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# Syntheses, biological evaluation and SAR of ingenol mebutate analogues for treatment of actinic keratosis and non-melanoma skin cancer

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ABSTRACT

Ingenol mebutate is the active ingredient in Picato® a new drug for the treatment of actinic keratosis. A number of derivatives related to ingenol mebutate were prepared by chemical synthesis from ingenol with the purpose of investigating the SAR and potency in assays relating to pro-inflammatory effects (induction of PMN oxidative burst and keratinocyte cytokine release), the potential of cell death induction, as well as the chemical stability. By modifications of the ingenol scaffold several prerequisites for activity were identified. The chemical stability of the compounds could be linked to an acyl migration mechanism. We were able to find analogues of ingenol mebutate with comparable in vitro properties. Some key features for potent and more stable ingenol derivatives have been identified.



A search for bioactive compounds in plants used in traditional medicine has led to the identification of ingenol mebutate (PEP005, ingenol 3-angelate, **1**, Fig. 1) from *Euphorbia peplus*.[1,2] This compound has been developed as the active ingredient in Picato®, a new drug for field treatment of actinic (solar) keratosis (AK) offering a short treatment schedule (2–3 days), providing effective and sustained clearance of AK lesions with a predictable onset and short duration of local skin responses.[1,3]

Ingenol mebutate (**1**) is a 3-mono-ester of the diterpene ingenol and angelic acid. More than thirty different 3-mono-esters of ingenol have been isolated from the plant genus *Euphorbia*, mainly with non-cyclic unbranched lipophilic aliphatic acids like hexadecanoic, decadienoic, decatrienoic and dodecatetraenoic acid.[4,5] 3-Mono-esters of ingenol display high affinity towards protein kinase C (PKC),[6] a family of related serine/threonine kinases mediating a number of important cellular signal transduction responses.[7] Ingenol mebutate (**1**) is an activator of novel (δ, ε, η, θ) and classical ($\alpha$, $\beta_I$, $\beta_{II}$, $\gamma$) PKC isoenzymes,[8] The therapeutic effect of ingenol mebutate in the treatment of actinic keratosis is believed to be caused by a dual mechanism of action: (i) induction of aberrant keratinocyte cell death and (ii) induction of a lesion-directed immune response, at least partially mediated by PKC.[9]

In our search for novel analogues of ingenol mebutate (**1**) with improved properties, such as chemical stability, potency in activation of the immune system and therapeutic efficacy, we have investigated the consequences of minor structural changes of **1**. The ingenol scaffold is a very rigid structure with four hydroxyl groups in the southern part of which only the 3-OH is esterified with angelic acid in **1**. We have explored the importance of the 5- and 20-OH groups as well as the geometry of the seven-membered ring. Furthermore, we have prepared transposed esters at O-3, and investigated cyclic ether lactones connecting O-3 and O-4 in order to understand the importance of the ester function in relation to biological activity. The results from these studies led us to focus on aliphatic ester analogues of **1** having intact ingenol scaffolds. Herein we report the preparation, the chemical and biological characterization of these novel ingenol 3-angelate analogues and the identification of compounds having improved properties.

With a few modifications we have adopted a previously reported method[10] for the preparation of the new 3-acyl derivatives of ingenol (Scheme 1). The formation of ingenol 5,20-acetonide (**3**), previously described,[11] can potentially lead to several mono

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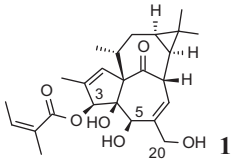


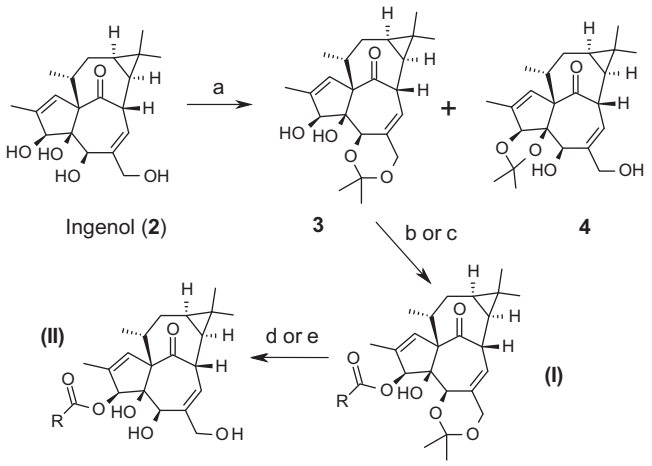
**Figure 1.** Ingenol mebutate (**1**, PEP005).

acetonide products and a bis-3,4-5,20-acetonide. In our hands, the main product **3** was isolated after crystallization in 73% yield. By chromatographic isolation from the mother liquor, we obtained a minor 3,4-regioisomer (**4**), which was used to prepare some 5-O and 20-*O*-methyl ether analogues of ingenol 3-angelate (cf. Schemes 2 and 3). The angeloylation to form **1** required special conditions to avoid isomerization induced by Michael addition by nucleophilic catalysts.[12] For other 3-acyl-ingenols not associated with this issue, standard procedures, such as Steglich coupling conditions or using acyl chlorides, could be applied in the preparation of **I**. The final acid catalyzed deprotection step to produce **II** was a minor modification of a previously described method.[10,13]

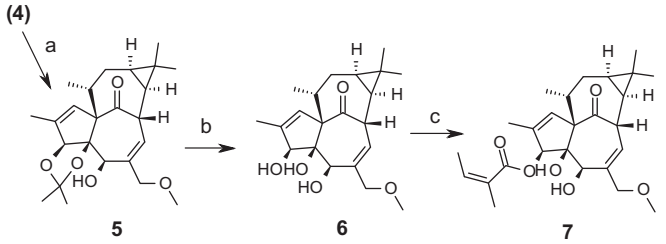
20-*O*-Methyl-ingenol mebutate (**7**) was synthesized from the 3,4-protected ingenol **4** as described in Scheme 2. Treatment of **4** with dimethyl sulfate and lithium bis(trimethylsilyl)amide as base provided **5** in a reasonable yield. Removal of the protecting group followed by angeloylation gave **7**.

The synthesis of 5-*O*-methyl-ingenol mebutate (**11**) was also based on **4** (Scheme 3). The primary alcohol was selectively protected as a triphenylmethyl (Tr) ether. Methylation of **8** followed by removal of the two protecting groups under mild acidic conditions delivered 5-*O*-methyl-ingenol (**9**). The primary alcohol of **9** again required protection prior to esterification which was realized with a *tert*-butyldimethylsilyl ether. Finally, angeloylation of **10** followed by removal of the silyl group under mild conditions provided **11**.

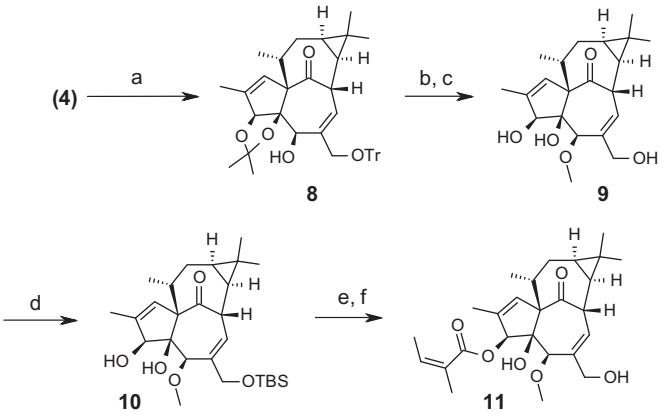
Scheme 4 depicts the preparations of the derivatives **13** and **14** with modified seven-membered rings. The synthesis of the fluoro derivative **13** was inspired by Appendino et al.[14] Selective acetylation of the primary alcohol in ingenol mebutate provided **12**. Treatment of **12** with diethylaminosulfur trifluoride followed by removal of the acetyl group delivered **13**. The two double bonds of ingenol show different reactivities due to the steric hindrance.[11] Thus, the epoxide **14** was prepared by oxidation of **1** with *m*-chloroperoxybenzoic acid and the stereochemistry was assigned from a $^1$H NOESY spectrum showing the distance between H-7 and H-8 to be about 4 Å in three dimensional space, which is consistent with a trans-configuration.



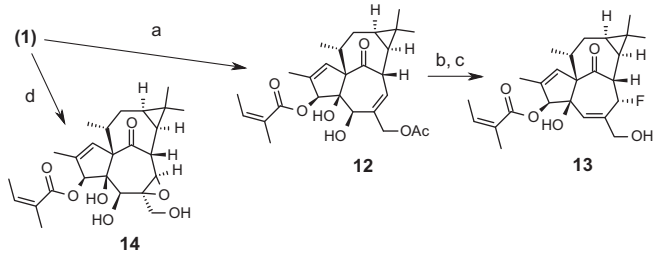

**Scheme 1.** General synthetic route from ingenol to ingenol 3-acylates (**II**). Reagents and conditions: (a) acetone, PTSA (cat), rt, 0.5 h, 73% for **3**, 4.5% for **4**; (b) $RCO_2H$, DCC, DMAP, $CH_3CN$, rt → 140 °C; (c) RCOCl, DMAP, DIPEA, $CH_3CN$, rt → 140 °C; (d) aq HCl/THF; (e) aq HCl/MeOH, rt.




**Scheme 2.** Synthesis of 20-*O*-methyl-ingenol mebutate (**7**). Reagents and conditions: (a) $(CH_3O)_2SO_2$ (1.2 equiv), LiHMDS (1.05 equiv), THF, 0 °C → rt, overnight, 35%; (b) aq HCl (4 M), THF, rt, 20 h, 51%; (c) angelic anhydride (1 equiv), $Cs_2CO_3$ (1.1 equiv), $CH_3CN$, 0–5 °C, overnight, 47%.




**Scheme 3.** Synthesis of 5-*O*-methyl-ingenol mebutate (**11**). Reagents and conditions: trityl chloride (1.1 equiv), DMAP, $Et_3N$, $CH_2Cl_2$, rt, 62%; (b) $(CH_3O)_2SO_2$ (3 equiv), LiHMDS (2 equiv), THF, −78 °C → rt, 1.2 h; (c) MeOH, concd HCl (cat), rt, 1 h, 7% over two steps; (d) *t*$BuMe_2SiCl$ (2.4 equiv), DMAP (2.9 equiv), $CH_2Cl_2$, 89%; (e) angelic anhydride (3.7 equiv), $Cs_2CO_3$ (4 equiv), $CH_3CN$, rt, 1 h, 70 °C, 1 h; (f) MeOH, concd HCl (cat), rt, 0.5 h, 28% over two steps.




**Scheme 4.** Syntheses of fluoro analogue **13** and epoxide **14**. Reagents and conditions: (a) $Ac_2O$ (3 equiv), $Et_3N$, $CH_2Cl_2$, 88%; (b) DAST (2 equiv), $CH_2Cl_2$, −78 °C, 2 h; (c) $Na_2CO_3$, MeOH, rt; (d) MCPBA (4.2 equiv), saturated aq $NaHCO_3$, $CH_2Cl_2$, rt, 3 h.

The 3-ether **15** and 3-ether-4-lactone **17** modifications were made under the same conditions in two steps (Scheme 5). Alkylation of the 5,20-acetonide **3** with ethyl 2-chloroacetate led to a lactone as main product via a two-step reaction. However, alkylation with the bulky *tert*-butyl ester prevents formation of lactone. Hydrolysis under acidic conditions provided the two 3-ether derivatives **15** and **17**. A cyclic 3,4-carbonate **16** was prepared by reacting **3** with 1,1′-carbonyldiimidazole followed by deprotection as described above.