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Bioorganic & Medicinal Chemistry Letters 15 (2005) 2966–2969
Bioorganic & Medicinal Chemistry Letters

Synthetic lanostane-type triterpenoids as inhibitors of DNA topoisomerase II

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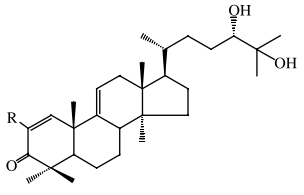
Received 17 March 2005; revised 22 April 2005; accepted 25 April 2005

Abstract—DNA topoisomerase (Topo) II is one of the target enzymes for chemotherapeutic drug development. Lanostane-type triterpenoids with various functional groups (–Cl, –Br, –OMe, –CHO, –CN, –COOH, and –COOMe) at C-2 were synthesized from 3-oxolanost-9(11)-en-24*S*,25-diol (**9**) isolated from *Pinus luchuensis* and their inhibitory effects on Topo II activity and cytotoxic activities against A549 cells were examined. All the derivatives showed Topo II inhibitory effects with IC_{50} values ranging from 1.86 to 149.97 μM and cytotoxic activities with ED_{50} values ranging from 3.96 to 38.15 μM.

DNA topoisomerases (Topos) are ubiquitous enzymes that govern the topological interconversions of DNA, thereby playing a crucial role in many aspects of DNA metabolism such as replication, transcription, recombination, and chromosome segregation at mitosis.[1] Topos are mechanistically divided into two main classes: the type I enzymes (Topos I) catalyze the ATP-independent relaxation of DNA supercoils by transiently breaking and religating single-stranded DNA, and the type II topoisomerases (Topos II) relax supercoiled DNA through catalysis of a transient breakage of double-stranded DNA in an ATP-dependent manner. To date, many Topo inhibitors have been developed and Topos have been identified as a target for anticancer chemotherapeutic drug development.[2] It has been reported that some triterpenoids inhibit Topo II activity and that a carboxylic moiety in the triterpenoid backbone may play an important role in the inhibition of Topo II.[3–6] Recently, Mizushina and co-workers reported that in the computer simulation, Topo II has a three-dimensional triterpenoid-binding region that appears in the form of a pocket into which specific triterpenoids can insert, consequently inhibiting Topo II activity.[5,6] In the binding region, Lys720 in Topo II may play an important role to interact with the carboxyl group of triterpenoids through electrostatic interaction. We reported that 3-oxolanost-9(11)-en-24*S*,25-diol (**9**), which was isolated from *Pinus luchuensis*, showed slight Topo II inhibitory effect (IC_{50} = 186 μM).[7] It was expected that if a carboxyl group was introduced into it for interaction with Lys720 in Topo II, its inhibitory effect would be enhanced. Therefore, we tried to introduce carboxyl groups into **9** and observed its Topo II inhibitory effect and cytotoxicity against human lung carcinoma A549 cells. In addition, the other functional groups were also introduced and the structure–activity relationships were studied.

Schemes 1 and 2 summarize our synthesis of **1**–**8** (Fig. 1).[8] The synthesis was executed according to the method of Honda and co-workers.[9,10] Compounds **1**–**4** were prepared in four steps from starting compound **9**



1 R = H **5** R = CHO
2 R = Cl **6** R = CN
3 R = Br **7** R = COOH
4 R = OMe **8** R = COOMe

Figure 1. Synthetic lanostane-type triterpenoids.

Keywords: Lanostane; Triterpenoid; DNA topoisomerase.
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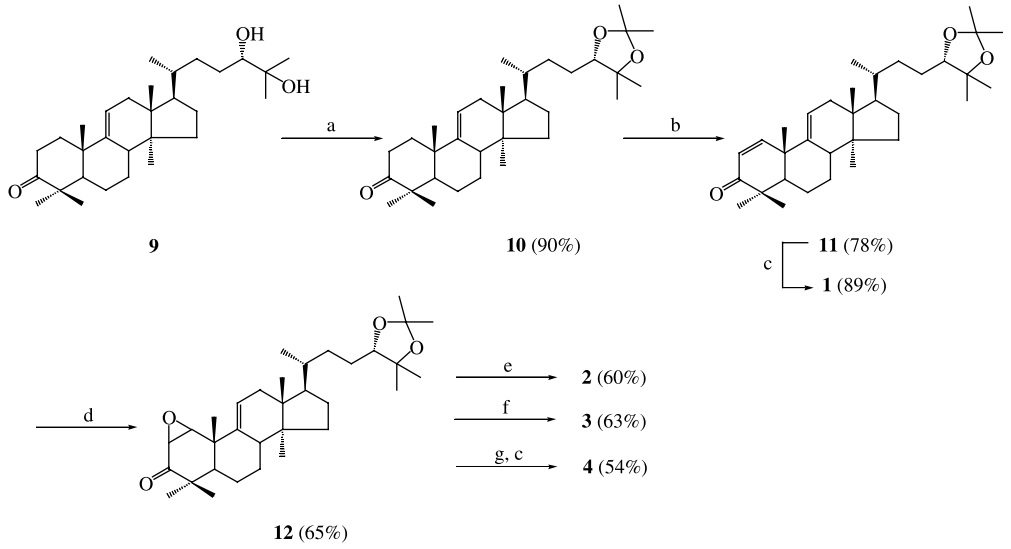
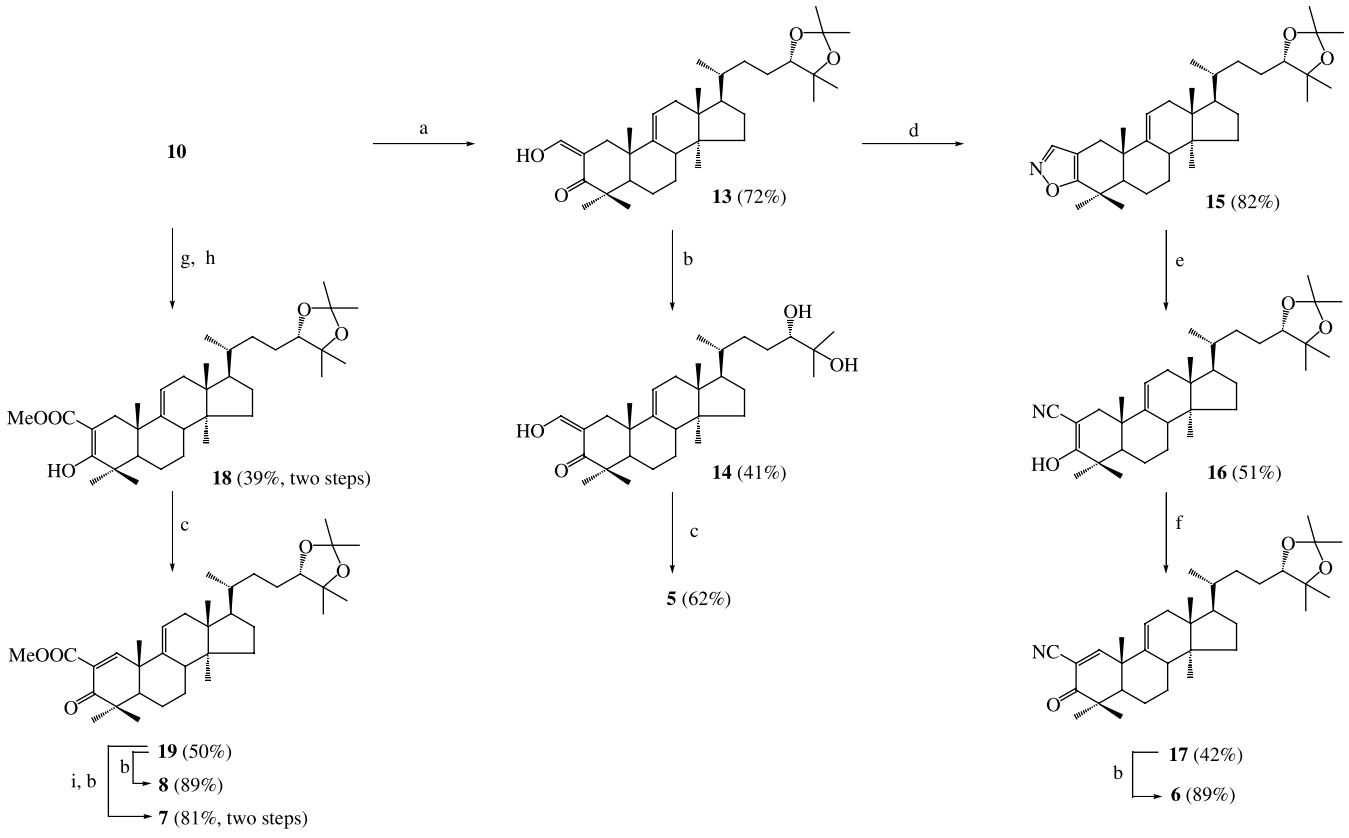
doi:10.1016/j.bmcl.2005.04.052



Scheme 1. Reagents: (a) Dimethoxypropane, TsOH, CH_2Cl_2; (b) PhSeCl, EtOAc, then H_2O_2, THF; (c) 1 N HCl, CH_2Cl_2, MeOH; (d) H_2O_2, NaOH, THF; (e) HCl, AcOH, $CHCl_3$; (f) HBr, AcOH, $CHCl_3$; (g) NaOMe, MeOH.



Scheme 2. Reagents: (a) HCOOEt, NaOMe, benzene; (b) 1 N HCl, CH_2Cl_2, MeOH; (c) PhSeCl, pyridine, CH_2Cl_2, then H_2O_2, THF; (d) NH_2OH–HCl, EtOH; (e) NaOMe, Et_2O; (f) DDQ, benzene; (g) Stile's reagent (methoxymagnesium methyl carbonate), DMF; (h) CH_2N_2, Et_2O–THF; (i) KOH, aq MeOH.

(Scheme 1). Protection of the C-24,25 dihydroxyl group by forming acetonide (**10**) was followed by the introduction of a double bond at C-1 with phenylselenyl chloride and the subsequent addition of hydrogen peroxide to give enone **11**. Removal of the protective group of **11** with hydrochloric acid yielded **1**. Epoxidation of **11** was followed by treatment with hydrochloric acid and hydrobromic acid to afford **2** and **3**, respectively, and with sodium methoxide to yield **4** after cleavage of the acetonide. Preparation of **5**–**8** was accomplished, as shown in Scheme 2. To introduce aldehyde and cyano groups at C-2, **10** was first formylated with ethyl for-

mate to give hydroxy methylene **13**. After hydrolysis of the acetonide, enal **5** was prepared according to the same method as that for **11**. On the other hand, isoxazole **15** was synthesized by condensation of **13** with hydroxylamine. Cleavage of the isoxazole part gave a mixture containing main component **16**, which was used directly in the next reaction. Oxidation of **16** with DDQ followed by hydrolysis of the acetonide gave **6**. Next, **10** was treated with Stile's reagent (methoxymagnesium methyl carbonate),[11] and this was followed by methylation with diazomethane to obtain main compound **18** as a mixture. Without further purification, ester **19** was synthesized from **18** according to the same method as that for **11**. Hydrolysis of the acetonide gave **8**. Ester **19** was hydrolyzed with potassium hydroxide, followed by deprotection of the acetonide to yield **7**.

The IC_{50} values of **1**–**8** against Topo II activity are shown in Table 1. All the compounds inhibited Topo II activity with IC_{50} values ranging from 1.86 to 149.97 μM. As expected, acid **7** significantly inhibited Topo II activity, whereas methyl ester **8** showed weaker Topo II inhibitory effect. Aldehyde **5** showed the highest Topo II inhibitory effect, probably due to the formation of a Schiff base between the ε-amino group of Lys720 and the aldehyde group of **5** in the binding pocket, in contrast to the electrostatic interaction between Lys720 and acid **7**. These results suggest that it might be possible for the synthetic triterpenoids to be inserted into the binding pocket, thereby inhibiting the Topo II activity. Compounds **3** and **4** showed similar effect to acid **7**, whereas **1**, **2**, and **6** showed weak inhibitory effect. The role of these functional groups remains ambiguous, however. As regards cytotoxic activity,[12] all these compounds exhibited the activity with ED_{50} values ranging from 3.96 to 38.15 μM. Acid **7** and methyl ester **8** exhibited potent activity, however, in contrast to the Topo II results, methyl ester **8** is more potent than acid **7**. It is surmised that due to the polarity of the carboxyl group of acid **7**, **7** may exhibit poor membrane permeabilization. Aldehyde groups were also found to reduce the cytotoxic activity, although the reason for this is unclear. The relationship between the Topo II inhibitory effects and the cytotoxicity by synthetic lanostane-type triterpenoids could not be clearly observed in this study. To achieve the cytotoxic effects of Topo II inhibitors, it is required that these molecules pass through plasma and nuclear membranes and, consequently, are located in nuclei. We consider that, probably, these triterpenoids could not efficiently reach the nuclei. Also, it might be possible for these compounds to inhibit the other cellular functional sites together with Topo II activities.

Table 1. IC_{50} values against topoisomerase II activity and ED_{50} values against A549 cells

Compound	IC_{50}[a] (μM)	ED_{50}[a] (μM)
1	149.97 ± 2.33	26.90 ± 4.58
2	106.80 ± 5.98	38.15 ± 5.18
3	18.27 ± 2.20	14.27 ± 4.81
4	22.77 ± 6.06	29.57 ± 1.60
5	1.86 ± 2.57	13.05 ± 2.59
6	83.23 ± 5.65	25.49 ± 3.54
7	23.80 ± 3.35	7.33 ± 1.31
8	66.51 ± 5.51	3.96 ± 0.31
Etoposide	50.40 ± 3.29	3.39 ± 0.61

[a] IC_{50} and ED_{50} values are means from at least three independent experiments (average ± SD).

In conclusion, from these data, we cannot indicate the relationships between structural characteristics such as lipophilicity and electron-withdrawing effects[13] and biological activities in this study. However, we can show that the introduction of functional groups into the triterpenoid skeleton increased the Topo II inhibitory effect and the cytotoxic activity and therefore, triterpenoids may serve as lead structures for new drug development.

Acknowledgments

We are grateful to the Resource Center for Biomedical Research, Institute of Development, Aging and Cancer, Tohoku University, for providing A549 cells.

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8. *3-Oxolanosta-1,9(11)-dien-24S,25-diol* (**1**): Colorless crystals; mp 196–198 °C (MeOH–$CHCl_3$); $[\alpha]_D^{22}$ +46 (*c* 0.26, $CHCl_3$); IR (KBr): ν_{max} = 3431, 1676, 1472, 1373 cm^{-1}; UV (EtOH): λ_{max} (log ε) = 207 (3.88), 227 (4.01) nm; SI-MS: *m*/*z* (rel. int.) = 457 (30) $[M+H]^+$; HR-SI-MS: *m*/*z* = 457.3680 ($C_{30}H_{49}O_3$, requires 457.3679); ^1H NMR ($CDCl_3$): δ_H = 7.30 (1H, d, *J* = 10.5 Hz), 5.93 (1H, d, *J* = 10.5 Hz), 5.47 (1H, d, *J* = 5.7 Hz), 3.29 (1H, d, *J* = 9.3 Hz), 1.28 (3H, s), 1.22 (3H, s), 1.17 (3H, s), 1.15 (3H, s), 1.06 (3H, s), 0.92 (3H, d, *J* = 6.3 Hz), 0.77 (3H, s), 0.69 (3H, s) ppm.
2-Chloro-3-oxolanosta-1,9(11)-dien-24S,25-diol (**2**): Colorless crystals; mp 195–197 °C (MeOH–$CHCl_3$); $[\alpha]_D^{22}$ +29 (*c* 0.01, $CHCl_3$); IR (KBr): ν_{max} = 3430, 1689, 1470, 1374 cm^{-1}; UV (EtOH): λ_{max} (log ε) = 204 (3.55), 245 (3.79) nm; SI-MS: *m*/*z* (rel. int.) = 491 (18) $[M+H]^+$; HR-SI-MS: *m*/*z* = 491.3286 ($C_{30}H_{48}ClO_3$, requires 491.3289); ^1H NMR ($CDCl_3$): δ_H = 7.52 (1H, s), 5.48 (1H, d, *J* = 6.0 Hz), 3.29 (1H, d, *J* = 10.2 Hz), 1.33 (3H, s), 1.22 (3H, s), 1.22 (3H, s), 1.17 (3H, s), 1.11 (3H, s), 0.92 (3H, d, *J* = 6.3 Hz), 0.77 (3H, s), 0.69 (3H, s) ppm.
2-Bromo-3-oxolanosta-1,9(11)-dien-24S,25-diol (**3**): Colorless amorphous solid; $[\alpha]_D^{22}$ +6.0 (*c* 0.06, $CHCl_3$); IR (KBr): ν_{max} = 3440, 1688, 1468, 1374 cm^{-1}; UV (EtOH): λ_{max} (log ε) = 204 (3.79), 253 (3.78) nm; SI-MS: *m*/*z* (rel. int.) = 535 (73) $[M+H]^+$; HR-SI-MS: *m*/*z* = 535.2782 ($C_{30}H_{48}BrO_3$, requires 535.2784); ^1H NMR ($CDCl_3$):

δ_H = 7.80 (1H, s), 5.48 (1H, d, J = 5.7 Hz), 3.29 (1H, d, J = 9.3 Hz), 1.33 (3H, s), 1.22 (3H, s), 1.22 (3H, s), 1.17 (3H, s), 1.11(3H, s), 0.92 (3H, d, J = 6.3 Hz), 0.77 (3H, s), 0.69 (3H, s) ppm.

2-Methoxy-3-oxolanosta-1,9(11)-dien-24S,25-diol (**4**): Colorless oil; $[\alpha]_D^{22}$ +37 (c 0.30, $CHCl_3$); IR (film): ν_{max} = 3488, 1679, 1461, 1385 cm^{-1}; UV (EtOH): λ_{max} (log ε) = 203 (3.65), 261 (3.83) nm; SI-MS: m/z (rel. int.) = 487 (46) $[M+H]^+$; HR-SI-MS: m/z = 487.3784 ($C_{31}H_{51}O_4$, requires 487.3784); ^1H NMR ($CDCl_3$): δ_H = 6.22 (1H, s), 5.51 (1H, d, J = 6.0 Hz), 3.66 (3H, s), 3.29 (1H, d, J = 9.9 Hz), 1.30 (3H, s), 1.22 (3H, s), 1.18 (3H, s), 1.17 (3H, s), 1.10 (3H, s), 0.92 (3H, d, J = 6.3 Hz), 0.78 (3H, s), 0.70 (3H, s) ppm.

2-Formyl-3-oxolanosta-1,9(11)-dien-24S,25-diol (**5**): Colorless oil; $[\alpha]_D^{22}$ +11 (c 0.16, $CHCl_3$); IR (film): ν_{max} = 3489, 1703, 1673, 1467, 1373 cm^{-1}; UV (EtOH): λ_{max} (log ε) = 206 (3.84), 234 (3.80) nm; SI-MS: m/z (rel. int.) = 485 (1) $[M+H]^+$; HR-SI-MS: m/z = 485.3632 ($C_{31}H_{49}O_4$, requires 482.3628); ^1H NMR ($CDCl_3$): δ_H = 10.11 (1H, s), 8.18 (1H, s), 5.59 (1H, d, J = 5.7 Hz), 3.29 (1H, d, J = 9.9 Hz), 1.33 (3H, s), 1.23 (3H, s), 1.20 (3H, s), 1.17 (3H, s), 1.11 (3H, s), 0.92 (3H, d, J = 6.0 Hz), 0.76 (3H, s), 0.70 (3H, s) ppm.

2-Cyano-3-oxolanosta-1,9(11)-dien-24S,25-diol (**6**): Colorless crystals; mp 259–262 °C (MeOH–$CHCl_3$); $[\alpha]_D^{22}$ +40 (c 0.12, $CHCl_3$); IR (KBr): ν_{max} = 3422, 1689, 1467, 1373 cm^{-1}; UV (EtOH): λ_{max} (log ε) = 208 (3.88), 235 (4.06) nm; SI-MS: m/z (rel. int.) = 482 (61) $[M+H]^+$; HR-SI-MS: m/z = 482.3635 ($C_{31}H_{48}NO_3$, requires 482.3631); ^1H NMR ($CDCl_3$): δ_H = 8.07 (1H, s), 5.47 (1H, d, J = 5.7 Hz), 3.29 (1H, d, J = 9.0 Hz), 1.36 (3H, s), 1.23 (3H, s), 1.21 (3H, s), 1.17 (3H, s), 1.10 (3H, s), 0.92 (3H, d,J = 6.3 Hz), 0.77 (3H, s), 0.69 (3H, s) ppm.

2-Carboxy-3-oxolanosta-1,9(11)-dien-24S,25-diol (**7**): Colorless needles; mp 200–203 °C (MeOH–$CHCl_3$); $[\alpha]_D^{22}$ +11 (c 0.09, $CHCl_3$); IR (KBr): ν_{max} = 3445, 1760, 1657, 1440, 1374 cm^{-1}; UV (EtOH): λ_{max} (log ε) = 206 (3.91), 231 (3.88) nm; SI-MS: m/z (rel. int.) = 501 (82) $[M+H]^+$; HR-SI-MS: m/z = 501.3580 ($C_{31}H_{49}O_5$, requires 501.3578); ^1H NMR ($CDCl_3$): δ_H = 8.85 (1H, s), 5.63 (1H, d, J = 5.1 Hz), 3.29 (1H, d, J = 9.0 Hz), 1.36 (3H, s), 1.25 (3H, s), 1.23 (3H, s), 1.17 (3H, s), 1.15 (3H, s), 0.92 (3H, d, J = 6.3 Hz), 0.76 (3H, s), 0.70 (3H, s) ppm.

2-Methoxycarbonyl-3-oxolanosta-1,9(11)-dien-24S,25-diol (**8**): Colorless needles; mp 115–118 °C (MeOH–$CHCl_3$); $[\alpha]_D^{22}$ −13 (c 0.01, $CHCl_3$); IR (KBr): ν_{max} = 3437, 1714, 1694, 1437, 1373 cm^{-1}; UV (EtOH): λ_{max} (log ε) = 204 (3.65), 261 (3.83) nm; SI-MS: m/z (rel. int.) = 515 (100) $[M+H]^+$; HR-SI-MS: m/z = 515.3732 ($C_{32}H_{51}O_5$, requires 515.3734); ^1H NMR ($CDCl_3$): δ_H = 8.07 (1H, s), 5.58 (1H, d, J = 5.1 Hz), 3.82 (3H, s), 3.29 (1H, d, J = 9.9 Hz), 1.29 (3H, s), 1.22 (3H, s), 1.17 (3H, s), 1.16 (3H, s), 1.12 (3H, s), 0.92 (3H, d, J = 6.3 Hz), 0.76 (3H, s), 0.69 (3H, s) ppm.

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