

Synthesis of novel [3,2-*b*]indole fused oleanolic acids as potential inhibitors of cell proliferation

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Dedicated to Dr. Joseph M. Muchowski on the occasion of his 65th birthday, and in recognition of his numerous outstanding contributions to indole and pyrrole chemistry

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Abstract

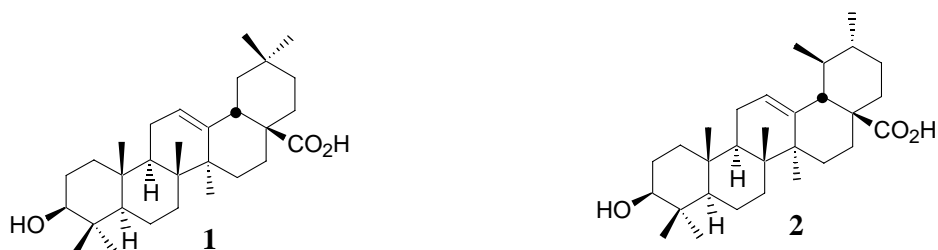
Seven new indole-fused oleanolic acid derivatives were synthesized from oleanolic acid for their ability to inhibit cell proliferation in NRP.152 cells.

Keywords: Oleanolic acid, cell proliferation, Fischer indole synthesis, indolotriterpenoids

Introduction

Triterpenoids are a diverse and ubiquitous group of C₃₀ pentacyclic compounds¹ that are derived biosynthetically from squalene cyclization.² Many triterpenoids display interesting biological and pharmacological profiles,³ which include the selective inhibition of inducible nitric oxide synthase (iNOS)⁴⁻⁷ and cyclooxygenase-2 (COX-2),^{5,6,8} modulation of collagen synthesis,⁹⁻¹¹ inhibition of tumorigenesis,^{12,13} and the ability to affect cell proliferation.^{14,15}

As part of our efforts to synthesize and screen for biological activity novel derivatives of oleanolic (**1**) and ursolic acid (**2**),^{5,6,16-20} we reported the ability of some 70 synthetic triterpenoids to affect cell proliferation in epithelial nonmalignant NRP.152 and malignant NRP.154 prostate cells.²¹ These NRP.152 prostate cells demonstrate sensitivity to retinoids and 1 α ,25-dihydroxyvitamin D₃ and may be used for analysis of normal prostate growth and prostatic carcinogenesis.^{22,23} Compounds that inhibit nonmalignant prostate cell proliferation mediated by the induction of TGF- β demonstrate potential as chemopreventive agents for prostate (and breast) cancer.²⁴



Results and Discussion

In the present paper we describe the synthesis of seven new indole-fused oleanolic acid derivatives, **3–9**, for evaluation in the NRP.252 cell assay. Fused heterocyclic derivatives of steroids and alkaloids are well documented,²⁵ and biologically active indole-fused examples are of particular interest.²⁶ Furthermore, several indole-fused steroids have been synthesized for electron-transfer studies.²⁷⁻³⁰ In contrast, only one research group has described the synthesis of indole-fused triterpenoids.^{31,32} Interestingly, a number of indole-fused diterpenes, such as the penitremes, are *Penicillium* fungal metabolites.³³ Our syntheses of the target compounds **3–9** (Figure 1) are based on the Fischer indole synthesis,^{34,35} and are depicted in Schemes 1–4.

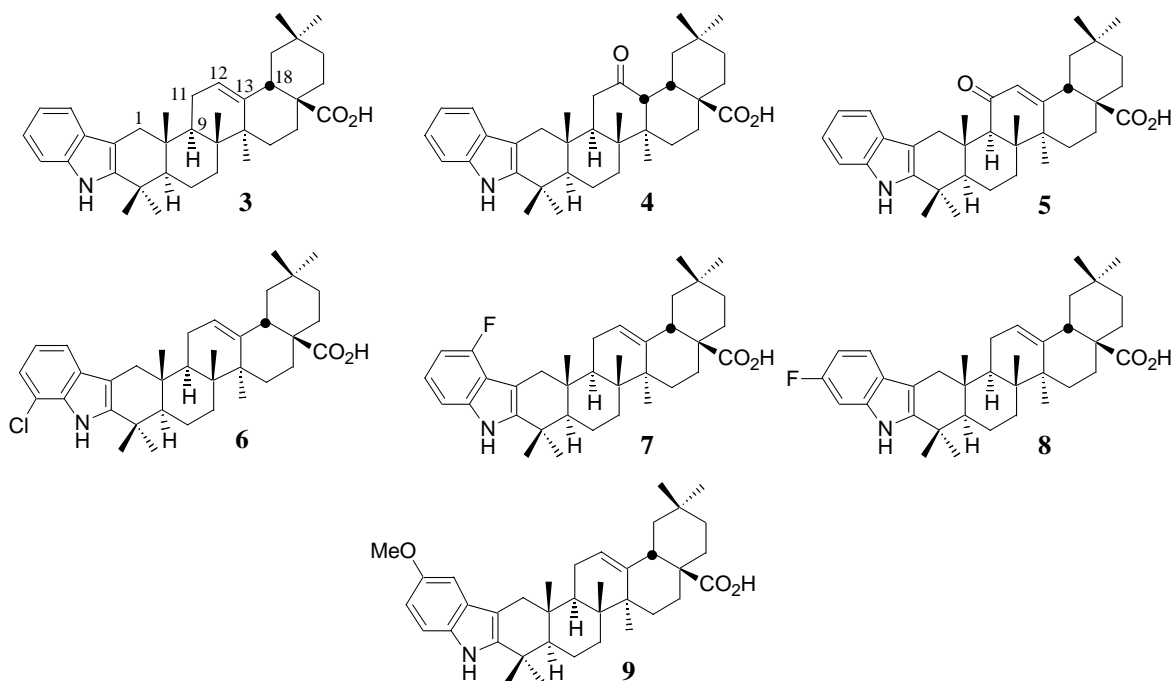
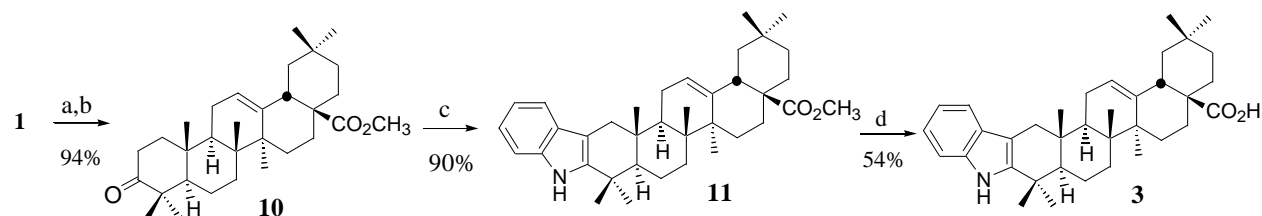


Figure 1

As we have previously described,¹⁶ sequential diazomethane treatment and Jones oxidation of oleanolic acid (**1**) furnished keto ester **10** in 94% yield (Scheme 1). Fischer indolization of **10**

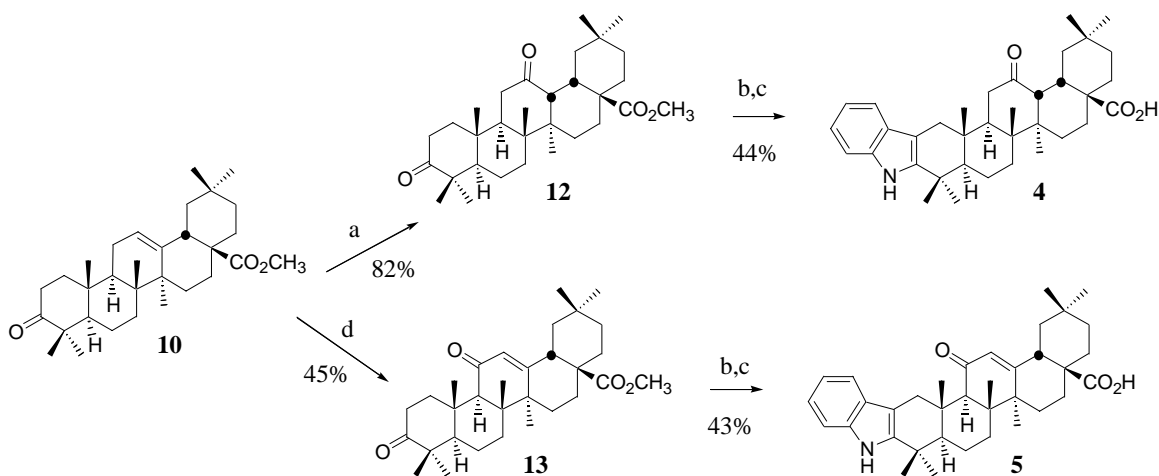
with phenylhydrazine in acetic acid gave the known³² fused indole ester **11** in 90% yield. Cleavage of this hindered methyl ester with lithium iodide in DMF³⁶ afforded **3** in 54% yield. The corresponding C-3 ketone obtained from **1** also underwent Fischer indolization to give **3** in 61% yield, but a persistent yellow contaminant could not be removed from **3** by either crystallization or silica gel chromatography.



(a) $\text{CH}_2\text{N}_2/\text{Et}_2\text{O}/\text{THF}$; (b) Jones oxidation; (c) phenylhydrazine/AcOH; (d) LiI/DMF.

Scheme 1

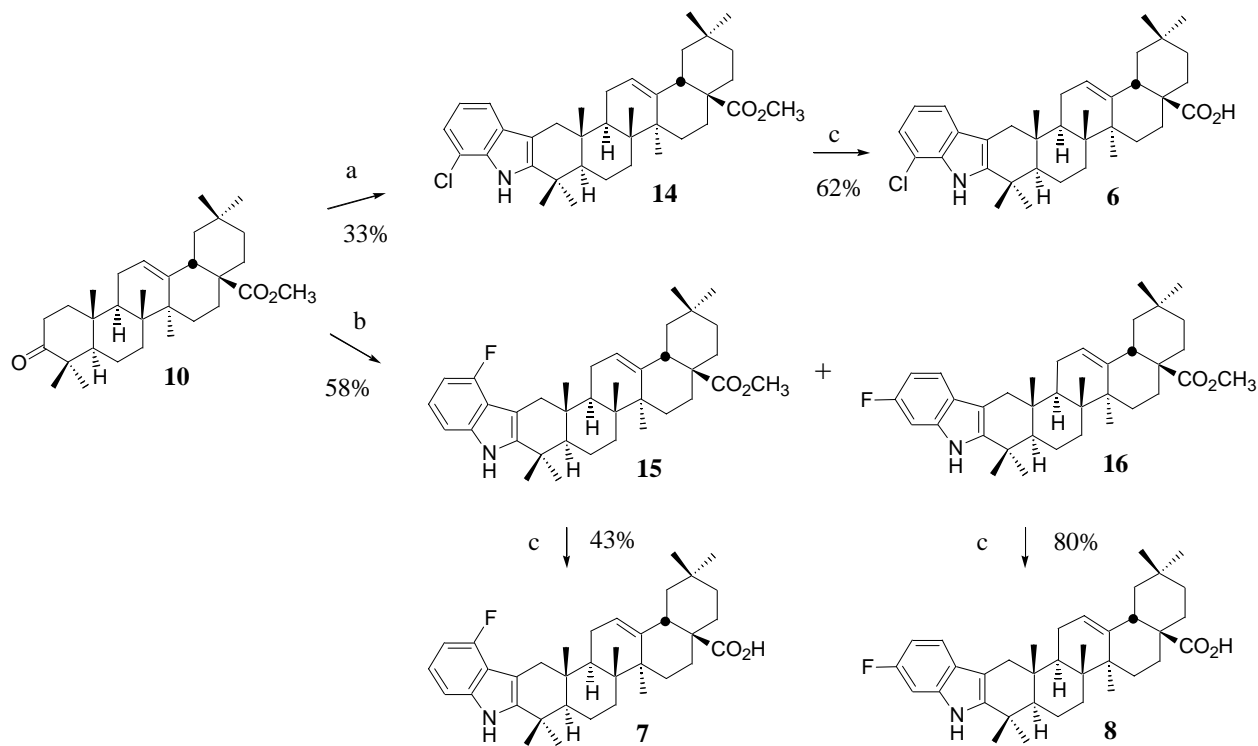
Due to the susceptibility of the indole ring in **3** to side reactions, particularly oxidation, modifications to the C-ring were performed prior to indolization. Thus, as shown in Scheme 2, and as we have previously described,²⁰ the synthesis of 3,12-diketone **12** was accomplished via an acid mediated epoxide rearrangement that occurred upon treatment of **10** with *m*-CPBA. Fischer indolization of **12** (74%) followed by ester cleavage (59%) gave the desired fused indole **4**. The highly hindered C-12 ketone in **12** remains unaffected under these Fischer indole reaction conditions.³⁷ Likewise, as we have reported,²⁰ allylic oxidation of **10** gave the known C-12,13 enone **13** (45% yield), which, upon Fischer indolization (79% yield) and ester cleavage (55% yield), afforded fused indole **5**.



(a) *m*CPBA/ CHCl_3 ; (b) phenylhydrazine/AcOH; (c) LiI/DMF; (d) $\text{CrO}_3/\text{tBuOOH}$.

Scheme 2

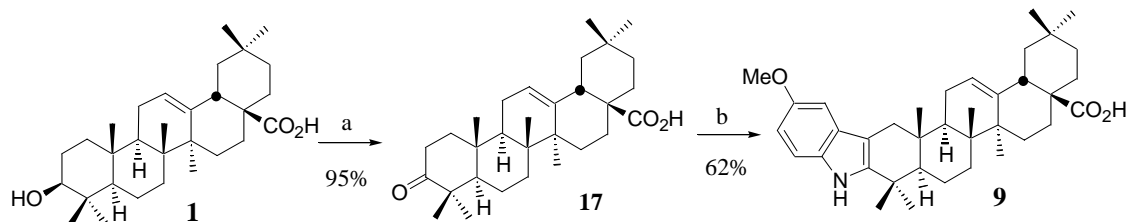
The indole ring substituted analogs **6–9** were synthesized by employing the appropriate substituted phenylhydrazine in the Fischer indolization (Scheme 3). Thus, treatment of **10** with 2-chlorophenylhydrazine³⁸ gave indole **14** that could be converted to indole **6** by ester cleavage. This indolization reaction was accompanied by 31% of uncyclized hydrazone. A sequence starting with 3-fluorophenylhydrazine yielded a mixture of indoles **15** and **16**, which were separated by sequential column and preparative silica gel chromatography in a 2:1 ratio, respectively. Cleavage of the methyl esters afforded **7** and **8**.



(a) 2-chlorophenylhydrazine/AcOH; (b) 3-fluorophenylhydrazine/AcOH; (c) LiI/DMF.

Scheme 3

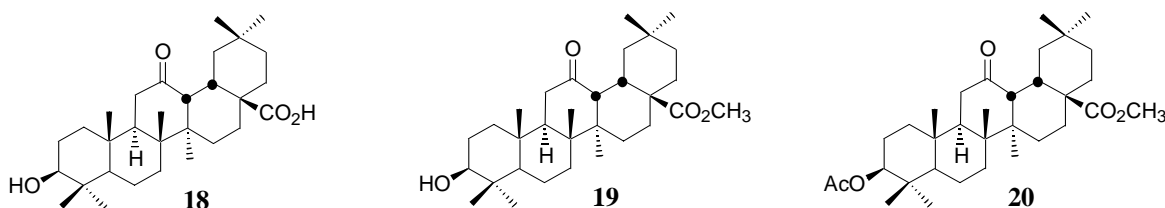
Finally, the 5-methoxyindole derivative **9** was synthesized directly from 3-keto acid **17** by Fischer indolization in 62% yield. The known keto acid **17** was prepared from oleanolic acid (**1**) by Jones oxidation (95% yield) as previously described.¹⁶ Interestingly, the corresponding methyl ester analog that was prepared by indolization of **10** decomposed under the lithium iodide ester cleavage conditions.



(a) Jones oxidation; (b) 4-methoxyphenylhydrazine/AcOH.

Scheme 4

Unfortunately, attempts to effect indolization of C-12 ring C ketone derivatives were unsuccessful, presumably due to the hindered nature of this position. For example, we could not prepare the phenylhydrazone of ketones **18** and **19**, or effect indolization of ketones **19** and **20** with 2-iodoaniline using the palladium-annulation method of Chen *et al.*³⁹



Oleanolic acid (**1**), indoles **3-9**, and **10**, **12**, and **13** were screened *in vitro* for their ability to inhibit proliferation of premalignant, non-tumorigenic prostate cells. Of the compounds prepared in the present study, only **4** and **5** showed some activity ($IC_{50} < 5 \mu M$). All of the others were essentially inactive in this assay ($> 5 \mu M$). For comparison, TGF- β has $IC_{50} = 0.000014 \mu M$.⁴⁰ Therefore, in view of the disappointing activity in this assay of this series of fused-indole oleananes, we are not currently pursuing the study of additional examples of indole-fused triterpenoids.

Experimental Section

General Procedures. Flash column chromatography was done with Select Scientific silica gel (230–400 mesh). 1H (300 MHz) and ^{13}C (75 MHz) NMR spectra were recorded on a Varian XL-300 spectrometer in $CDCl_3$ solvent; chemical shifts are reported with reference to the δ 7.27 signal of $CHCl_3$ (1H NMR) and δ 77.23 signal of $CDCl_3$ (^{13}C NMR) as an internal standard.

General procedure for Fischer indolization

A mixture of ketone **10** (89.3 mg, 0.191 mmol), phenylhydrazine (0.02 mL, $d = 1.1$, 1.05 eq), and glacial acetic acid (2 mL) was heated at reflux under N_2 for 30 min. During this period the color changed from colorless to bright yellow. The reaction mixture was pipetted into distilled

water (50 mL) and extracted with ether (4 x 20 mL). The combined ether extracts were washed with 5% aqueous NaOH (2 x 20 mL) and brine (2 x 20 mL), dried (Na₂SO₄), and concentrated in vacuo to afford a yellow solid. Flash chromatography over silica gel and elution with hexane-ethyl acetate afforded indole **11** (92.4 mg, 90%) as an amorphous pale yellow solid. The synthesis of indole **9** from ketone **17** was worked up by simply pouring into water, extracting with ethyl acetate, and processing in the usual way to give an amorphous product after flash chromatography. Indoles **9**, **11**, and **14–16** were all amorphous solids and were directly converted into the corresponding acids as described below.

General procedure for ester cleavage

A mixture of indole ester (0.09 mmol) and lithium iodide (0.45 mmol) in DMF (1.5 mL) under N₂ was heated at reflux for 15 h. The mixture was allowed to cool, treated with water (20 mL) and 10% aqueous hydrochloric acid (5 mL), and extracted with dichloromethane (3 x 20 mL). The organic layer was washed with water, dried (MgSO₄), and concentrated in vacuo to afford the crude acid. Purification was effected by preparative TLC (hexane/ethyl acetate, 4:1) to give **3–8** as amorphous solids, for which melting points could not be obtained. The amounts of compounds, which were needed for biological screening, were insufficient for crystallization. Spectra data of **3–9** are tabulated in Tables 1–3.

Table 1. High-Resolution Mass Spectral Data of **3–6, 9** [*m/z*]

	Compound	M ⁺ , Calculated	M ⁺ , Observed
3	C ₃₆ H ₄₉ NO ₂	527.3763	527.3751
4	C ₃₆ H ₄₉ NO ₃	543.3712	543.3672
5	C ₃₆ H ₄₇ NO ₃	541.3556	541.3582
6	C ₃₆ H ₄₈ NO ₂ Cl	561.3374	561.3385
9	C ₃₇ H ₅₁ NO ₃	557.3869	557.3884

Table 2. ¹H NMR data of **3–9**: δ [ppm]; coupling constants *J* [Hz]

	Me	H-1	H-12	H-18	NH	Aromatic	Other
3	0.85, 0.90, 0.94 (2), 1.15, 1.17, 1.27	2.75; 15.3 2.18; 15.9	5.38 1.15	2.87; 9.9	7.70	7.41; 6.9 7.28; 7.5 7.07 (2)	-
4	0.87, 0.92 1.00 (2), 1.07, 1.15, 1.28	2.69; 14.7 2.14; 14.7		2.80	7.75	7.40; 7.5 7.27; 7.2 7.08; (2)	2.49; 5.1, 17.1 2.33; 13.1 6.5 (H-11) 2.75; 4.2 (H-13)
5	0.94, 0.95, 0.99, 1.13, 1.15, 1.26, 1.39	3.96; 15.6 2.25; 15.6	5.72	3.01	7.72	7.49; 7.2 7.27; 7.2 7.07 (2)	2.62 (H-9)
6	0.84, 0.90,	2.73; 15.0	5.37	2.86; 9.9	7.84	7.30; 7.5	

Table 2. Continued

	0.93 (2), 1.17, 1.19, 1.30	2.18; 15.0				7.09; 1.2, 7.8 6.97; 7.8, 7.8	
7	0.84, 0.90, 0.94, 0.96, 1.14, 1.17, 1.26	2.71; 14.7 2.16; 14.7	5.37	2.87; 13.5	7.71	7.29; 5.4, 8.7 6.96; 2.4, 9.9 6.80; 2.4, 9.0, 9.9	
8	0.84, 0.90, 0.94, 0.96, 1.15, 1.16, 1.26	2.98; 15.6 2.35; 15.6	5.37	2.86; 13.5	7.76	7.04; 8.1 6.98 5.1, 7.8 6.67; 7.5, 10.8	
9	0.84, 0.90, 0.94 (2), 1.14, 1.17, 1.25	2.70; 14.7 2.16; 15.3	5.37	2.86; 12.6	7.59	7.16; 8.4 6.87; 2.4 6.75; 8.7, 2.4	3.83 (OMe)

Table 3. ^{13}C NMR Data of **3–9**: δ [ppm]

	CO ₂ H	Ketone	C=C	Aromatic
3	184.5		145.6 123.1	141.0, 136.3, 128.4, 121.2, 119.1, 118.2, 110.5, 107.1
4	184.7	211.8		140.7, 136.3, 128.2, 121.4, 119.3, 118.2, 110.6, 106.5
5	178.4	199.6	169.5	141.3, 137.6, 129.0, 120.9,
6	184.4		143.6	141.9, 133.4, 129.9, 120.6,
7	184.6		143.6 125.0	161.3, 158.1, 143.6, 141.3, 136.3, 136.1, 123.0, 118.7, 107.3, 107.0, 97.0
8	184.5		143.4 123.2	155.6, 140.8, 139.0, 138.9, 121.4, 116.9, 111.5, 106.6, 105.7, 104.6
9	184.3		143.6 123.1	154.0, 142.1, 131.4, 128.8, 111.2, 110.9, 107.0, 100.6

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References and Notes

1. Connolly, J. D.; Hill, R. A. *Nat. Prod. Rep.* **2000**, *19*, 494 and previous reviews in this series.
2. Prestwich, G. D.; Abe, I.; Zheng, Y. F.; Robustell, B. J.; Tongyun, D. *Pure Appl. Chem.* **1999**, *71*, 1127.
3. Tang, W.; Eisenbrand, G. *Chinese Drugs of Plant Origin*; 1992, Springer-Verlag, p 1056.
4. Dirsch, V. M.; Kiemer, A. K.; Wagner, H.; Vollmar, A. M. *Eur. J. Pharmacol.* **1997**, *336*, 211.
5. Suh, N.; Honda, T.; Finlay, H. J.; Barchowsky, A.; Williams, C.; Benoit, N. E.; Xie, Q. W.; Nathan, C.; Gribble, G. W.; Sporn, M. B. *Cancer Res.* **1998**, *58*, 717.
6. Suh, N.; Wang, Y. P.; Honda, T.; Gribble, G. W.; Dmitrovsky, E.; Hickey, W. F.; Maue, R. A.; Place, A. E.; Porter, D. M.; Spinella, M. J.; Williams, C.; Wu, G.; Dannenberg, A. J.; Flanders, K. C.; Letterio, J. J.; Mangelsdorf, D. J.; Nathan, C. F.; Nguyen, L.; Porter, W. W.; Ren, R. F.; Roberts, A. B.; Roche, N. S.; Subbaramaiah, K.; Sporn, M. B. *Cancer Res.* **1999**, *59*, 336.
7. Honda, T.; Honda, Y.; Favalaro, Jr., F. G.; Gribble, G. W.; Suh, N.; Place, A. E.; Rendi, M. H.; Sporn, M. B. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 1027.
8. Ringbom, T.; Segura, L.; Noreen, Y.; Perera, P.; Bohlin, L. *J. Nat. Prod.* **1998**, *61*, 1212.
9. Cha, H. J.; Bae, S. K.; Lee, H. Y.; Lee, O. K.; Sato, H.; Seiki, M.; Park, B. C.; Kim, K. W. *Cancer Res.* **1996**, *56*, 2281.
10. Kweifio-Okai, G.; De Munk, F.; Macrides, T. A.; Smith, P.; Rumble, B. A. *Drug Dev. Res.* **1995**, *36*, 20.
11. Tenni, R.; Zanaboni, G.; De Agostini, M. P.; Rossi, A.; Bendotti, C.; Cetta, G. *Ital. J. Biochem.* **1988**, *37*, 69.
12. Huang, M. T.; Ho, C. T.; Wang, Z. Y.; Ferraro, T.; Lou, Y. R.; Stauber, K.; Ma, W.; Georgiadis, C.; Laskin, J. D.; Conney, A. H. *Cancer Res.* **1994**, *54*, 701.
13. Wang, Z. Y.; Agarwal, R.; Zhou, Z. C.; Bickers, D. R.; Mukhtar, H. *Carcinogenesis* **1991**, *12*, 187.
14. Umehara, K.; Takagi, R.; Kuroyanagi, M.; Ueno, A.; Taki, T.; Chen, Y. J. *Chem. Pharm. Bull.* **1992**, *40*, 401.
15. Paper, D. H. *Planta Med.* **1998**, *64*, 686.
16. Honda, T.; Finlay, H. J.; Gribble, G. W.; Suh, N.; Sporn, M. B. *Bioorg. Med. Chem. Lett.* **1997**, *7*, 1623.
17. Honda, T.; Rounds, B. V.; Gribble, G. W.; Suh, N.; Wang, Y.; Sporn, M. B. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 2711.
18. Honda, T.; Rounds, B. V.; Bore, L.; Favalaro, Jr., F. G.; Gribble, G. W.; Suh, N.; Wang, Y.; Sporn, M. B. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 3429.
19. Honda, T.; Gribble, G. W.; Suh, N.; Finlay, H. J.; Rounds, B. V.; Bore, L.; Favalaro, Jr., F. G.; Wang, Y.; Sporn, M. B. *J. Med. Chem.* **2000**, *43*, 1866.

20. Honda, T.; Rounds, B. V.; Bore, L.; Finlay, H. J.; Favalaro, Jr., F. G.; Suh, N.; Wang, Y.; Sporn, M. B.; Gribble, G. W. *J. Med. Chem.* **2000**, *43*, 4233.
21. Finlay, H. J.; Honda, T.; Gribble, G. W.; Danielpour, D.; Benoit, N. E.; Suh, N.; Williams, C.; Sporn, M. B. *Bioorg. Med. Chem. Lett.* **1997**, *7*, 1769.
22. Danielpour, D.; Kadomatsu, K.; Anzano, M. A.; Smith, M.; Sporn, M. B. *Cancer Res.* **1994**, *54*, 3413.
23. Danielpour, D. *J. Cell Physiol.* **1996**, *166*, 231.
24. Sporn, M. B. *Cancer Res.* **1991**, *51*, 6215.
25. Camoutsis, C.; Nikolaropoulos, S. *J. Heterocycl. Chem.* **1998**, *35*, 731.
26. Brown, D. W.; Mahon, M. F.; Ninan, A.; Sainsbury, M. *J. Chem. Soc., Perkin Trans. 1* **1997**, 2329.
27. Antaki, H.; Petrow, V. *J. Chem. Soc.* **1951**, 901.
28. Warnhoff, E. W.; Nanonggai, P. *J. Org. Chem.* **1962**, *27*, 1186.
29. Ban, Y.; Sato, Y. *Chem. Pharm. Bull.* **1965**, *13*, 1073.
30. Haugland, R. P.; Yguerabide, J.; Stryer, L. *Proc. Natl. Acad. Sci.* **1969**, *63*, 23.
31. Tolstikov, G. A.; Kim, K.; Goryaev, M. I. *Zh. Obshch. Khim.* **1967**, *37*, 1690.
32. Kim, K.; Tolstikov, G. A.; Shumov, I. P.; Nasonova, A. M. *Zh. Org. Khim.* **1969**, *5*, 1987.
33. (a) de Jesus, A. E.; Steyn, P. S.; Van Heerden, F. R.; Vleggaar, R.; Wessels, P. L.; Hull, W. E. *J. Chem. Soc., Chem. Commun.* **1981**, 289. (b) de Jesus, A. E.; Steyn, P. S.; Van Heerden, F. R.; Vleggaar, R.; Wessels, P. L.; Hull, W. E. *J. Chem. Soc., Perkin Trans. 1* **1983**, 1847. (c) de Jesus, A. E.; Steyn, P. S.; Van Heerden, F. R.; Vleggaar, R.; Wessels, P. L.; Hull, W. E. *J. Chem. Soc., Perkin Trans. 1* **1983**, 1857. (d) Penn, J.; Biddle, J. R.; Mantle, P. G.; Bilton, J. N.; Sheppard, R. N. *J. Chem. Soc., Perkin Trans. 1* **1992**, 23. (e) Musuku, A.; Selala, M. I.; de Bruyne, T.; Claeys, M.; Schepens, P. J. C.; Tsatsakis, A.; Shtilman, M. I. *J. Nat. Prod.* **1994**, *57*, 983.
34. Robinson, B. *The Fischer Indole Synthesis*, Wiley-Interscience: New York, 1982.
35. Gribble, G. W. *J. Chem. Soc., Perkin Trans. 1* **2000**, 1045.
36. Dean, P. D. G. *J. Chem. Soc.* **1965**, 6655.
37. Gillies, C. W. *J. Am. Chem. Soc.* **1975**, *97*, 1276.
38. This was prepared in 41% yield by diazotization and SnCl₂ reduction of 2-chloroaniline using the general procedure reported: Witte, J.; Boekelheide, V. *J. Org. Chem.* **1972**, *37*, 2849.
39. Chen, C.-Y.; Lieberman, D. R.; Larsen, R. D.; Verhoeven, T. R.; Reider, P. J. *J. Org. Chem.* **1997**, *62*, 2676.
40. Unpublished results from our laboratory.