Skeletal Transformations of Terpenoid Forskolin Employing an Oxidative Rearrangement Strategy

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Abstract

The skeletal transformations of diterpenoid forskolin were achieved employing an oxidative rearrangement strategy. A library of 52 forskolin analogues, including 12 CTD compounds and 40 SAR compounds with unique scaffolds/ring systems, was produced during the course of this work. Compounds 2c and 10c exhibited inhibition of nitric oxide (NO) production in lipopolysaccharide (LPS)-stimulated RAW 264.7 cells, with IC50 values of 0.3μM and 4.1μM.

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Keywords

oxidative rearrangement; diterpenoid; forskolin; complexity to diversity

Comprehensive Summary

Background and Originality Content

Natural products (NPs) have been an important source of new drugs since ancient times. In the past three decades, approximately 50% of newly approved small molecule drugs were NPs or derivatives of NPs.[1,2] With the continuous development of powerful tools such as genomics,[3,4] proteomics,[5,6]metabolomics,[7] metabolomics,[8,9]synthesis and combinatorial chemistry,[10] more and more novel methods can be used to directly detect the interaction between NPs and target proteins, which has made modern drug research more focused on the development and utilization of NPs, particularly terpenoids in medicinal plants.[11,12] The antitumoral diterpene paclitaxel, the anti-malaria sesquiterpene lactone artemisinin, the anti-inflammatory tricyclic diterpene triptolide, and the antibacterial diterpene lactone andropanolide, along with other terpenoid compounds, have been extensively used in clinical settings. People's enthusiasm for the study of terpenoids has been restored because it offers new and intriguing frameworks with improved physical, chemical, pharmacokinetic, and pharmacodynamic properties.[13]

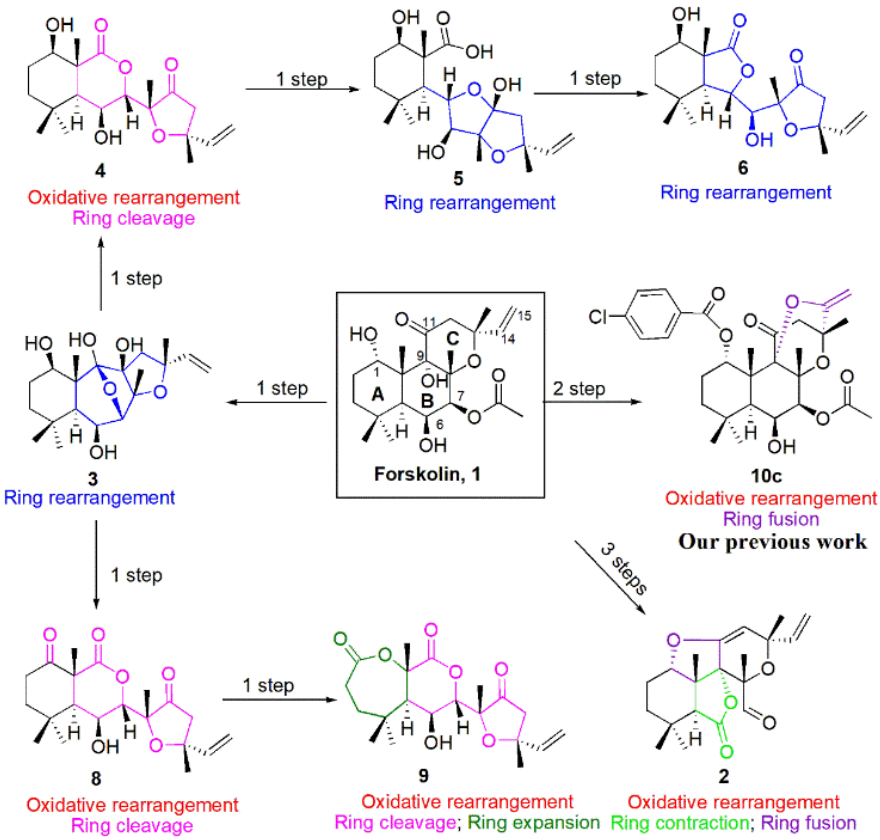
High-throughput screening as a driver of drug discovery has stimulated the development of methods to increase the structural diversity of NPs.[14] These methods include Diversity-Oriented Synthesis (DOS),[15,16] Biology-Oriented Synthesis (BIOS),[17] Pseudo-Natural Products Strategy,[18] and Complexity to Diversity (CtD) Strategy.[19]The CtD strategy, proposed by Paul J. Hergenrother, has been successfully applied to the structure diversification of several terpenoids, including abietic acid,[20] gibberellic acid,[19]limonin,[21] and pleuromutilin.[22]

The oxidation reactions of terpenoids, such as the Criegee diol oxidative cleavage reaction and the Baeyer-Villiger oxidative ring expansion reaction, often produce new structures along with additional rearrangements (Figure S1). The biosynthesis of terpenoid natural products is primarily driven by the carbocation cyclization/rearrangement reactions mediated by terpene synthases. Additionally, enzymes like cytochrome P450s facilitate the incorporation of heteroatoms, particularly oxygen atoms. Research has indicated that structures with intermediate oxidation levels have lower rearrangement barriers. Specifically oriented noncovalent interactions can lower the barriers for these structures to undergo the biologically oxidative rearrangement.[23]

Forskolin is a labdane diterpenoid found in the roots of the Indian plant Coleus forskohlii. Forskolin, as an adenylate cyclase agonist, has been used in traditional medicine for centuries. It has also shown promise and effectiveness in modern medicine. Pharmacological activities of forskolin have been discovered, such as the inhibition of platelet activating factor,[24] anticancer properties,[25-28] anti-inflammatory effects,[29,30] antioxidative properties,[31,32] anti-obesity effects,[33] reduction of intraocular pressure,[34,35] and stimulation of nerve regeneration.[36]

The chemical structure of forskolin includes acetyl group, carbonyl group, double bonds, and eight stereocenters, which contribute to its diverse chemical properties. It is particularly rich in functional groups, making it an excellent candidate for oxidative rearrangement.

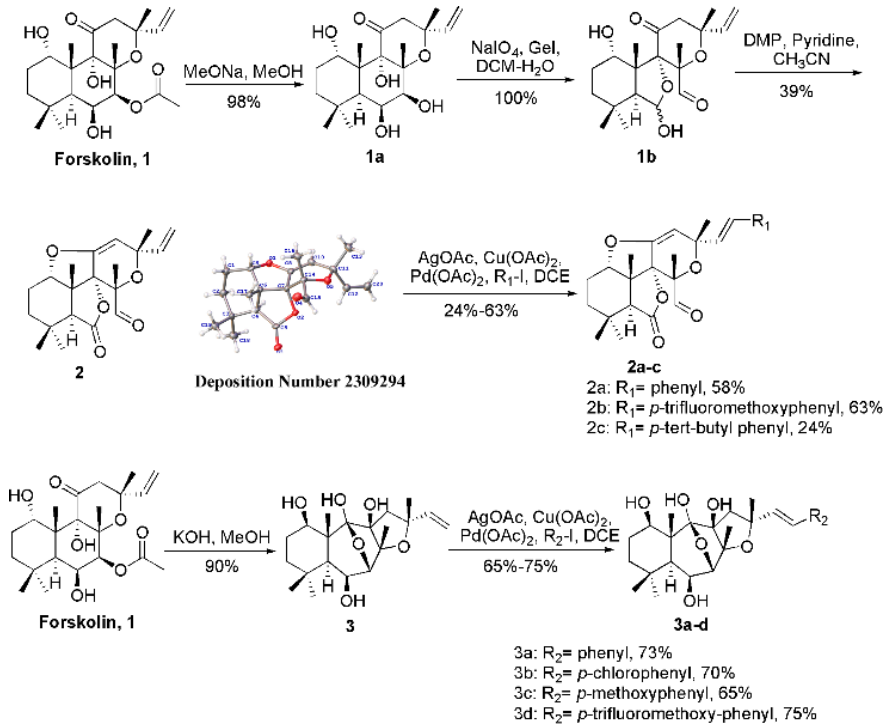
Therefore, we propose an oxidative rearrangement strategy that is suitable for synthesizing the diverse structures of forskolin. Series of novel analogues of forskolin were effectively synthesized employing this strategy. An overview of these transformations is provided in**Scheme 1** .



Scheme 1 . Overview of scaffolds generated from forskolin

Results and Discussion

Firstly, the deacetylation reaction of forskolin yielded diol**1a** by utilizing sodium methylate to remove the B-ring 7-acetyl group in 98% yield. Then, **1a** was oxidized with sodium periodate to yield an unstable aldehyde intermediate, which was not separated and underwent a dehydration reaction to form hemiacetal**1b** as a racemate mixture. Next, hemiacetal **1b** was oxidized with Dess-Martin periodinane to produce a lactone. Simultaneously, a fused ether ring was formed, resulting in the formation of tetracyclic compound **2** (**Scheme 2**). After screening a series of conditions, the oxidative rearrangement of hemiacetal **1b** with DMP in pyridine gave compound **2** in 39% yield (**Figure S3** and **Table S1**). With compound**2** in hand, several alkene derivatives were prepared. Standard Heck reaction conditions are not suitable for compound **2**because it is sensitive to alkaline conditions. Therefore, we turned to a novel $Pd(OAc)_2$ and AgOAc catalyzed Heck-like reaction.[37] With this condition, the derivatives**2a-c** of compound **2** were successfully prepared in 24-63% yields. The E configuration of the double bond was determined by the steric hindrance of the groups attached to both sides of the double bond and judged by the coupling constant. In the preparation of lactone diterpene **2** , two oxidation rearrangement reactions, including Malaprade oxidation and Dess-Martin oxidation, were employed, along with structural rearrangements (**Scheme 2**).

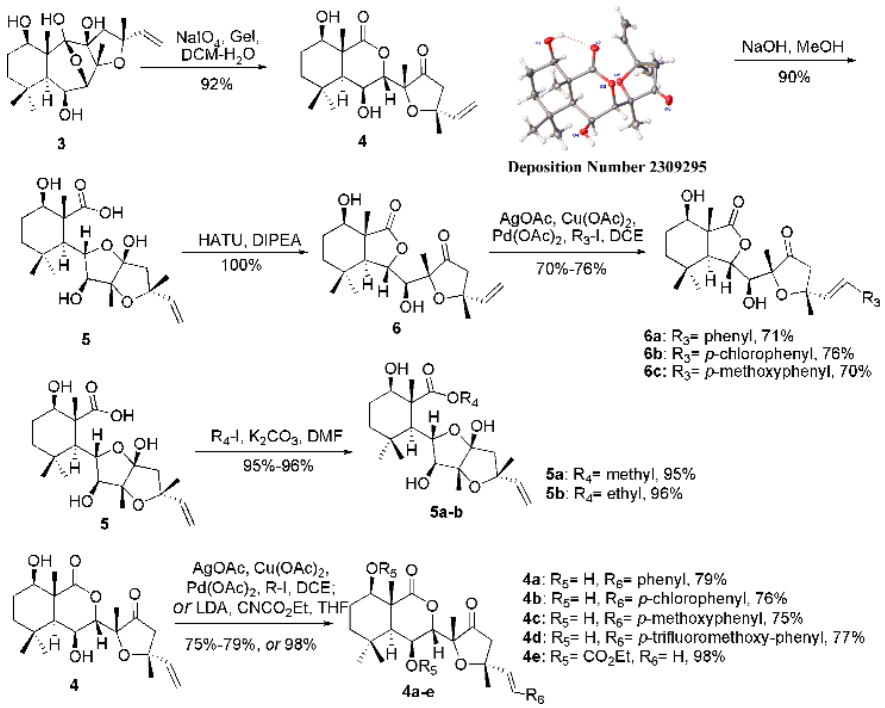


Scheme 2. Synthesis of forskolin analogues **2** ,**3** and their derivatives.

Previously, a series of forskolin analogues have been reported, [38-41] including the redox rearrangement products,[42] acid-catalyzed rearrangement products, and base-catalyzed rearrangement products (**Figure S10**).[42,44] The base-catalyzed rearrangement product **3** bears a unique 6/7/5 tricyclic carbon skeleton, an oxygen bridging ring, making it an ideal precursor for next oxidative rearrangements (**Scheme 2**). Four derivatives**3a-3d** of compound **3** were synthesized using the same conditions as compound **2** in 65-75% yields.

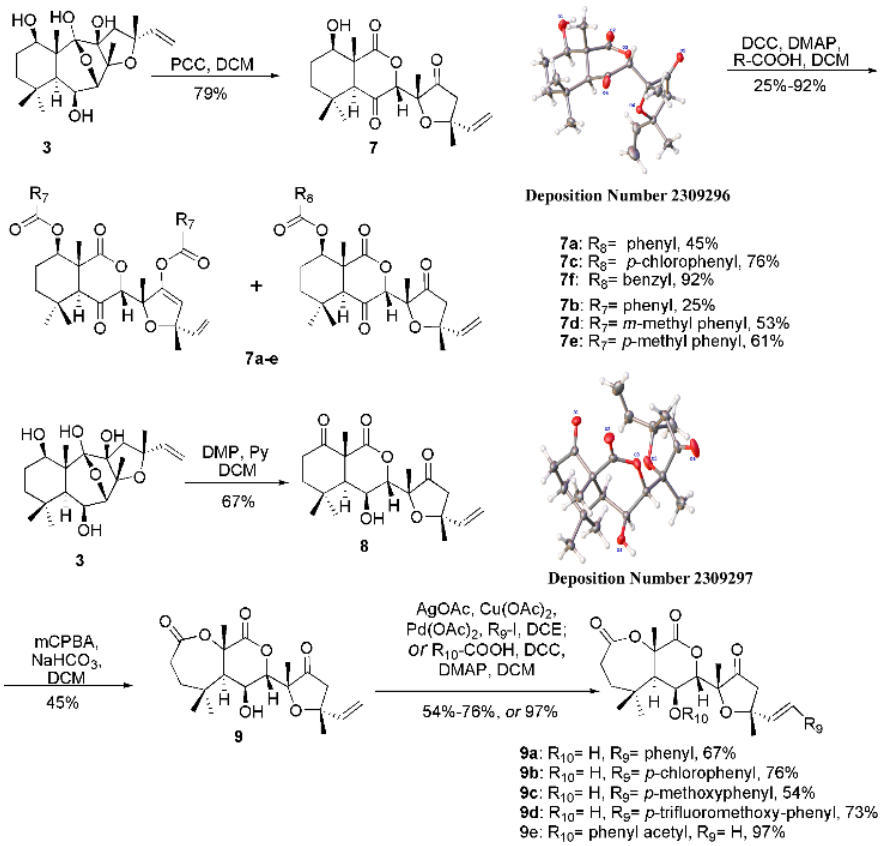
Compound **3** was treated with various oxidants, such as $NaIO_4$, PCC, and DMP (**Scheme 3** and **4**), resulting in the formation of different products through oxidative rearrangement, namely compounds **4** , **7** , and **8** . The relative configurations of these three compounds were elucidated using X-ray crystallography. (**Figures S11 -S14**). Compound **4** was treated with sodium hydroxide to undergo hydrolysis, and the resulting product, which was not separated, subsequently underwent an intramolecular nucleophilic attack to form compound **5** in 90% yield (**Figure S9**). The configuration of **5** was confirmed by COSY and NOESY spectra. In the COSY spectrum of compound **5** , H-8 at δ 3.85 (t, J=7.9 Hz) showed COSY with H-9 at δ 3.77 (dd, J=7.9, 5.4 Hz) and H-5 at δ 2.11 (d, J=7.8 Hz). H-9 at δ 3.77 (dd, J=7.9, 5.4 Hz) showed COSY with 9-OH at δ 4.94 (d, J=5.8 Hz). In the NOESY spectrum of compound 5, H-5 at δ 2.11 showed NOESY with H-9 at δ 3.77, indicating that C-5 is S configuration. H-8 at δ 3.85 showed no NOESY with H-5 at δ 2.11 but showed COSY with 10-CH3 at δ 1.08, 9-OH at δ 4.94, and 11-OH at δ 6.18. That indicates that the configurations of C-8 and C-11 are S and R, respectively.

Compound **5** was treated with HATU and DIPEA, or DCC and DMAP, which resulted in the formation of the intramolecular ester condensation product **6** with quant yield. Then, compound **5** was reacted with iodoalkanes to obtain esters **5a** and **5b** in 95-96% yields. (**Scheme 3**) Three derivatives **6a-6c** of compound **6** were synthesized using the same conditions as compound **2** in 70-76% yields.



Scheme 3. Synthesis of forskolin analogues **4** ,**5** , **6** and their derivatives.

In the esterification process of the rearrangement compound **7** , the carbonyl group in the C ring is susceptible to enol tautomerization. As a result, the aliphatic carboxylate and benzoate attached to the electron-withdrawing group yield monosubstituted products **7a** ,**7c** , and **7f** in 45-92% yields, while the benzoate attached to the electron-donating group produces doubly substituted products **7b** , **7d** , and **7e** in 25-61% yields. (**Scheme 4**).



Scheme 4. Synthesis of forskolin analogues **7** ,**9** and their derivatives.

Oxidative rearrangement compound **9** was obtained through the oxidative ring expansion reaction of compound 8 using Baeyer-Villiger oxidation (**Scheme 4**). The position of the oxygen atom insertion was determined by the HMBC spectrum. In the HMBC spectra of compound**8** , H-5 at δ 2.39 and 10-CH_3 at δ 1.70 showed HMBC correlations with C-9 at δ 169.58 and C-1 at δ 206.38. While in the HMBC spectrum of compound **9** , H-5 at δ 3.49 and 10-CH_3 at δ 1.71 showed HMBC correlations with C-9 at δ 171.92, the HMBC correlations of H-5 and 10-CH_3 with C-1 are no longer present. Therefore, it can be concluded that the oxygen atom is inserted between C-1 and C-10. Subsequently, five derivatives**9a -e** of compound **9** were synthesized using the same conditions as compound **2** in 54-78% yields. The relative mechanisms of synthesis of compounds **2** , **3** , **4** ,**5** , **6** , **7** , **8** were proposed in SI (**Figure S9**).

By utilizing oxidative rearrangement and other ring distortion reactions, we can produce 38 molecules in this work, as well as 14 forskolin analogues that we reported before. While standard visual examination is an effective way to detect structural variation, we employ hierarchical clustering for a more quantitative comparison using ChemMine Tools.[45-48] The similarity metric, also known as the Tanimoto coefficient, was subtracted from one to create the distance matrix, which ranges from 0 to 1. Higher values indicate a decrease in similarity between the chemicals. It is evident that the resulting compounds differed significantly from one another and from the parent NPs in terms of structure (**Figure 1**).

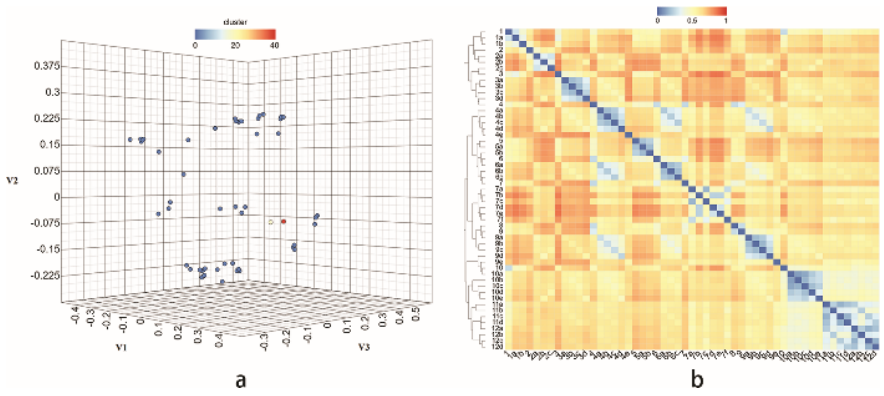


Figure 1. Comparison of compounds created using the CtD method. a, Distance Matrix for Full Compounds (3D), with a similarity cutoff of 0.5. b, Distance Matrix for Full Compounds (2D), distance matrix for CtD compounds relative to the NPs and to each other (0 represents perfect similarity).

The cytotoxicity of the compounds on HelaS3, MCF-7 and A549 cancer cell lines was measured using CCK-8 assay. The results indicated that these compounds showed no apparent cytotoxicity. However, we found these compounds possessed potent anti-inflammatory activity.

Table 1 . Anti-inflammatory activity of the investigated compounds against RAW 264.7 mouse monocyte-macrophage

Compounds	$IC_{50}(\mu M)$	Compounds	$IC_{50}(\mu M)$
2c	0.3	**7c**	>50
3b	9.0	**9a**	>50
4d	>50	**10c**	4.1
5a	11.2	forskolin	25.0
6a	>50	dexamethasone	8.6

The results of the anti-inflammatory tests are shown in **Table 1** . The preliminary bioassay test suggested that most of tested forskolin analogues showed strong anti-inflammatory activity. In particular, compounds **2c** and **10c** exhibited better activity than the positive control, dexamethasone. Among them, compound**2c** showed the highest inhibitory activity with an IC_{50} value of 0.3 μM. They showed potential to be developed as anti-inflammatory drugs, and the mechanism of action needs to be further investigated.

Conclusions

In conclusion, we have demonstrated that oxidative rearrangements can serve as a practical strategy for the skeletal transformations of terpenoids. A library of 52 forskolin analogues, including 12 CTD compounds and 40 SAR compounds with unique scaffolds/ring systems, was produced during the course of this work. Biological assays showed that some of these forskolin analogues had the potential to be developed as anti-inflammatory drugs. We believe that oxidative rearrangement could serve as a novel strategy for increasing the diversity of terpenoids and enriching the toolbox of complexity to diversity strategy. A novel, mild, $Pd(OAc)_2$ and AgOAc catalyzed Heck-like reaction was found to be suitable for late-stage functionalization of NPs and their analogues. We anticipate that the compounds disclosed herein, and others generated from different natural products, will find utility in a wide variety of biological investigations.

Supporting Information

The supporting information for this article is available on the WWW under

https://doi.org/10.1002/cjoc.2023xxxxx.

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The skeletal transformations of diterpenoid forskolin were achieved employing an oxidative rearrangement strategy. A librar