 425.
56. Ding, G. J.; Fischer, P. A.; Boltz, R. C.; Schmidt, J. A.; Colaianne, J. J.; Gough, A.; Rubin, R. A.; Miller, D. K. *J. Biol. Chem.* **1998**, *273*, 28897.
57. Teo, S. K. *Aaps. J.* **2005**, *7*, E14.
58. Schey, S. A.; Fields, P.; Bartlett, J. B.; Clarke, I. A.; Ashan, G.; Knight, R. D.; Streetly, M.; Dalgleish, A. G. *J. Clin. Oncol.* **2004**, *22*, 3269.