Available online at www.sciencedirect.com
ScienceDirect
Bioorganic & Medicinal Chemistry Letters 18 (2008) 1884–1888
Bioorganic & Medicinal Chemistry Letters

# New nordihydroguaiaretic acid derivatives as anti-HIV agents

Jih Ru Hwu,[a,b,*] Ming-Hua Hsu[a,c] and Ru Chih C. Huang[c,*]

[a]*Department of Chemistry, National Tsing Hua University, 101, Section 2, Kuang-fu Road, Hsinchu 30013, Taiwan, ROC*
[b]*Department of Chemistry, National Central University, Jhongli 32001, Taiwan, ROC*
[c]*Department of Biology, The Johns Hopkins University, Baltimore, MD 21218, USA*

Received 13 November 2007; revised 1 February 2008; accepted 7 February 2008
Available online 10 February 2008

**Abstract**—Reaction of nordihydroguaiaretic acid with various alkyl chloride, 1-piperidinecarbonyl chloride, methyl chloroformate, or 1,1′-carbonyldiimidazole under alkaline conditions produced the corresponding phenol ethers, carbamates and carbonates, respectively, in 67–83% yields. Among these derivatives, the nitrogen-containing compounds were converted to the corresponding hydrochloride salts. Having good solubility, these NDGA derivatives were found stable in aqueous solution. These new compounds exerted appealing activity against HIV Tat-regulated transactivation in human epithelial cells. The most potent compound *meso*-2,3-dimethyl-1,4-bis(3,4-[2-(piperdino)ethoxyphenyl])butane tetrakishydrochloride salt (**5b**) showed $IC_{50}$ value of 0.88 μM.


Nordihydroguaiaretic acid (NDGA, **1** in Fig. 1) is a lignan found in the leaves and twigs of the shrub *Larrea tridentata*. Being a lipoxygenases inhibitor, NDGA can induce cystic nephropathy in the rat.[1] In addition, it shows various bioactivities, including inhibition of protein kinase C,[2] induction of apoptosis,[3] alterations of membrane,[4] elevation of cellular $Ca^{2+}$ level[5] and activation of $Ca^{2+}$ channels in smooth muscle cells,[6] breakdown of pre-formed Alzheimer's β-amyloid fibrils in vitro,[7] anti-oxidation,[8] etc. Although having been banned in some countries (e.g., USA), this natural product is used commercially as a food additive to preserve fats and butter. Recently, the derivatives of the plant lignan NDGA have been used for block of viral replication through the inhibition of viral transcription.[9–16] These compounds can inhibit production of HIV,[9–13] herpes simplex virus,[14,15] and HPV transcripts[16] by deactivation of their Sp1-dependent promoters. Moreover, (tetra-*O*-methyl)nordihydroguaiaretic acid ($M_4N$, **2**) can function as an anti-HIV proviral transcription inhibitor; this NDGA derivative also causes growth arrest of a variety of transformed human and mouse cells in culture and in mice.[17,18]

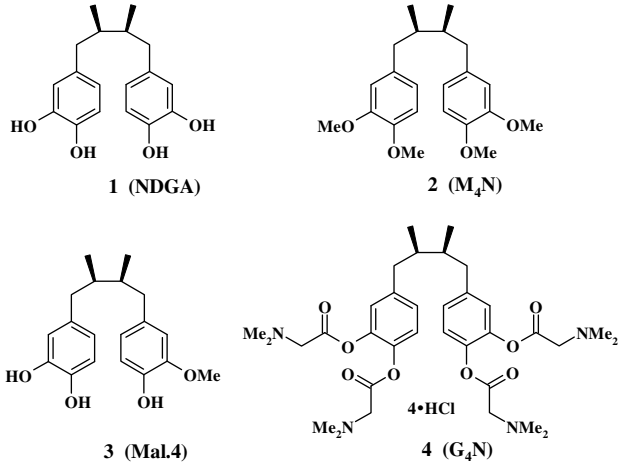



**Figure 1.** The structures of NDGA derivatives.

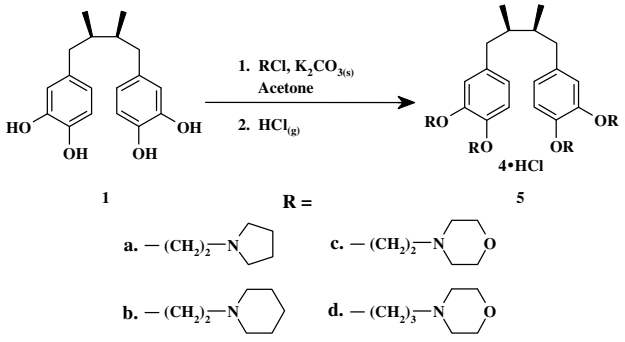
We have been focusing on design of NDGA derivatives that can selectively arrest growth and induce apoptosis of cancer cells with minimal toxicity to normal tissues. Huang et al.[12] reported that (3′-*O*-methyl)nordihydroguaiaretic acid (Mal.4, **3**) can directly and specifically interfere wit21h the binding of Sp1 protein to the Sp1 sites of the HIV long terminal repeat. The $M_4N$ (**2**) appears to induce cell cycle arrest in mammalian cell lines[18] and thus inhibits tumor cell growth. Nevertheless, $M_4N$ and other methylated NDGA show poor water solubility, which limits their applicability. To circum-

*Keywords*: Anti-HIV; NDGA; Nordihydroguaiaretic acid; DNA; HIV Tat-regulated transactivation.
* Corresponding authors. Tel.: +1 886 35 725813; fax: +1 886 35 721594; e-mail: jrhwu@mx.nthu.edu.tw

0960-894X/$ - see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2008.02.018

vent this problem, we designed and synthesized (tetra-*O*-glycyl)nordihydroguaiaretic acid ($G_4N$, **4**), a water soluble derivative of NDGA.[11] It can act as mutation-insensitive transcription inhibitor to HSV-1.[10,15] The high water solubility associated with $G_4N$ allows it to work efficiently in inhibition of HSV despite its short half-life in aqueous solution. The instability comes from the ester bonds therein, which connect the glycine moieties onto the NDGA main skeleton. Accordingly, we planned to synthesize a new series of NDGA derivatives with appealing anti-HIV activity as well as good water solubility and stability.

Being a potent transcription activator encoded by the human immunodeficiency virus-1 (HIV-1), HIV Tat is required for replication of the deadly virus.[19] Tat-regulated transactivation has since become an attractive strategy for the development of anti-HIV drugs.[20] On the other hand, Berger et al.[21] reported a novel eukaryotic reporter gene, the secreted alkaline phosphatase (SEAP). By adopting these methods, we screened our newly synthesized NDGA derivatives against HIV by using HIV long terminal repeat (LTR) promoter, SEAP, and CMV promoter driven Tat. We found that compound **5b** was able to inhibit of HIV Tat-regulated transactivation in a great extent and represents a new lead of anti-HIV drugs.



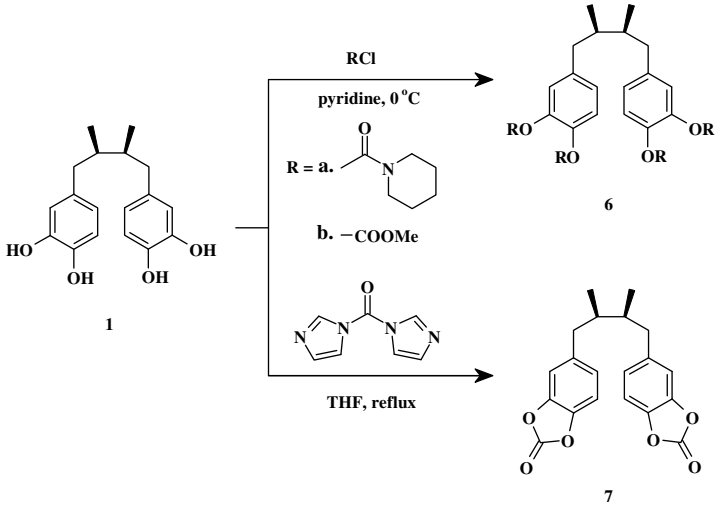

**Scheme 1.** Synthesis of new NDGA derivatives.

We first treated NDGA (**1**) with an alkyl chloride bearing a hydrocarbon spacer and a nitrogen—containing five- or six-membered ring in the presence of sodium carbonate and acetone (see Scheme 1). These intermediates were then allowed to react with $HCl_{(g)}$ in situ to give tetra-*O*-alkylated NDGAs **5a**–**d** in 67–82% overall yields. Their solubility in aqueous solution was found 379–541 mg/mL.

Furthermore, we prepared NDGA derivatives in the families of carbamate and carbonate as shown in Scheme 2. Treatment of NDGA with 1-piperidinecarbonyl chloride in the presence of pyridine at 0 °C produced the NDGA carbamate **6a** in 72% yield. Under the same conditions, NDGA reacted with methyl chloroformate or 1,1′-carbonyldiimidazole afforded carbonates **6b** and **7**, respectively. The latter product was generated through an intramolecular cyclization process.

These new NDGA derivatives were found stable in aqueous solution; >99% of these compounds remained intact after 28 days (see Fig. 2(b)). In a sharp contrast, >96% $G_4N$ (**4**) decomposed in aqueous solution within 24 h (see Fig. 2(a)).

We used the transactivation assay involving transfection of plasmid constructs[21] to test the effect of synthesized NDGA derivatives on Tat-regulated secreted alkaline phosphatase (SEAP). This protein was produced in COS cells as described previously.[12,13,21] The plasmid constructs included a cytomegalovirus (CMV) promoter driven *tat* gene and an HIV LTR promoter driven reporter gene (i.e., SEAP). A standard SEAP assay in the absence of drug was initially run to find out efficiency of transfection. Our results shown in Figure 3 indicate a nearly 5 times increment in Tat-induced SEAP expression after 60 min in comparison with the control (i.e.,




**Scheme 2.** Synthesis of new NDGA derivatives.

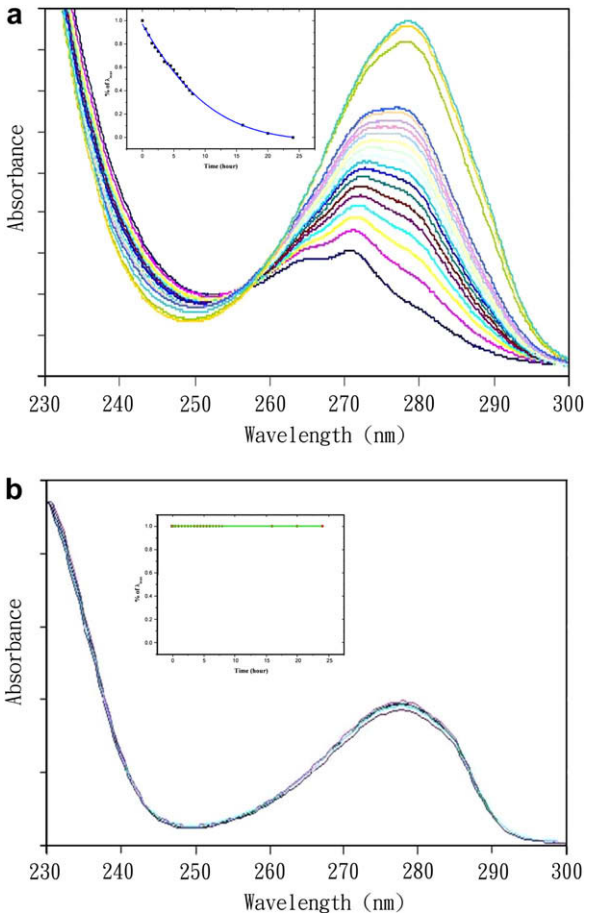



**Figure 2.** UV–visible spectra for indication of stability of NDGA derivatives (a) $G_4N$ (**4**) degradation in Milli-Q Water. (b) $P_4N$ (**5b**) present in Milli-Q Water after 28 days.

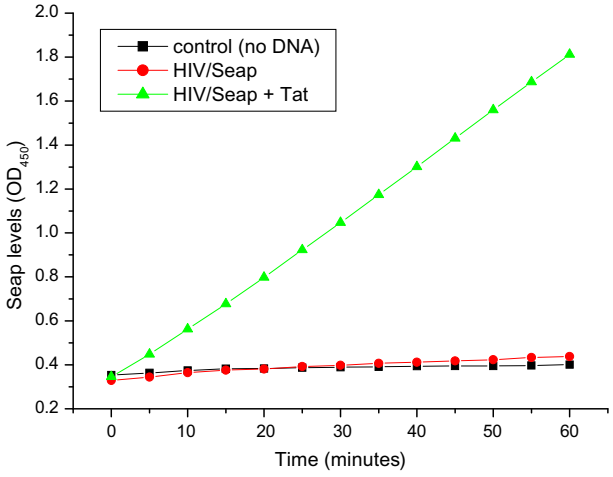



**Figure 3.** Induction of SEAP expression in SEAP standard assay.

without DNA) levels of the enzyme and the non-selected gene (HIV/SEAP) alone. Both of them were close to the baseline.

We treated the NDGA derivatives **5a**–**d**, **6a**, **b**, and **7** with the SEAP assay, of which the results displayed a dose-dependent inhibitory activity of Tat transactivation (see Table 1 and Fig. 4). At 80 μM, all of these NDGA derivatives inhibited the Tat-regulated SEAP production to the level >90%; at 20 μM, the inhibition was still >50%. All of these newly synthesized derivatives exhibited a greater inhibitory activity than the parent NDGA (**1**, $IC_{50}$ = 20 μM) and Mal.4 (**3**, $IC_{50}$ = 25 μM).[12] Except **5a** ($IC_{50}$ = 17.2 μM) and **5c** ($IC_{50}$ = 17.3 μM), all other NDGA derivatives showed greater potency than $M_4N$ (**2**, $IC_{50}$ = 11.1 μM).[9] Compound **5b** ($IC_{50}$ = 0.88 μM) was found to be the strongest inhibitor among all of the new NDGA derivatives for the HIV Tat-regulated transactivation.

For determining cell cytotoxicity in vitro, we adopted the MTT assay[22] by growing cultured cancer cells in the presence of the NDGA derivatives. Remaining viable cells were then measured by a spectrophotometric method involving the reduction of the yellow 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (i.e., MTT) to a purple–blue formazan by mitochondrial dehydrogenases of metabolically active cancer cells. The $IC_{50}$ value indicates the concentration that resulted in a 50% decrease in cell growth relative to an untreated control.[22] Our results are shown in Table 2, which includes the cell growth inhibition by the NDGA derivatives to COS cells without transfected by HIV/SEAP.

In comparison with the NDGA derivatives **6a**, **b** and **7**, the ether linkage in compounds **5a**–**d** enabled them much more stable in aqueous medium. On the other hand, our results from their inhibition of the HIV Tat-regulated transactivation indicate that the piperidine derivative **5b** possessed greater activity than the pyrrolidine derivative **5a**. The angle strain in five-membered rings is bigger than that in six-membered ring, which may limit the bioactivity. On the other hand, placement of an oxygen atom at the 4-position in the six-membered ring decreased the anti-cancer activity; the morpholine derivative **5c** ($IC_{50}$ = 17.34 μM) was less potent than piperidine derivative **5b** ($IC_{50}$ = 0.88 μM). In the morpholine series (cf. **5c** and **5d**), we found that the activity was increased by elongating the spacer from two methylene units to three units. The longer spacer in **5d** than that in **5c** could allow the morpholine to have a greater flexibility for reaching the blocking side. Among all of these NDGA derivatives, compound **5b** showed most potent activity in HIV Tat-regulated transactivation. It could be regarded as a lead in the development of new anti-HIV agents.

Johansen et al.[23] reported the binding of Sp1 family proteins with DNA at a GC box through the major groove. The NDGA derivative **5b** could also block the GC box of DNA through the major groove to form a complex as shown in Figure 5. We performed this graphic molecular modeling by using the programs Builder and Biopolymer for the construction of structures. The energies for conformations were minimized by use of the program Discover with the consistent valence forcefield (CVFF). The guest **5b**, however, was expelled from the minor groove of the host DNA because of steric congestion.

**Table 1.** Inhibition (%) of HIV Tat-regulated transactivation in COS cells by NDAG derivatives

| Compound | Concentration[a] (μM) | | | | | | | | | | | $IC_{50}$[b] (μM) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 0.10 | 0.25 | 0.50 | 1.0 | 2.5 | 5.0 | 10 | 20 | 40 | 80 | 100 | |
| **5a** | 18.1 | 15.4 | 27.2 | 19.6 | 31.0 | 14.1 | 26.3 | 59.1 | 94.8 | 97.1 | 100 | 17.23 |
| **5b** | 8.7 | 4.3 | 29.0 | 56.8 | 90.6 | 97.0 | 99.8 | 100 | 100 | 100 | 97.7 | 0.88 |
| **5c** | 5.0 | 16.5 | 9.9 | 8.1 | 4.6 | 13.3 | 25.4 | 58.9 | 86.0 | 96.8 | 100 | 17.34 |
| **5d** | 15.0 | 15.9 | 10.6 | 9.5 | 52.7 | 89.5 | 96.0 | 99.9 | 99.8 | 100 | 89.5 | 2.41 |
| **6a** | 0 | 3.5 | 5.8 | 11.7 | 46.2 | 78.6 | 84.0 | 86.1 | 83.8 | 90.1 | 89.8 | 2.79 |
| **6b** | 9.8 | 12.3 | 16.0 | 13.1 | 15.1 | 38.0 | 51.6 | 58.4 | 70.8 | 93.4 | 88.9 | 9.41 |
| **7** | 7.5 | 18.1 | 4.2 | 5.9 | 23.7 | 47.1 | 51.2 | 68.8 | 85.7 | 96.3 | 91.6 | 8.54 |

[a] All data represent the average of three experiments.
[b] Concentrations exhibiting 50% inhibitory ($IC_{50}$) represented the mean of the triplicate determinations with standard deviations.

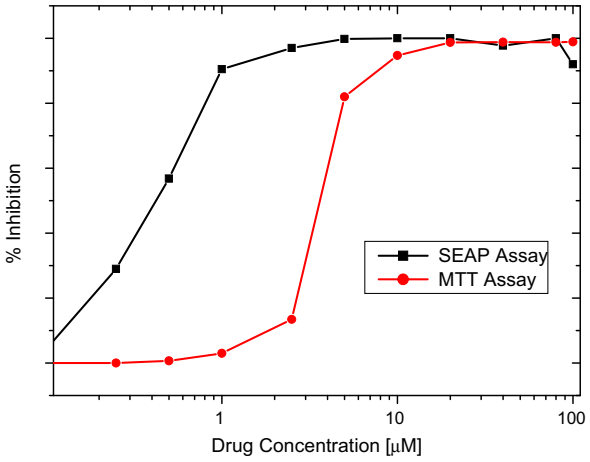



**Figure 4.** Inhibition of HIV Tat-regulated transactivation in COS cells by compound **5b**. The COS cells were co-transfected with a Tat-producing plasmid. A plasmid containing the HIV LTR linked to the SEAP reporter gene was treated with various concentrations of **5b**. After 48 h, aliquots of media were removed and analysis of SEAP activity was performed. The percent inhibition of SEAP activity by **5b** was calculated in comparison with that presented in the medium of untreated cells. In a separate experiment, COS-7 cells were exposed for the same 48 h time period to media containing increasing concentrations of compound **5b** and assayed for viability by use of the MTT colorimetric method. The data are presented as percent inhibition of cell viability, which was calculated relative to the value for untreated cells.

**Table 2.** Comparative potency ($TC_{50}$ and $IC_{50}$)[a,b] and relative safety (selective indexes,[c]SI) in COS cells by NDAG derivatives

| Compound | $TC_{50}$ (μM) | $IC_{50}$ (μM) | SI |
|---|---|---|---|
| **5a** | 27.32 | 17.23 | 1.59 |
| **5b** | 3.83 | 0.88 | 4.35 |
| **5c** | 19.71 | 17.34 | 1.14 |
| **5d** | 1.79 | 2.41 | 0.74 |
| **6a** | 5.53 | 2.79 | 1.98 |
| **6b** | 34.75 | 9.41 | 3.69 |
| **7** | 9.84 | 8.54 | 1.15 |

[a] Toxicity concentration ($TC_{50}$) and inhibition concentration ($IC_{50}$) obtained as the average of triplicate determination.
[b] $TC_{50}$ and $IC_{50}$ values were determined after a 48 h exposure to the test compound.
[c] The selective index was defined as the ratio: $TC_{50}/IC_{50}$.

In conclusion, the NDGA derivatives in the family of carbamate, carbonates, and heterocycle-containing ethers were synthesized by a chemical method. Among the seven new NDGA derivatives, heterocycle-containing ether NDGAs were found stable in aqueous medium and possessed inhibitory activity toward HIV Tat-regulated transactivation in human epithelial cells. The piperidine containing NDGA **5b** with $IC_{50}$ 0.88 μM exhibited the greatest potency for inhibiting Tat-induced transactivation. This compound could be regarded as an ideal lead for anti-HIV drug.

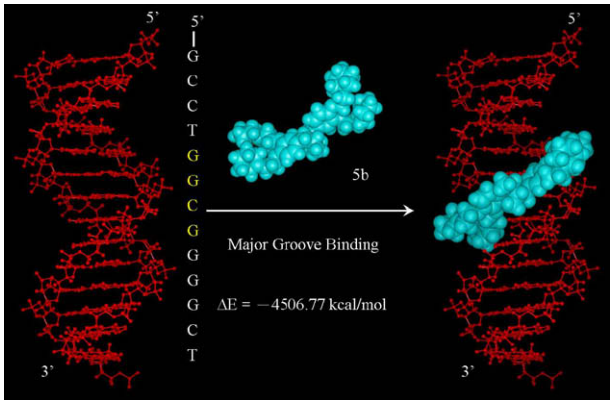



**Figure 5.** Possible complexation between NDGA derivative **5b** and a segment of DNA at its GC box through the major groove. The graphical results were obtained by CVFF calculations through local energy minimization.

## Acknowledgment

This work was supported by the National Science Council of Republic of China.

## Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2008.02.018.

## References and notes

1. Trang, T.; Stutak, M.; Quirion, R.; Jhamandas, K. *Br. J. Pharmacol.* **2003**, *140*, 295.
2. Nakadate, T. *Jpn. J. Pharmacol.* **1989**, *49*, 1.

3. Hausott, B.; Greger, H.; Marian, B. *J. Cancer Res. Clin. Oncol.* **2003**, *129*, 569.
4. Fujiwara, T.; Misumi, Y.; Ikehara, Y. *Biochem. Biophys. Res. Commun.* **2003**, *301*, 927.
5. (a) Cheng, J. S.; Jan, C. R. *Toxicol. In Vitro* **2002**, *16*, 485; (b) Huang, J. K.; Chen, W. C.; Huang, C. J.; Hsu, S. S.; Chen, J. S.; Cheng, H. H.; Chang, H. T.; Jiann, B. P.; Jan, C. R. *Life Sci.* **2004**, *75*, 2341.
6. Yamamura, H.; Nagano, N.; Hirano, M.; Muraki, K.; Watanabe, M.; Imaizumi, Y. *J. Pharmacol. Exp. Ther.* **1999**, *291*, 140.
7. Ono, K.; Hasegawa, K.; Yoshiike, Y.; Takashima, A.; Yamada, M.; Naiki, H. *J. Neurochem.* **2002**, *81*, 434.
8. Lee, C. H.; Jang, Y. S.; Her, S. J.; Moon, Y. M.; Baek, S. J.; Eling, T. *Exp. Cell. Res.* **2003**, *289*, 335.
9. Hwu, J. R.; Tseng, W. N.; Gnabre, J.; Giza, P.; Huang, R. C. *J. Med. Chem.* **1998**, *41*, 2994.
10. Huang, R. C.; Li, Y.; Giza, P. E.; Gnabre, J. N.; Abd-Elazem, I. S.; King, K. Y.; Hwu, J. R. *Antiviral Res.* **2003**, *58*, 57.
11. King, K. Y.; Hakimelahi, G. H.; Huang, R. C.; Hwu, J. R. *J. Genetics Mol. Biol.* **2002**, *13*, 248.
12. Gnabre, J. N.; Brady, J. N.; Clanton, D. J.; Ito, Y.; Dittmer, J.; Bates, R. B.; Huang, R. C. *Proc. Natl. Acad. Sci. U.S.A.* **1995**, *92*, 11239.
13. Gnabre, J.; Ito, Y.; Ma, Y.; Huang, R. C. *J. Chromatogr. A* **1996**, *719*, 353.
14. Chen, H.; Teng, L.; Li, J.; Park, R.; Mold, D. E.; Gnabre, J.; Hwu, J. R.; Tseng, W. N.; Huang, R. C. *J. Med. Chem.* **1998**, *41*, 3001.
15. Park, R.; Giza, P. E.; Mold, D. E.; Huang, R. C. *Antiviral Res.* **2003**, *58*, 35.
16. Craigol, J.; Callahan, M.; Huang, R. C.; DeLucia, A. *Antiviral Res.* **2000**, *47*, 19.
17. Chang, C.-C.; Heller, J. D.; Kuo, J.; Huang, R. C. *Proc. Natl. Acad. Sci. U.S.A.* **2004**, *101*, 13239.
18. Heller, J. D.; Kuo, J.; Wu, T. C.; Kast, W. M.; Huang, R. C. *Cancer Res.* **2001**, *61*, 5499.
19. Arya, S. K.; Guo, C.; Josephs, S. F.; Wong-Staal, F. *Science* **1985**, *229*, 69.
20. Hsu, M. C.; Schutt, A. D.; Holly, M.; Slice, L. W.; Sherman, M. I.; Richman, D. D.; Potash, M. J.; Volsky, D. J. *Science* **1991**, *254*, 1799.
21. Berger, J.; Hauber, J.; Hauber, R.; Geiger, R.; Cullen, B. *Gene* **1988**, *66*, 1.
22. Ducki, S.; Hadfield, J. A.; Hepworth, L. A.; Lawrence, N. J.; Liu, C. Y.; McGown, A. T. *Bioorg. Med. Chem. Lett.* **1997**, *7*, 3091.
23. Sjøttem, E.; Andersen, C.; Johansen, T. *J. Mol. Biol.* **1997**, *267*, 490.