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Novel routes for the generation of structurally diverse labdane diterpenes from andrographolide†

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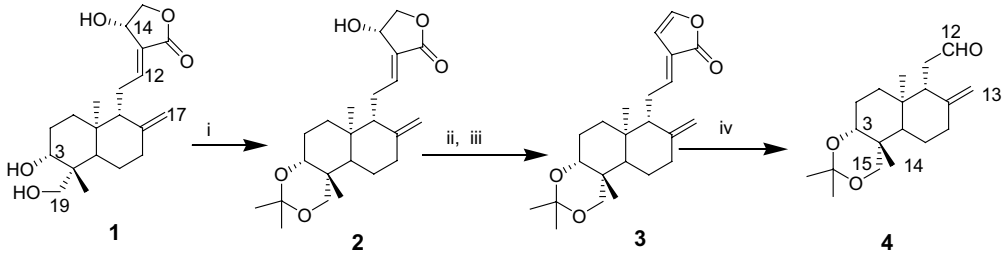
Abstract—Andrographolide **1**, the major constituent of the Indian medicinal plant *Andrographis paniculata* (Acanthaceae) was converted into the key intermediate **4** by selective oxidative degradation of the C-12,13 olefin bond. The aldehyde functional group present in **4** has been utilized for synthesizing a number of structurally diverse labdane diterpenes. Synthesis and in vitro cytotoxic activity results of the compounds prepared are discussed.

The utilization of natural products as a source of structural or functional templates for the design and synthesis of a wide variety of novel molecules has been an important aspect of new drug design. The isolation of a number of biologically active di- and triterpenes such as podolactones,[1] quassinoids,[2] labdane diterpenes[3] and compounds of mixed biosynthetic pathways viz., wiedendiol[4] has greatly stimulated the development of efficient synthetic methodologies for their preparation owing to their presence in only small quantities in the natural sources. Several groups have utilized the naturally occurring sclareol,[5,6] sclareolide,[7,8] communic acids[9,10] and manool[11] as starting materials for these syntheses. Most of the methods used involved side-chain degradations of labdanes. In this study, we have developed an efficient semi-synthetic procedure for the preparation of structurally diverse labdanes from andrographolide **1**, the major constituent of the Indian medicinal plant *Andrographis paniculata* (Acanthaceae).[12]

The method involved conversion of **1** into the key intermediate **4**, which served as a chiral intermediate (Scheme 1). Initially, the hydroxyl groups at C-3 and 19 of **1** were protected as an isopropylidene to yield **2**. Our



Scheme 1. Reagents and conditions: (i) 2,2-dimethoxypropane/PPTS/benzene/reflux, 95%; (ii) Ac_2O/TEA/DCM/rt, 95%; (iii) DMAP/DCM/rt, 80%; (iv) aq $KMnO_4$/THF, rt, 62%.

Keywords: Andrographolide; *Andrographis paniculata*; Labdane diterpenes.
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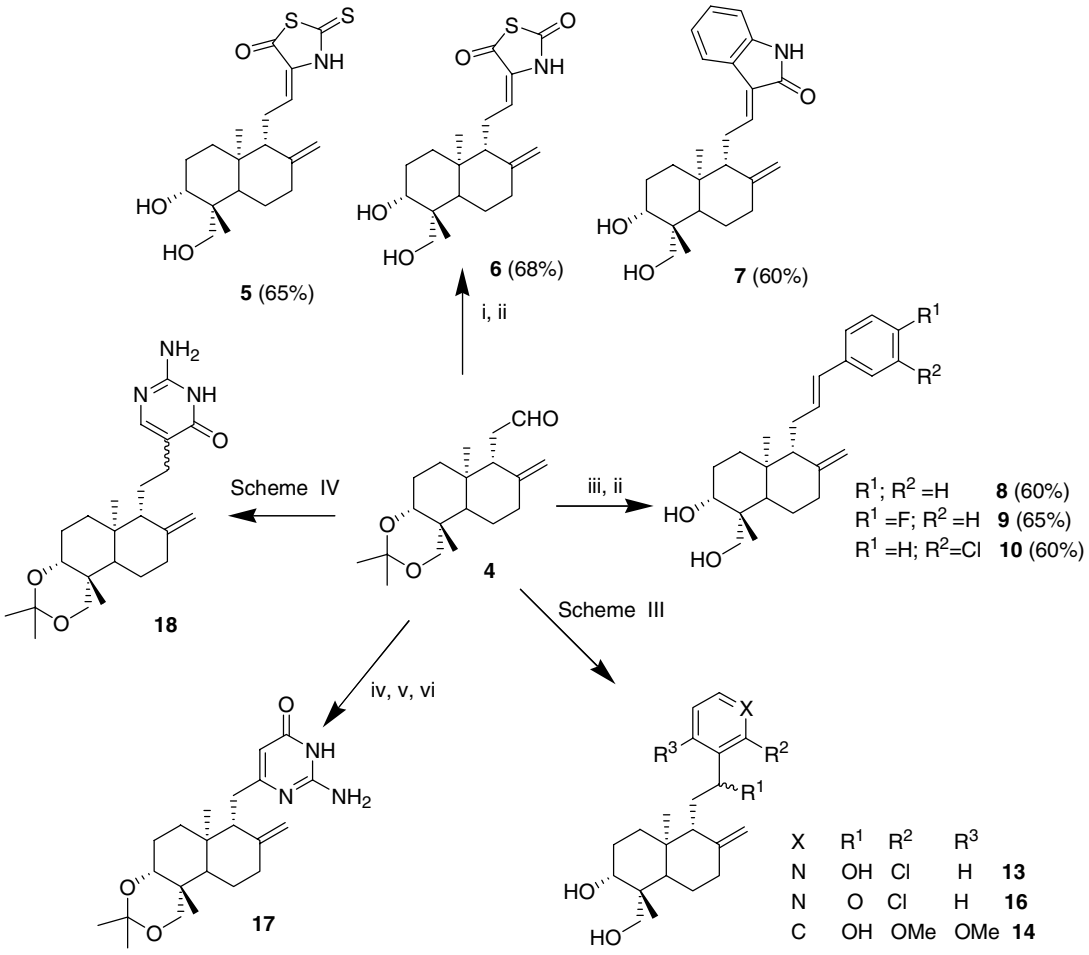
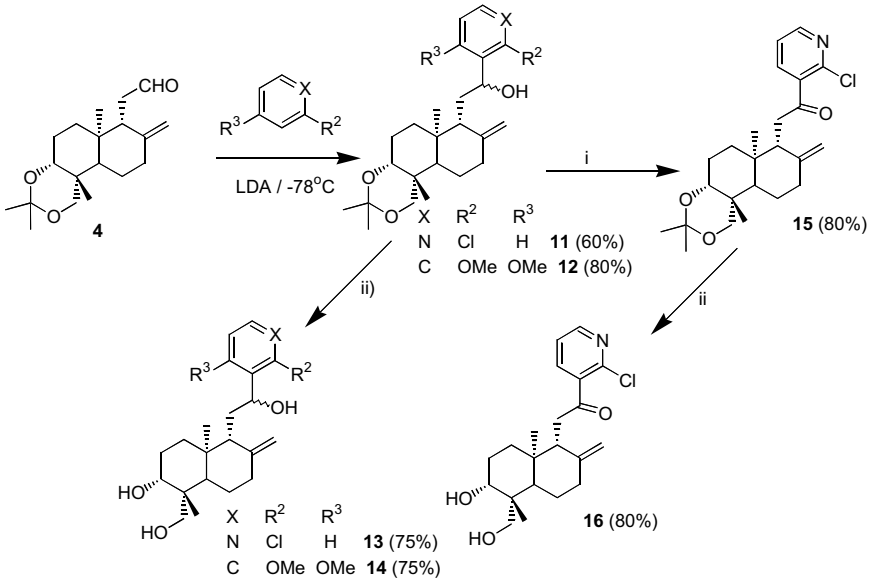
efforts to oxidize the C-12,13 olefin bond present in **2** with $KMnO_4$ to yield **4** did not give satisfactory results. However, quantitative preparation of **4**[13] could be achieved by oxidation of **3**. Compound **3** was synthe-



Scheme 2. Reagents and conditions: (i) rhodanine/thiazolidinedione/oxindole, β-alanine/benzene/reflux; (ii) $AcOH/H_2O$ (7:3), rt; (iii) Wittig salts of substituted benzyl bromides/NaH/benzene/rt; (iv) $BrCH_2CO_2Et$/Zn/benzene/reflux, (70%); (v) PDC, DCM/rt, (90%); (vi) guanidinium chloride/NaOMe/MeOH/reflux, (60%).



Scheme 3. Reagents and conditions: (i) PDC/DCM, (ii) $AcOH/H_2O$ (7:3).

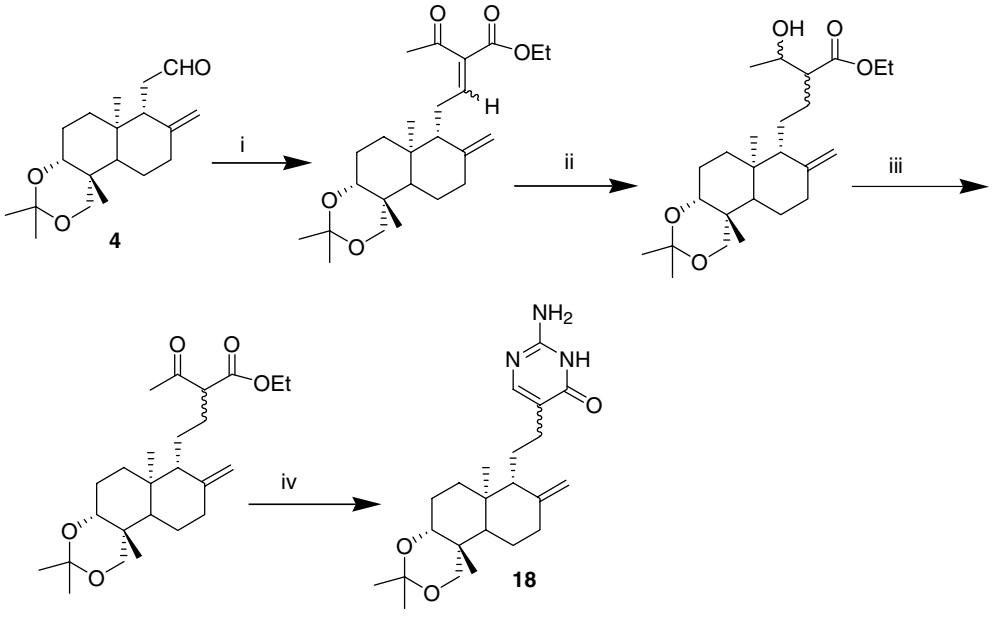
sized from **2** by elimination of the hydroxyl at C-14 as its acetate.

The key intermediate **4** obtained above was converted into a number of labdane diterpenes by utilizing its aldehyde functionality (Scheme 2). Knoevenagel condensation of **4** with rhodanine, thiazolidinedione and oxindole followed by deprotection of the C-3,19-isopropylidene protection gave compounds **5**, **6** and **7**, respectively. Compounds **8**, **9** and **10** were prepared by Wittig olefination of **4** with the ylides generated from various benzyl bromides.

Reaction of **4** with different aryllithiums yielded **11** and **12** (Scheme 3). Compound **11** was oxidized to the 12-keto derivative **15** and subsequent removal of isopropylidene protection gave **16**. Deprotection of the cyclic acetals of **11** and **12** gave compounds **13** and **14**, respectively.

Compound **17**, which has an aminopyrimidone moiety on the side chain was prepared by Reformatsky reaction of **4** with ethyl bromoacetate followed by PDC oxidation and condensation with guanidine (Scheme 2). The corresponding homologue **18** was prepared as shown in Scheme 4.

All the labdane derivatives synthesized from **4** were evaluated for their in vitro cytotoxic activity against nine human cancer cell lines using the NCI standard protocol[14] and the GI_{50} values obtained are given in Table 1. Among the Knoevenagel condensation products of **4**,compound **7** exhibited moderate cytototoxic activity against all the cell lines. Compound **6** was found



Scheme 4. Reagents and conditions: (i) ethyl acetoacetate/$TiCl_4$/py, (70%); (ii) $NaBH_4$/MeOH (95%); (iii) PDC/DCM, (95%); (iv) guanidine hydrochloride/NaOMe/MeOH/reflux (60%).

Table 1. In vitro anticancer screening results of the labdanes synthesized

Compd No	Cytotoxicity (GI_{50} μM)[a]								
	Breast		Colon		Lung	Melanoma	Ovarian	Prostate	
	MCF-7/ADR	MCF-7	SW 620	HT29	H 522	UACC62	OVCAR8	DU145	PC3
5	>100	>100	>100	>100	41	31	40	27	84
6	52	72.7	66	55	nd[b]	65	24	0.78	100
7	27	24	35	24	29	36	nd[b]	nd[b]	18
8	14	10	19	20	11	19	4	17	20
9	4	14	20	16	3	17	2	7	14
10	13	27	19	14	14	18	8	18	18
13	>100	65	>100	>100	nd[b]	>100	42	26	50
14	>100	43.2	>100	90	nd[b]	>100	37	0.17	42
16	>100	>100	>100	51	nd[b]	39	6.2	>100	60
17	>100	43.2	>100	90	nd[b]	>100	37	0.2	42
18	52	0.01	>100	34	nd[b]	54	29	20	41

[a] Cytotoxicity GI_{50} values are the concentrations corresponding to 50% growth inhibition.
[b] nd—not done.

to exhibit potent activity against prostate cell line (DU145). The Wittig olefination products **8**, **9** and **10** showed good activity against all the cell lines listed (GI_{50} values 2–20 μM). Compounds **14** and **17** demonstrated activity selectively against the DU145 cell line at 0.17 and 0.2 μM concentrations, respectively. Potent activity against MCF-7/ADR cell line was observed for **18**.

In summary, we have developed an efficient synthetic methodology for the preparation of **4** from **1** by selective oxidative degradation of the andrographolide side chain. The aldehyde functional group on **4** has been utilized for synthesizing a number of structurally diverse labdane diterpenes, which have exhibited potent cytotoxic activity.

References and notes

1. Connolly, J. D.; Hill, R. A. In *Dictionary of Terpenoids*; Chapman & Hall: London, UK, 1991; Vol. 2, pp 853–857.
2. (a) Polonsky, J. F. *Chem. Org. Naturst.* **1973**, *30*, 101; (b) Polonsky, J. F. *Chem. Org. Naturst.* **1985**, *47*, 222.
3. Meenakshi, S.; Mahesh, P.; Sharma, R. P. *Planta Med.* **1999**, *65*, 2.
4. Coval, S. J.; Conover, M. A.; Mierzwa, R.; King, A.; Puar, M. S.; Phife, D. W.; Pai, J. K.; Burrier, R. E.; Ahn, M. S.; Boykow, G. C.; Patel, M.; Pomponi, S. A. *Bioorg. Med. Chem. Lett.* **1995**, *5*, 605.
5. Barrero, A. F.; Alvarez-Manzaneda, E. J.; Rachid, C. C.; Gonzalez, D. *Synlett* **2000**, *11*, 1561.
6. Barrero, A. F.; Alvarez-Manzaneda, E. J.; Rachid, C. C. *Tetrahedron* **1998**, *54*, 5635.
7. Rosselli, S.; Bruno, M.; Pibiri, I.; Piozzi, F. *Eur. J. Org. Chem.* **2002**, *24*, 4169.
8. Oh, S.; Jeong, I. H.; Shin, W.-S.; Lee, S. *Bioorg. Med. Chem. Lett.* **2003**, *12*, 13.
9. Barrero, A. F.; Simeon, A.; Jose, F. Q. M.; Herrador, M. M.; Valdivia, M.; Jimenez, D. *J. Org. Chem.* **2002**, *67*, 2501.
10. Barrero, A. F.; Alvarez-Manzaneda, E. J.; Alvarez-Manzaneda, R.; Chahboun, R.; Meneses, R.; Cuerva, J. M.; Aparicio, M.; Romera, J. L. *Org. Lett.* **2001**, *3*(5), 647.
11. Jose, V.; Juan, F.; Franklin, S.; Eleonora, T.; Randolph, A. *J. Nat. Prod.* **2003**, *66*, 1623.
12. Rangaswamy, S.; Subba Rao, V. *J. Sci. Ind. Res.* **1951**, *10B*, 201.
13. Compound **4**: ^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) = 9.65 (s, 1H, CHO), 4.85 (s, 1H, H-13a), 4.44 (s, 1H, H-13b), 3.97 (d, 1H, H-15a, $J = 11.6$ Hz), 3.50 (m, 1H, H-3), 3.19 (d,1H, H-15b, $J = 11.6$ Hz), 2.57 (m, 1H), 2.45 (m, 2H), 2.35 (d, 1H), 2.10–1.95 (m, 2H), 1.85–1.70 (m, 2H), 1.65 (m, 1H), 1.40 (s, 3H), 1.38 (s, 3H), 1.35–1.25 (m, 3H), 1.22 (s, 3H), 0.92 (s, 3H). ^{13}C NMR (50 MHz, $CDCl_3$) δ (ppm) = 202.4, 147.4, 108.6, 98.8, 76.3, 76.1, 63.6, 51.9, 50.2, 39.7, 37.6, 37.1, 34.3, 26.9, 25.9, 25.1, 24.8, 22.7, 16.2. Mass (CI) m/z: 307 (M^++1), 249, 232, 205. IR ν_{max} cm^{-1} (neat): 3081, 2935, 2716, 1725, 1644, 1457, 1376, 1226, 1095, 996, 834, 757.
14. Cell growth assay: Exponentially growing cells were seeded (10,000 cells/well) in a 96-well plate. Cell culture plates were incubated with different concentrations of test compounds at 37 °C in a 5% CO_2 incubator. After 48 h the cells were fixed by adding ice-cold 50% trichloroacetic acid (TCA), washed with distilled water and stained with SRB solution. The plates were washed with 1% acetic acid, the bound SRB stain was solubilized with 10 mM tris buffer and the optical densities were read on a spectrophotometric plate reader at a wavelength of 515 nm. The percentage growths were calculated and the GI_{50} values were interpolated from the growth curves.