5-2-2003

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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

(received 18 Oct 02; accepted 24 Apr 03; published on the web 02 May 03)

Abstract

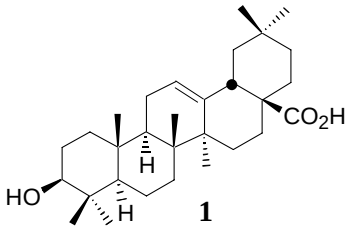
Seven new indole-fused oleanolic acid derivatives were synthesized from oleanolic acid for their ability to inhibit cell proliferation in NRP.152 cells.

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

Introduction

Triterpenoids are a diverse and ubiquitous group of C_{30} pentacyclic compounds[1] 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 1α,25-dihydroxyvitamin D_3 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]



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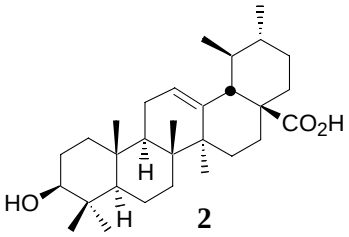

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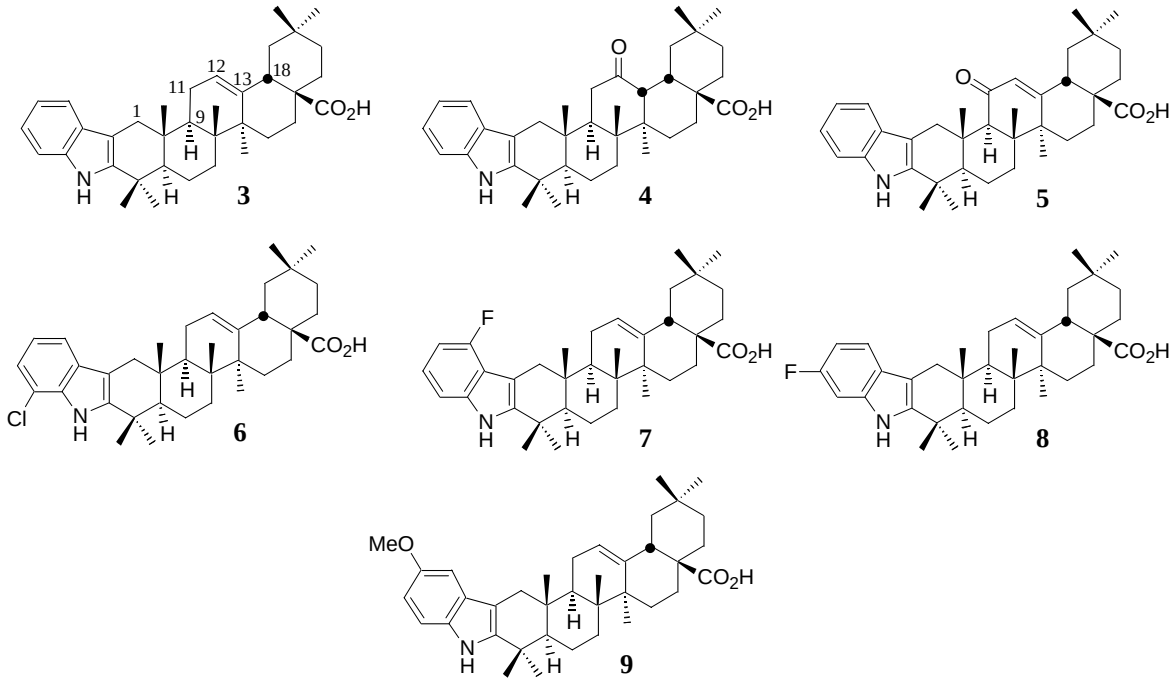
Results and Discussion

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 penitrems, 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.

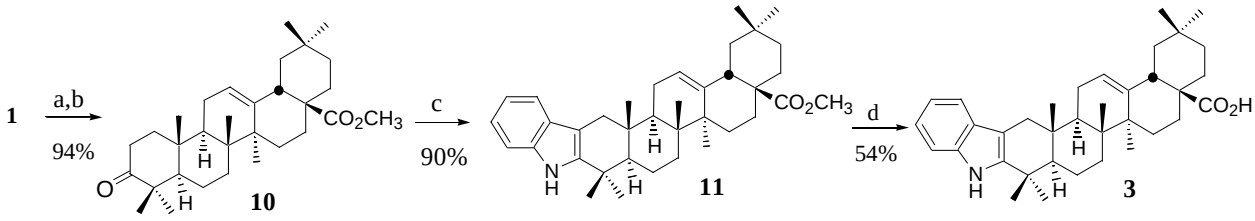


3 **4** **5** **6** **7** **8** **9**

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**

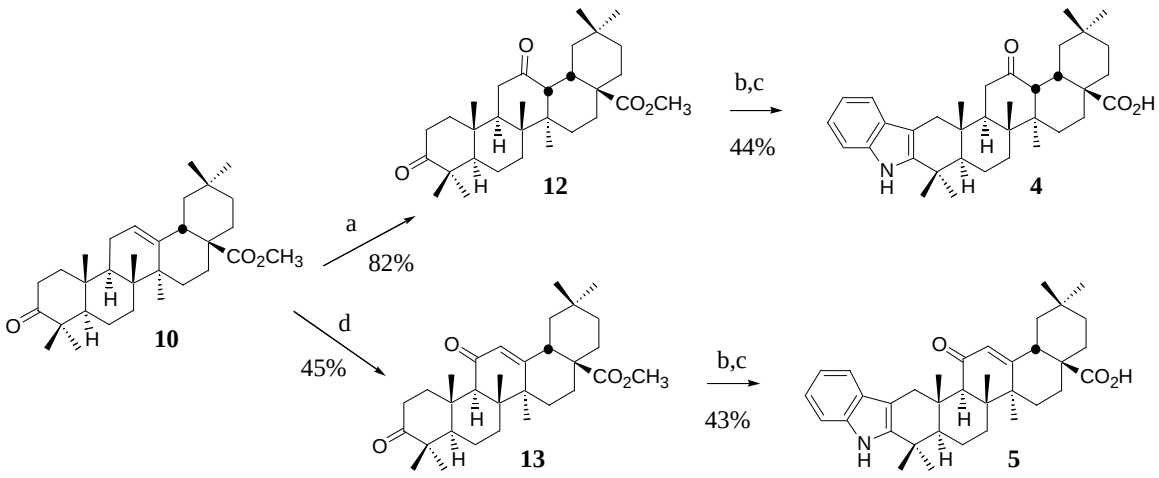
with phenylhydrazine in acetic acid gave the known[32] fused indole ester **11** in 90% yield. Cleavage of this hindered methyl ester with lithium iodide in DMF[36] afforded **3** in 54% yield. The corresponding C-3 ketone obtained from **1** also underwent Fischer indolization to give **3** in 61% yield, but a persistent yellow contaminant could not be removed from **3** by either crystallization or silica gel chromatography.



(a) CH_2N_2/Et_2O/THF; (b) Jones oxidation; (c) phenylhydrazine/AcOH; (d) LiI/DMF.

Scheme 1

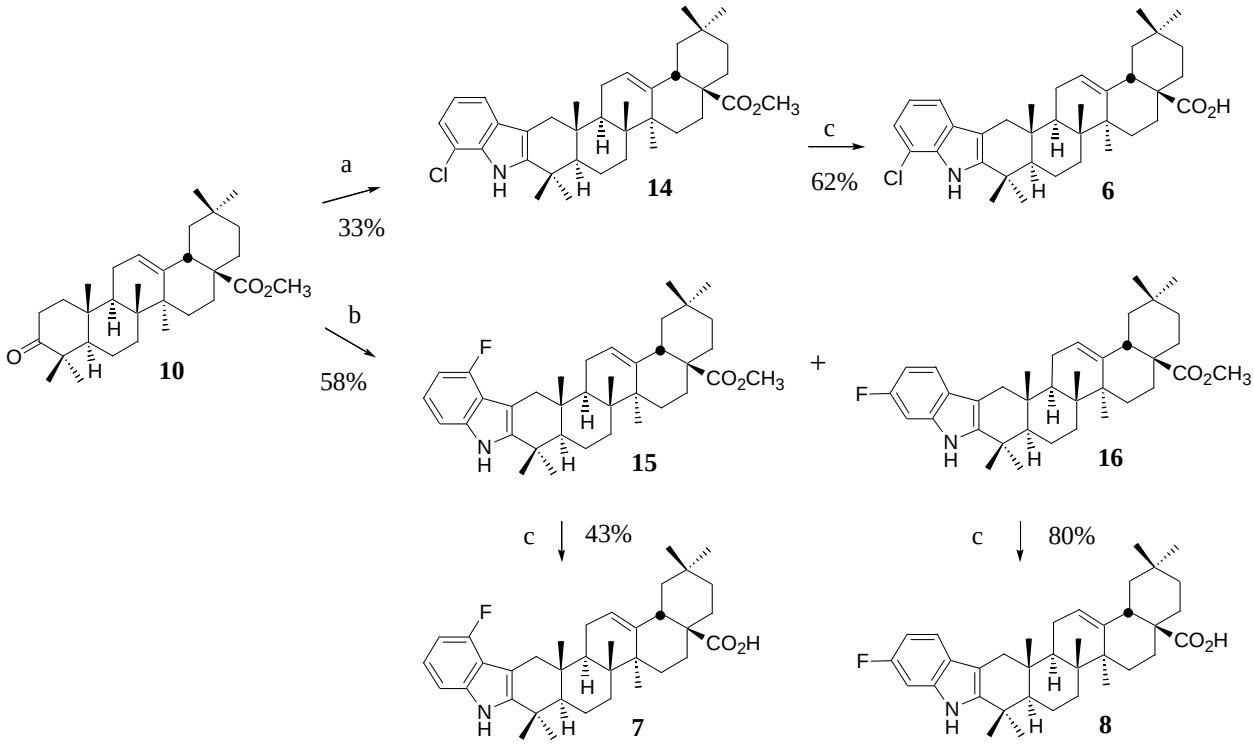
Due to the susceptibility of the indole ring in **3** to side reactions, particularly oxidation, modifications to the C-ring were performed prior to indolization. Thus, as shown in Scheme 2, and as we have previously described,[20] the synthesis of 3,12-diketone **12** was accomplished via an acid mediated 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) mCPBA/$CHCl_3$; (b) phenylhydrazine/AcOH; (c) LiI/DMF; (d) $CrO_3/^tBuOOH$.

Scheme 2

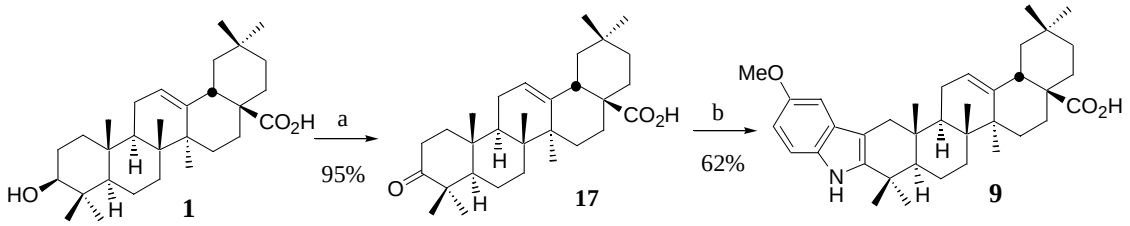
The indole ring substituted analogs **6**–**9** were synthesized by employing the appropriate substituted phenylhydrazine in the Fischer indolization (Scheme 3). Thus, treatment of **10** with 2-chlorophenylhydrazine[38] gave indole **14** that could be converted to indole **6** by ester cleavage. This 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

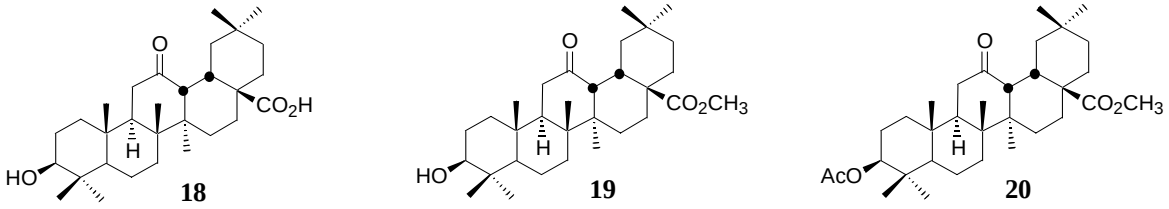
Finally, the 5-methoxyindole derivative **9** was synthesized directly from 3-keto acid **17** by Fischer indolization in 62% yield. The known keto acid **17** was prepared from oleanolic acid (**1**) by Jones oxidation (95% yield) as previously described.[16] Interestingly, the corresponding methyl 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 μM). All of the others were essentially inactive in this assay (>5 μM). For comparison, TGF-β has IC_{50} = 0.000014 μM.[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). ^{1}H (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 C*H*Cl_3 (^{1}H NMR) and δ 77.23 signal of *C*DCl_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 (Na_2SO_4), 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 N_2 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 ($MgSO_4$), 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	$C_{36}H_{48}NO_2Cl$	561.3374	561.3385
9	$C_{37}H_{51}NO_3$	557.3869	557.3884

Table 2. ^{1}H 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,	2.75; *15.3*	5.38	2.87; *9.9*	7.70	7.41; *6.9*	-
	0.94 (2), 1.15,	2.18; *15.9*	1.15			7.28; *7.5*	
	1.17, 1.27					7.07 (2)	
4	0.87, 0.92	2.69; *14.7*		2.80	7.75	7.40; *7.5*	2.49; *5.1*, *17.1*
	1.00 (2), 1.07,	2.14; *14.7*				7.27; *7.2*	2.33; *13*,*1 6.5* (H-11)
	1.15, 1.28					7.08; (2)	2.75; *4.2* (H-13)
5	0.94, 0.95,	3.96; *15.6*	5.72	3.01	7.72	7.49; *7.2*	2.62 (H-9)
	0.99, 1.13,	2.25; *15.6*				7.27; *7.2*	
	1.15, 1.26, 1.39					7.07 (2)	
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_2H	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

Acknowledgments

This investigation was supported by funds from the Norris Cotton Cancer Center. Mass spectral data were kindly furnished by Dr. Steven Mullen and Mary Young at the University of Illinois and Dr. Larry Reilly. We also thank Drs. Michael B. Sporn and Nanjoo Suh of the Dartmouth Medical School for performing the biological assays and for their continued interest and support of this project.

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