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

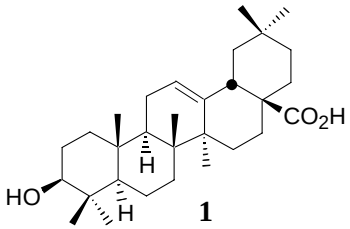
Seven new indole-fused oleanolic acid derivatives were synthesized from oleanolic acid for their ability to inhibit cell proliferation in NRP.152 cells.

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

Introduction

Triterpenoids are a diverse and ubiquitous group of 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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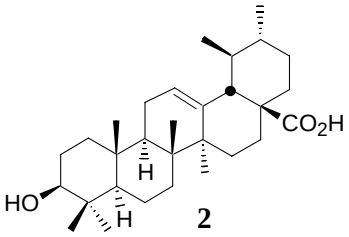

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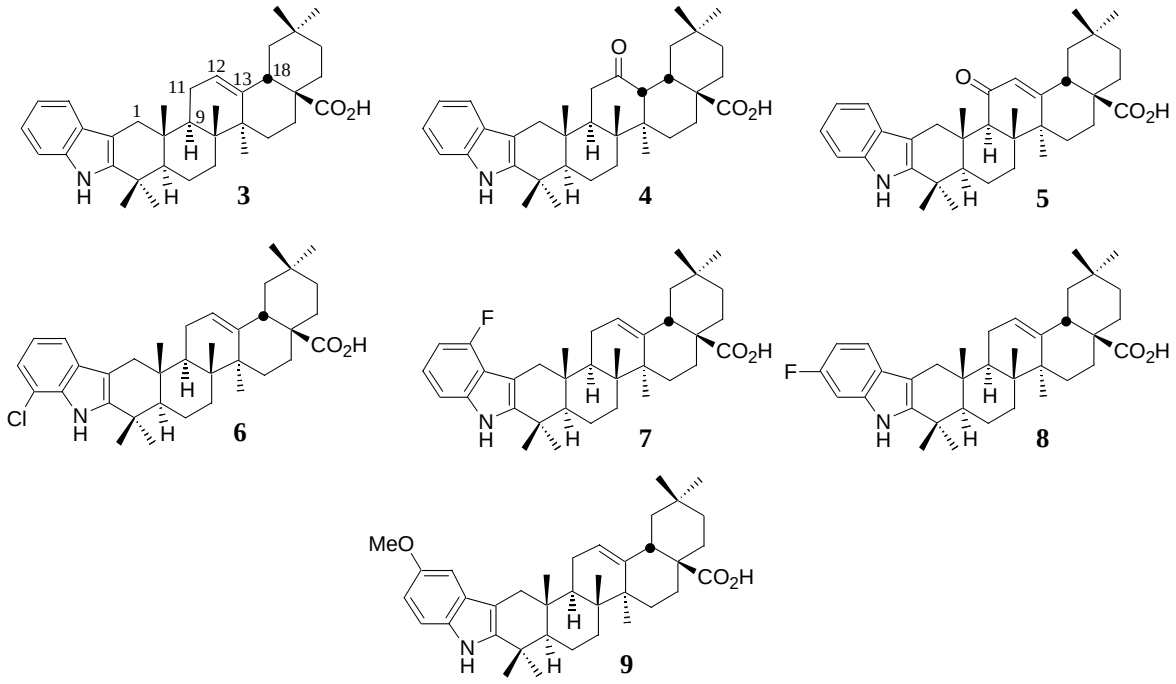
Results and Discussion

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.

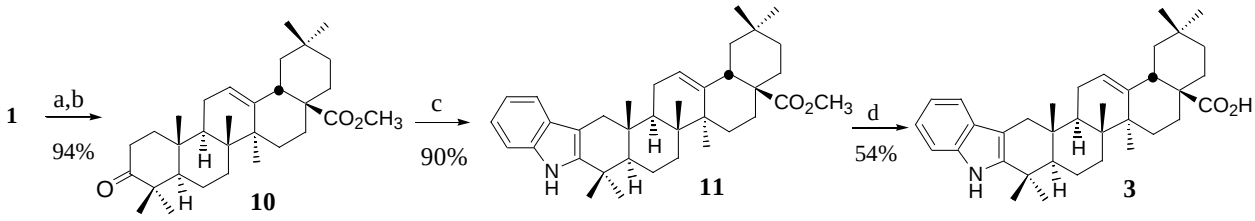


3 **4** **5** **6** **7** **8** **9**

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**

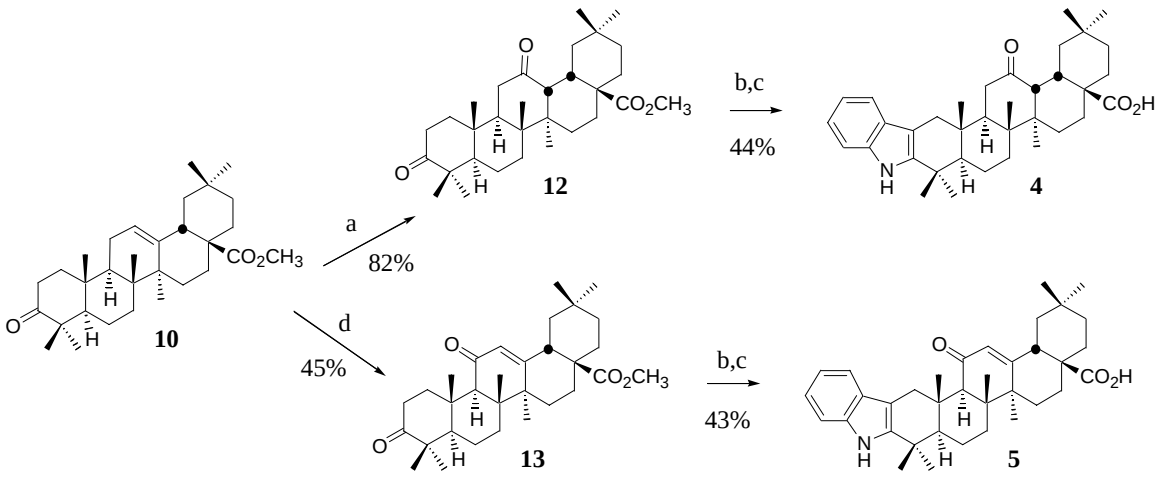
with phenylhydrazine in acetic acid gave the known[32] fused indole ester **11** in 90% yield. Cleavage of this hindered methyl ester with lithium iodide in DMF[36] afforded **3** in 54% yield. The corresponding C-3 ketone obtained from **1** also underwent Fischer indolization to give **3** in 61% yield, 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

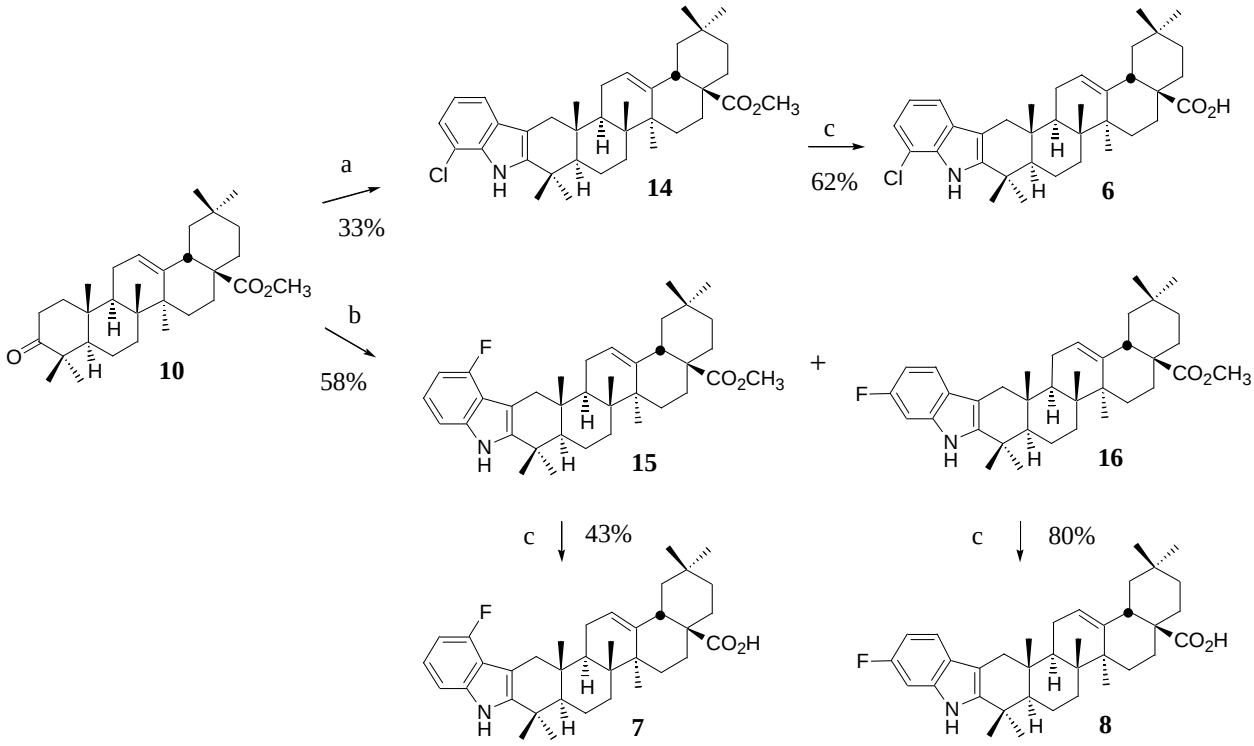
Due to the susceptibility of the indole ring in **3** to side reactions, particularly oxidation, modifications to the C-ring were performed prior to indolization. Thus, as shown in Scheme 2, and as we have previously described,[20] the synthesis of 3,12-diketone **12** was accomplished via an acid mediated 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

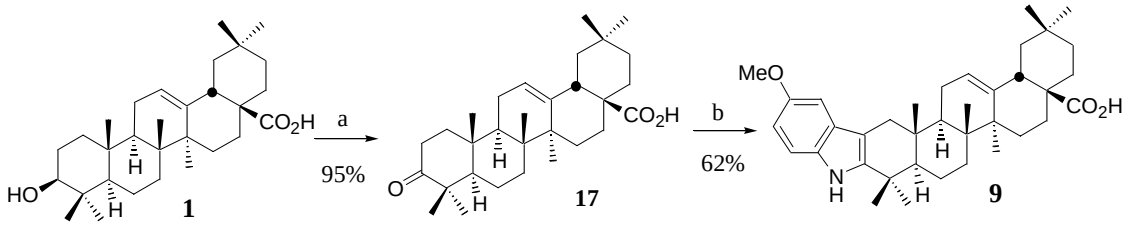
The indole ring substituted analogs **6**–**9** were synthesized by employing the appropriate substituted phenylhydrazine in the Fischer indolization (Scheme 3). Thus, treatment of **10** with 2-chlorophenylhydrazine[38] gave indole **14** that could be converted to indole **6** by ester cleavage. This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

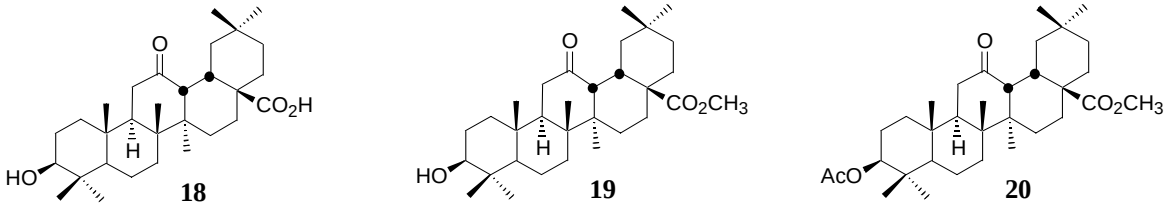
Finally, the 5-methoxyindole derivative **9** was synthesized directly from 3-keto acid **17** by Fischer indolization in 62% yield. The known keto acid **17** was prepared from oleanolic acid (**1**) by Jones oxidation (95% yield) as previously described.[16] Interestingly, the corresponding methyl 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

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