Inhibitors of DNA polymerase β from *Schoepfia californica*

Jie Chen, Yu-Huan Zhang, Li-Kai Wang, Steven J. Sucheck, Angela M. Snow and Sidney M. Hecht*

Departments of Chemistry and Biology, University of Virginia, Charlottesville, Virginia 22901, USA. E-mail: sidhecht@virginia.edu

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Fractionation of a hexane extract prepared from the plant *Schoepfia californica* was carried out by bioassay guided fractionation, affording five inhibitors of DNA polymerase β; four of these were shown to be anacardic acid and structurally related derivatives, while the fifth was oleic acid.

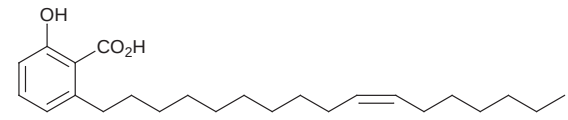
In addition to their role in DNA replication, at least three eukaryotic DNA polymerases participate in the repair of damaged DNA.[1] Given that elements in our environment such as sunlight and pollution provide a steady source of DNA damage, the repair of damaged DNA must be essential to maintain cell viability.[2] There is, however, at least one context in which DNA damage repair is not beneficial, namely following the clinical administration of antitumor agents that function by damaging DNA. The repair of DNA damage inflicted by these agents on cancer cells must mitigate their therapeutic potency.

DNA polymerase β is believed to have primary responsibility for supporting short patch DNA base excision repair.[3–5] This pathway has specifically been implicated in the repair of DNA damage caused by agents such as *cis*-platinum, bleomycin and neocarzinostatin.[3,4,6–8] To identify non-toxic compounds that can block the function of DNA polymerase β selectively, and which may thereby be able to potentiate the action of anticancer agents such as bleomycin, we have surveyed plant extracts for naturally occurring inhibitors of polymerase β.[9] Presently we describe five natural inhibitory principles from *Schoepfia californica* that can potentiate the action of bleomycin and *cis*-platinum.

A hexane extract prepared from the twigs, leaves and fruit of *Schoepfia californica* was partitioned between hexane and MeOH; the hexane phase contained most of the polymerase β inhibitory activity. Successive bioassay-guided fractionations on two silica gel columns and then by preparative silica gel TLC afforded an active fraction that effected 94% inhibition of polymerase β function at 5 μg ml^{-1} *vs.* 38% inhibition for the initial extract.† Fractionation (C_8 column, 80% MeOH) then afforded pure compounds **1–5**; all were obtained as colorless needles except **3**, which was a colorless oil.

The structures were determined by spectroscopic analysis. Compound **1** was found to have a molecular ion at *m/z* 374.2822, establishing the molecular formula as $C_{24}H_{38}O_3$. The ^{13}C NMR spectrum of **1** displayed six resonances (δ 110.31, 115.87, 122.74, 135.38, 147.76 and 163.65) assigned to an aromatic ring and a resonance at δ 175.67 assigned to a carboxylate carbon, thus accounting for five of the six units of unsaturation implied by the molecular formula. The presence of three aromatic resonances in the ^{1}H NMR spectrum at δ 6.77 (d, *J* 7.5), 6.87 (d, *J* 7.5) and 7.36 (dd, *J* 7.5, 7.5) indicated that the aromatic ring was trisubstituted; the multiplicities of the proton resonances were consistent with a 1,2,3-trisubstituted benzenoid system.‡

The chemical shift of the carboxylate at δ 175.67 was typical of a carboxylate attached to an aromatic ring; the downfield shift of the aromatic H at δ 7.36 (dd) suggested the *para* relationship of this H to the carboxylate moiety. That the remaining O atom was present as part of a phenolic substituent was suggested by the downfield shift of one ring carbon (δ 163.65). The *ortho* relationship of this OH to the ring carboxylate was supported by the upfield shift of the ring C atom (δ 110.31), consistent with the known (δ 12.7) shielding effect of *ortho* phenolic OH groups.[15]



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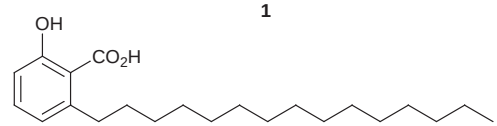

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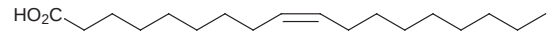

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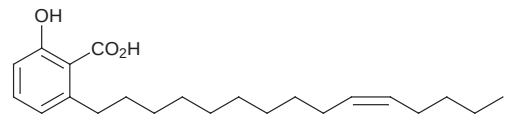

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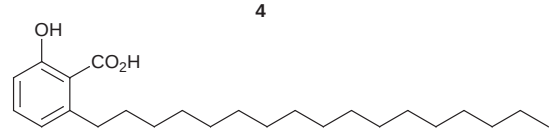

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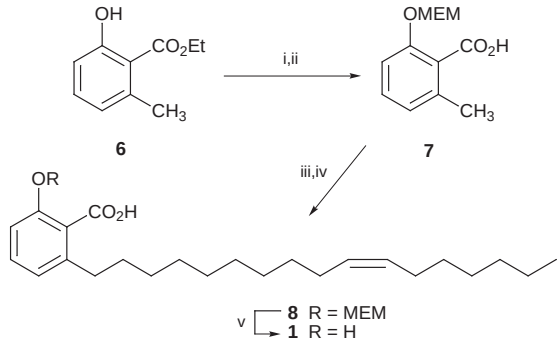
These data suggested that **1** was a 6-substituted salicylic acid; the ^{1}H and ^{13}C NMR spectra were consistent with an unbranched alkyl substituent containing a single double bond. The double bond configuration was assigned as *cis* based on the ^{13}C NMR resonances of the allylic carbon atoms.[16] Determination of the olefin position was made by modification of published procedures.[17]

The structure assigned to **1** was confirmed by chemical synthesis, as outlined in Scheme 1. Ethyl 2-hydroxy-6-methylbenzoate **6**[18] was protected as the corresponding MEM ether[19] and the ethyl ester was saponified, affording carboxylic acid derivative **7** as colorless prisms (71% yield). Treatment of **7** with LDA (2 equiv., THF), followed by admixture of (9*Z*)-hexadec-9-enyl bromide at 0 °C,[20] afforded homologated salicylic acid derivative **8** (68% yield). MEM deprotection[19] provided **1**[14] as colorless needles (80% yield). The synthetic and naturally derived samples were found to be identical as judged by their behavior on silica gel TLC, and by their identical ^{1}H and ^{13}C NMR and mass spectra, as well as their equal abilities to inhibit DNA polymerase β (*vide infra*).

Compounds **2**, **4**, and **5** were found to have spectroscopic properties most similar to **1**. The structures were assigned in the same fashion as for **1**, a process facilitated by previous reports



Scheme 1 *Reagents and conditions*: i, MEMCl, Pr^i_2NEt; ii, Bu^tOK, 61% over 2 steps; iii, LDA; iv, (9*Z*)-hexadec-9-enyl bromide, 68% over 2 steps; v, $ZnBr_2$, 80%.

of each of these compounds.[21] Verification of the structure assignments was accomplished by total chemical synthesis of each. The mass spectrum of **3** contained a molecular ion having *m/z* 282. The ^1H and ^{13}C NMR spectra of **3** were found to be identical with those of oleic acid.[13]

The activities of **1**–**5** as inhibitors of DNA polymerase β are shown in Table 1. Compound **1** exhibited an IC_{50} of 1.4 μM in the absence of bovine serum albumin (BSA). BSA reduced the IC_{50} to 9 μM, undoubtedly reflecting the binding of **1** to this (basic) protein. Unsaturated analogues **1** and **4** were found to have the greatest potencies in the presence of BSA (9 and 19 μM, respectively), and to be reproducibly more active than saturated derivatives **2** and **5**. Oleic acid, which is a simple fatty acid, was active only at significantly higher concentration. Methylation of the phenol or carboxylic acid moieties essentially eliminated inhibitory activity as did conversion of the carboxylate of **5** to the respective carboxamide, demonstrating that the anacardic acids are specific inhibitors rather than simple denaturants.§

Table 1 Inhibition of rat liver DNA polymerase by **1**–**5** and structurally related compounds[a]

Compound	IC_{50}/μM
1	1.4[b]
1	9
2	30
3	72
4	19
5	25

[a] Determined as described in footnote †. [b] Determined in the absence of bovine serum albumin.

Compound **1** and a few structurally related species were evaluated in more detail as DNA polymerase β inhibitors. In both short and long term mammalian cell culture, these compounds potentiated the action of DNA damaging agents such as bleomycin (Table 2) and *cis*-platinum, and inhibited bleomycin-induced unscheduled DNA synthesis. They also blocked DNA polymerase β-mediated gap filling of a DNA duplex substrate.[12] Thus specific inhibitors of DNA polymerase β may well be able to potentiate the effects of the DNA damage caused by clinically used antitumor agents that function at this locus.

Table 2 Potentiation of the cytotoxicity of bleomycin (BLM) by **1**[a]

Compounds present	Viable cells (% of control)
—	100
50 μM BLM	96
50 μM **1**	98
50 μM BLM + 50 μM **1**	68

[a] P388D, cells were cultured in suspension in the presence of the test compounds for 6 h, then assessed for viability by trypan blue exclusion staining.

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Notes and references

† Inhibition of polymerase β was assayed in 62.5 mM ammediol buffer, pH 8.6, containing 10 mM $MgCl_2$, 1 mM dithiothreitol, 50 μg ml^{-1} of BSA, 6.25 μM deoxynucleotide triphosphates including [^{3}H]thymidine triphosphate and 12.5 μg of DNase I-treated calf thymus DNA. The reactions were initiated with 0.2 μg of rat liver DNA polymerase β (ref. 10,11), incubated at 37 °C for 1 h, and monitored as described (ref. 12).

‡ The assignments of the H3, H4 and H5 resonances in the NMR spectra of **1**, **2**, **4** and **5** were further supported by HMQC spectroscopy. The spectra also proved useful for assigning proton and carbon resonances in the aliphatic substituents of **1**–**5**, used in combination with the full ^{13}C NMR assignments of a number of common fatty acids (ref. 13) and the ^{1}H-coupled ^{13}C NMR spectra of several compounds structurally related to **1**, **2**, **4** and **5** (ref. 14).

§ Also determined was the selectivity of inhibition. A number of derivatives were found to be essentially inactive as inhibitors of calf thymus DNA topoisomerase I, AMV reverse transcriptase, DNA polymerase I (Klenow fragment) and restriction endonuclease *Hin*dIII at concentrations at which those compounds strongly inhibited DNA polymerase β.

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