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A novel diterpenoid lactone-based scaffold for the generation of combinatorial libraries†

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Abstract—A novel labdane diterpenoid-based scaffold: 14-deoxyandrographolide has been identified for the generation of combinatorial libraries using solid-phase methods. To allow access to a larger pool of building blocks with wide structural diversity, 14-deoxy andrographolide has been linked to 2-chlorotrityl chloride resin. This was followed by a series of solid-phase reactions carried out on the C-3 hydroxyl and the C-8(17) double bond. The details of our synthetic strategies involving coupling of the resin, solid-phase acetylation under neutral conditions, esterification, oxidation, epoxidation reactions and formation of oxime esters from oximes are presented here. The utility of the scaffold has been demonstrated by synthesizing a small, 20 member, library of 14-deoxyandrographolide derivatives via esterification and oxime ester formation using five alkyl and five aryl carboxylic acids. © 2001 Published by Elsevier Science Ltd.

In recent years there has been an increasing interest in the design of libraries based on natural product templates.[1] Natural products have traditionally been an excellent and reliable source for the development of new drugs. Some natural products have been used directly while others have served as intermediates in semi-synthetic pathways or as the source of structural or functional templates for drug design. Previously, we have developed solid-phase syntheses and combinatorial chemistry of complicated structures such as the anti-malarial, anti-inflammatory triterpenoid lupeol,[2] betulinic acid, and ursolic acid.[3] The challenge in the design of combinatorial libraries based on natural product scaffolds lies in the adaptation and modification from solution-phase synthetic organic chemistry to synthesis on polymeric supports followed by screening and identification of the desired compounds. For natural product libraries, the chemistry has to be optimized for individual representative molecules before the construction of the library.[4]

The goal of this study was to generate a combinatorial library based on the 14-deoxyandrographolide scaffold **1** using solid-phase methodology. The motivation stemmed from the high profile biological activity of andrographolide. A phase I trial of andrographolide in HIV positive patients and normal volunteers indicated that andrographolide may inhibit HIV-induced cell cycle dysregulation, leading to a rise in CD4(+) lymphocyte levels in HIV infected patients.[5] Andrographolide and deoxyandrographolide are active principles of the medicinal plant *Andrographis paniculata*, used for the prevention and treatment of the common cold in Scandinavia and are known to have anti-inflammatory, antiviral, anti-thrombotic, hypotensive and anti-atherosclerotic activity.[6] Andrographolide has also been reported to exhibit a nitric oxide (NO) inhibitory property in endotoxin-stimulated macrophages,[7] immunostimulant behavior[8] as well as cell differentiation-inducing activity.[9] In our endeavors towards accelerating the synthesis and screening of a larger number of 14-deoxyandrographolide derivatives, we have carried out several classical reactions on solid-phase to provide a variety of novel scaffolds **2**, **3**, **4**, **5** and **6**. These novel scaffolds can serve as good precursors for obtaining structurally diverse derivatives of 14-deoxyandrographolide. The details of our synthetic strategies involve resin linkage at the C-19 position and chemical modification using acetylation under neutral conditions, esterification, oxidation, epoxidation reactions and formation of oxime esters from oximes.

Keywords: andrographolide; phase I trials; solid-phase methods; 14-deoxyandrographolide.

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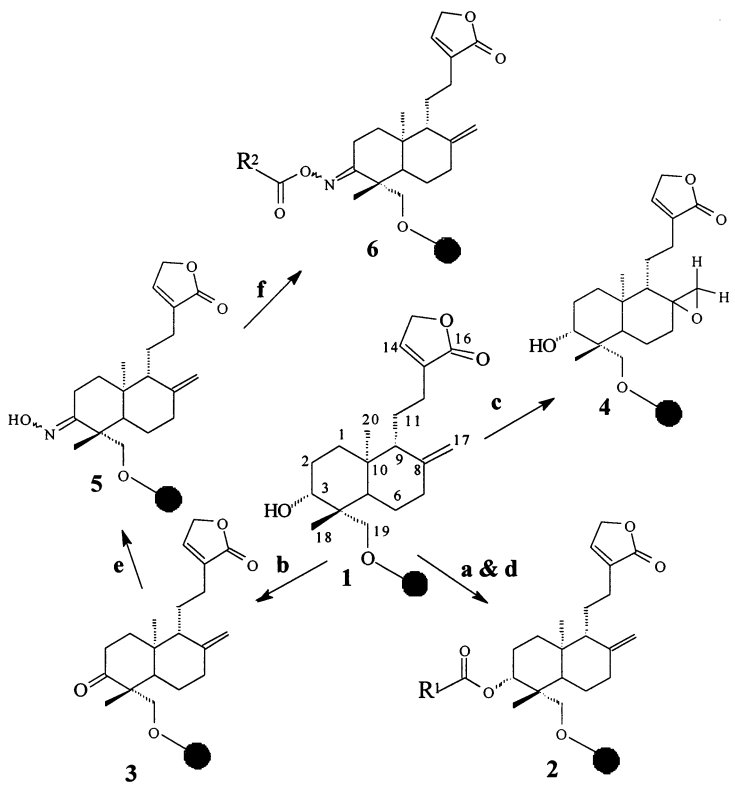
As part of our studies aimed at developing solid-phase synthetic strategies on the andrographolide skeleton,[10] the 14-deoxyandrographolide required for our studies was obtained from andrographolide triacetate treated with sodium borohydride followed by hydrolysis.[11] The first step in our synthetic strategy involve the coupling of the C-19 hydroxyl group of 14-deoxyandrographolide on to 2-chlorotrityl chloride resin (1.3 mmol/gram) in the presence of pyridine/DCM at 40°C for 24 h to provide the polymer linked scaffold **1**. The extent of loading was determined by HPLC after cleavage and was found to be 1.2 mmol/gram (loading ~90%). This was followed by a series of solid-phase reactions carried out on scaffold **1**, utilizing esterification with carboxylic acids to afford 3-*O*-esters **2**, an oxidation reaction in the presence of pyridinium dichromate (PDC) to furnish the potential 3-keto scaffold **3**, and a selective epoxidation reaction on the 8(17)-double bond to give the epoxy scaffold **4**. In addition acetylation of the C-3 hydroxyl group under neutral conditions has also been carried out. Though acetylations are routinely carried out using acid anhydrides or acid chlorides in the presence of alkyl amines or Lewis acids, we have used the reported solution phase method using acid anhydrides/*N*-bromosuccinimide (NBS).[12] Solid-phase acetylation of **1** with acetic anhydride in the presence of pyridine for 48 h at room temperature produced acetate scaffold **2b**; however, there were also several undesired by-products. To avoid formation of these undesired products during acetylation of scaffold **1**, we carried out acetylation under neutral conditions using acetic anhydride along with a catalytic amount of NBS to provide the 3-*O*-acetate **2b** as a single product. The solid-phase synthesis and the reaction conditions for obtaining various 14-deoxy andrographolide derivatives are outlined in Scheme 1.

The preparation of a phenolic ether derivative at the 3α-hydroxyl position of scaffold **1** with a substituted phenol using Mitsunobu methods and carbon–carbon bond formation utilizing the Wittig reaction on the 3-keto position of scaffold **3** failed to yield the desired products. Instead, under Mitsunobu conditions a new rearranged product was formed, which is being characterized. Resin bound intermediates and products were cleaved with 1–2% TFA and characterized using ^{1}H NMR and MS (FAB).[13] The products were obtained in high yields with purities ranging from 60 to 90% based on analytical HPLC.

Some of the intermediates synthesized above are indeed good substrates for developing the diversity of 14-deoxyandrographolide. This has been demonstrated by carrying out further diversification of scaffold **3**. The oxime esters **6** were prepared in two steps: condensation of the keto scaffold **3** with hydroxylamine hydrochloride to give the oxime scaffold **5** was followed by esterification with aliphatic and aromatic carboxylic acids in the presence of HOBt and DMAP in DMF. Resin bound intermediates and products were cleaved with 1–2% TFA and characterized using HPLC, ^{1}H NMR, MS (FAB).[14] The purities of these compounds



Scheme 1. (a) Aliphatic or aromatic carboxylic acids/DMAP/DIC in DMF at 40°C for 30 h; (b) PDC in dry DMF at 40°C 6 h; (c) *m*-CPBA/$NaHCO_3$/DCM, at 40°C for 48 h; (d) acetic anhydride/NBS; (e) $NH_2OH{\cdot}HCl$/pyridine at 40°C for 24 h; (f) aliphatic or aromatic carboxylic acid/DIC/DMAP/DMF at 40°C for 12 h.

Table 1. Structures, yields and purities of representative compounds of the derivative library

Sample number	R^1	Yield%	Purity	Sample number	R^2	Yield%	Purity
2a	$H_3C(p)C_6H_4CH_2$-	95	91	**6a**	$H_3CO(p)C_6H_4$-	65	61
2b	CH_3-	83	84	**6b**	$C_6H_5CH_2$-	70	63
2c	3-C_6H_4N-	76	78	**6c**	3-C_6H_4N-	60	69
2d	C_3H_7-	92	95	**6d**	C_3H_7-	67	61
2e	H_2NCH_2-	78	61	**6e**	H_2NCH_2-	62	60

ranged from 60 to 80% based on analytical HPLC. After optimizing the reaction conditions a small library of 20 derivatives based on scaffolds **1** and **3** was generated through automation using an Advanced Chemtech 496Ω Multiple Organic Synthesizer; the product yields and purities ranged from 60 to 90% (Table 1).

In summary, we have identified 14-deoxyandrographolide as a novel building block for the generation of 14-deoxyandrographolide scaffolds for the synthesis of combinatorial libraries. The utility of these templates have been demonstrated by synthesizing a small library of 20 derivatives using five alkyl and five aryl carboxylic acids on scaffolds **1** and **3**. The scaffolds **4**, **5** and **6** are new to the chemical literature and their structures have been characterized by detailed NMR and MS spectral studies. Reactions proceeded in good yields and with excellent regioselectivities as established by HPLC and NMR analyses after cleavage from the solid support. These solid-phase reactions can be used individually or in tandem, and can be combined with other standard reactions for solid-phase synthesis of diterpenoids to access an array of organic structures.

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13. Selected spectral data of novel representative compounds: (**2a**) MS (FAB) m/z 467 (M+1), ^{1}H NMR ($CDCl_3$) δ 0.62 (s, 3H), 0.98 (s, 3H), 3.66 (d, 1H, $J=14.1$ Hz), 4.11 (d, 1H, $J=11.7$ Hz), 4.28 (d, 1H, $J=11.7$ Hz), 4.59 (s, 1H), 4.77 (s, 2H), 4.87 (s, 1H), 7.08 (s, 1H) p-methylbenzyl moiety 2.30 (s, 3H), 3.64 (s, 2H), 7.17 (m, 4H). Compound **3**, MS (FAB) m/z 333 (M+1), ^{1}H NMR ($CDCl_3$) δ 0.79 (s, 3H), 1.25 (s, 3H), 3.46 (d, 1H, $J=11.1$ Hz), 3.91 (d, 1H, $J=11.1$ Hz), 4.68 (s, 1H), 4.78 (s, 2H), 4.96 (s, 1H), 7.11 (s, 1H). Compound **4**, MS (FAB) m/z 351 (M+1), ^{1}H NMR ($CDCl_3$) δ 0.78 (s, 3H), 1.24 (s, 3H), 3.32 (d, 1H, $J=12$ Hz), 3.42 (m, overlapped, 2H), 3.42 (m, overlapped, 1H), 4.17 (d, 1H, $J=12$ Hz), 4.79 (brs, 2H), 7.22 (s, 1H).
14. Spectral data for novel representative compounds: (**5**) MS (FAB) m/z 348 (M+1), ^{1}H NMR ($CDCl_3$) δ 0.75 (s, 3H), 1.26 (s, 3H), 3.46 (d, 1H, $J=10.5$ Hz), 3.81 (d, 1H, $J=10.5$ Hz), 4.66 (s, 1H), 4.78 (m, 2H), 4.94 (s, 1H), 7.11 (s, 1H). Compound **6a**, MS (FAB) m/z 482 (M+1), ^{1}H NMR ($CDCl_3$) δ 0.79 (s, 3H), 1.25 (s, 3H), 3.47 (d, 1H, $J=11.7$ Hz), 3.81 (d, 1H, $J=11.7$ Hz), 4.76 (s, 1H), 4.78 (s, 2H), 4.95 (s, 1H), 7.11 (s, 1H) p-methoxyphenyl moiety 3.88 (s, 3H), 6.95 (d, 2H, $J=8.7$ Hz), 8.01 (d, 2H, $J=8.7$ Hz).