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Synthesis and bioevaluation of 22-hydroxyacuminatine analogs

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Abstract—A series of 22-hydroxyacuminatine analogs was prepared by using different Friedländer condensations. Several of the new compounds were tested for antiproliferative activity on cancer cell lines and for topoisomerase I inhibitory activity.

Camptothecin-family alkaloids and their derivatives have attracted enormous attention from both academic and pharmaceutical research groups due to their potent antiproliferative activities.[1] The archetype of this family, camptothecin (**1**),[2] acts as a selective poison of DNA topoisomerase I (Fig. 1).[3] Several AB-ring substituted analogs of this molecule are among the most potent drugs used in cancer chemotherapy: Topotecan (**2**)[4] and Irinotecan (**3**)[5] have been approved as antineoplastic agents and a number of other analogs of **1** are currently in clinical trials.[6]

22-Hydroxyacuminatine (**4**)[7] is structurally similar to camptothecin, albeit lacking the lactone motif in the E ring. This alkaloid was isolated in extremely low yield (0.000006%) from *Camptotheca acuminata* and was shown to have significant in vitro cytotoxic activity against the murine leukemia P-388 and KB (ED_{50} 1.32 and 0.61 μg/mL, respectively).

So far, only a few total syntheses of this alkaloid have been described and most of them are not easily adapted for producing analogs.[8]

We have recently developed an efficient and flexible approach to 22-hydroxyacuminatine starting from hydroxy pyridone **5**.[8c] As summarized in Scheme 1, the synthesis of the natural product **4** was achieved by reduction of ester **8**, which could be produced by a Friedländer condensation of the key tricyclic compound **6** with an *o*-aminobenzaldehyde surrogate **7**.

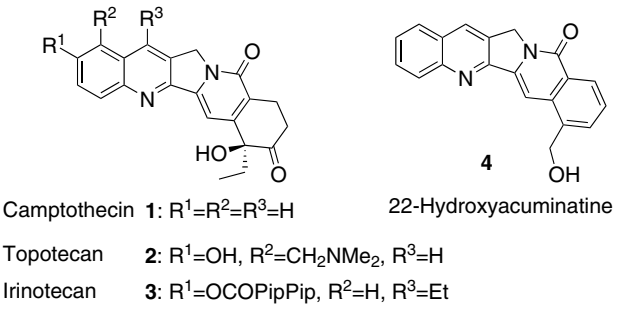
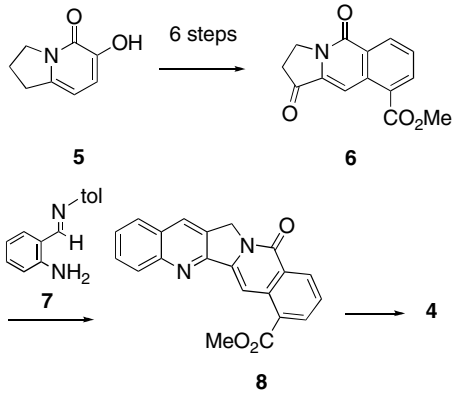


Figure 1. Structures of camptothecin, its analogs, and 22-hydroxyacuminatine.



Scheme 1. Reagents and conditions: see Ref. 8c.

Keywords: Camptothecin; 22-Hydroxyacuminatine analogs; Synthesis; Topoisomerase I; Cytotoxicity.
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In this paper, we report the synthesis of a series of analogs of **4**, taking advantage of the flexibility inherent in our previous work. For several derivatives, in vitro antiproliferative activities on human cancer cell lines and topoisomerase I inhibitory activity have been measured.

The synthesis of the keto pyridone **6** was conducted by using our previously described route, but with a modification of the oxidation at the benzylic-like position. The transformation of **9** into **6**, initially achieved in two steps, is now effected in a single step under aerobic conditions in the presence of *N*-hydroxyphthalimide (NHPI) and CuCl.[9] This new procedure avoids the use of toxic selenium dioxide and improves the reproducibility of the transformation (Scheme 2).

Next, a series of masked substituted *o*-aminobenzaldehydes **12a–d** was prepared by condensation of *p*-toluidine with *o*-nitrobenzaldehydes, followed by sodium sulfide reduction.[10] The resulting arylimines were then treated with keto pyridone **6** under Friedländer conditions to afford the corresponding pentacyclic esters **13a–d**[11] in serviceable yields (Scheme 3).

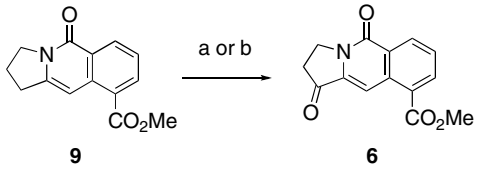
It had been hoped that these esters could be converted into the corresponding hydroxymethylated derivatives by using Dibal-H in dichloromethane at low temperature, as for the preparation of natural product **4**. However, due to their very poor solubility, the reduction of these esters proved to be inefficient. Only compound **13b** could be transformed into **14b** in reasonable yield.[12]

In order to synthesize also a series of analogs bearing substituents on both the A and B rings, a number of *o*-aminoketones **16a–g** were prepared. The synthesis of these substrates was effected by direct acylation of substituted anilines,[13] as outlined in Scheme 4. The Friedländer condensations were then performed as in the previous series, but the corresponding pentacyclic esters **17a–g** were obtained in higher yields.[14] Esters **17b**, **17e**, and **17g** were next reduced by exposure to Dibal-H to afford the corresponding alcohols.[15]

Some of the pentacyclic compounds herein obtained were selected for biological evaluation (see Table 1). The antiproliferative activities against three human cancer cell lines (DU145, Mia PaCa, and HT29) were compared with those of 22-hydroxyacuminatine (**4**) and SN-38.

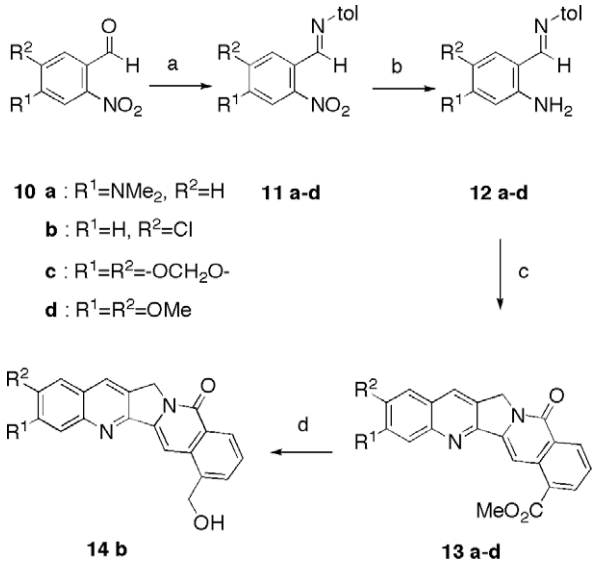
As can be seen, no effect on cellular proliferation could be detected against the colorectal cancer cell line HT29. Only compounds **18b** and **18g** showed slight activity against prostate cancer cell line DU145. However, most of the tested compounds were active against pancreatic carcinoma Mia PaCa, with IC_{50} values ranging from 0.81 to 1.99 μM.[16]

In the ester series, the introduction of substituents on the quinoline ring system does not seem to be beneficial

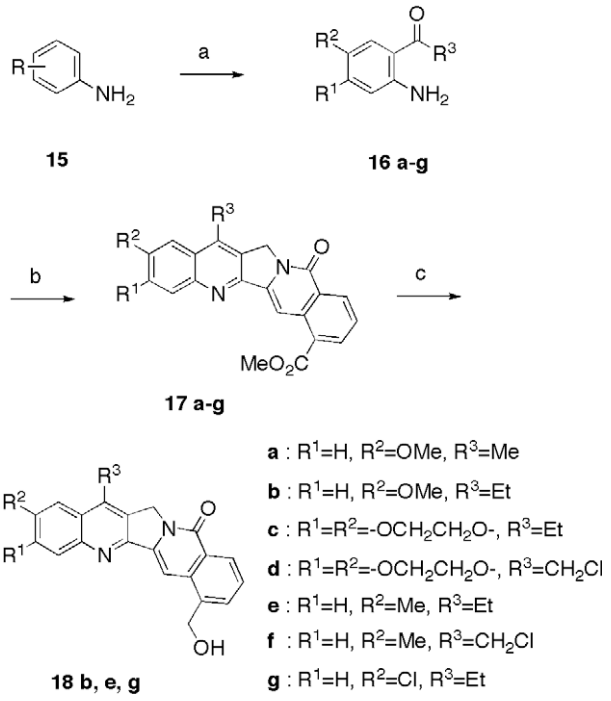


9 **6**

Scheme 2. Reagents and conditions: (a) SeO_2, dioxane, reflux; Dess–Martin periodinane, CH_2Cl_2 (75%, 2 steps); (b) O_2, CuCl, NHPI, CH_3CN, 35 °C (75%).



Scheme 3. Reagents and conditions: (a) *p*-toluidine, EtOH, reflux, 2 h (63–99%); (b) $Na_2S{\cdot}9H_2O$, EtOH, reflux, 2 min (60–69%); (c) **6**, PhMe, *p*-TsOH, reflux Dean–Stark (30–53%); (d) Dibal-H, CH_2Cl_2, −78 °C (56%).



Scheme 4. Reagents and conditions: (a) BCl_3, R^3CN, $GaCl_3$, CH_2Cl_2, reflux, 24 h (45–81%); (b) **6**, *p*-TsOH, PhMe, reflux Dean–Stark, 5 h (64–93%); (c) Dibal-H, CH_2Cl_2, −78 °C, 5 h (26–60%).

Table 1. In vitro cytostatic activity against various cell lines

Compound	DU145 IC_{50}[a] (μM)	Mia PaCa IC_{50}[a] (μM)	HT29 IC_{50}[a] (μM)
8	na[b]	1.01	na
14b	na	1.70	na
17a	na	1.99	na
17b	na	na	na
17c	na	1.40	na
17e	na	1.81	na
17f	na	1.35	na
17g	na	1.50	na
4	na	1.01	na
18b	1.81	0.81	na
18e	na	1.14	na
18g	1.69	0.81	na
SN-38	0.004	0.006	0.012

[a] Concentration necessary for 50% of cell growth inhibition after 96 h of incubation.

[b] Not active at concentrations up to 2 μM.

for biological activity, while in the hydroxymethyl series, the presence of these substituents showed negligible influence.

In addition, topoisomerase I inhibitory activity assays with the same derivatives[17] were carried out using the supercoiled DNA unwinding method.[18] Only compounds **8**, **17g**, and **18g** revealed some inhibitory activity, but much lower than that of camptothecin (used as the positive reference).

It should be pointed out that all of the tested compounds have low solubility in DMSO. As the maximum tested concentrations used in both biological studies were limited by the final DMSO concentrations in the assay mixtures (4% or 5%), in some cases the activity could not be quantified.

In summary we have used the flexibility of our synthetic approach to prepare a variety of 22-hydroxyacuminatine analogs. We have also performed the first structure–activity relationship study of this cytotoxic alkaloid. None of the tested compounds proved significantly more active than the natural product **4**.

Acknowledgments

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9. (a) A solution of 0.62 mmol of compound **9**, 0.62 mmol of *N*-hydroxyphthalimide and 0.031 mmol of CuCl in 11 mL of acetonitrile under O_2 was stirred at 35 °C for 24 h. After evaporation of the solvent under reduced pressure, the residue was purified by silica gel column chromatography to afford **6**; For a review of NHPI-catalyzed aerobic oxidation, see (b) Ishii, Y.; Sakaguchi, S.; Iwahama, T. *Adv. Synth. Catal.* **2001**, *343*, 393.
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11. (a) *Compound **13a:*** mp 264.5–266.5 °C, ^{1}H NMR ($CDCl_3$) δ 3.14 (s, 6H), 4.06 (s, 3H), 5.29 (s, 2H), 7.13 (m, 2H), 7.54 (t, 1H, J = 7.7 Hz), 7.63 (d, 1H, J = 8.9 Hz), 8.10 (s, 1H), 8.27 (d, 1H, J = 7.4 Hz), 8.56 (s, 1H), 8.73 (d, 1H, J = 8.2 Hz), HRMS *m/z* calcd for $C_{23}H_{20}N_3O_3$ (MH^+) 386.14992, found 386.15012; (b) *Compound **13b:*** mp 277–279 °C, ^{1}H NMR ($CDCl_3$) δ 4.07 (s, 3H), 5.35 (s, 2H), 7.58 (t, 1H, J = 7.5 Hz), 7.69 (d, 1H, J = 8.8 Hz), 7.83 (s, 1H), 8.13 (d, 1H, J = 8.8 Hz), 8.20 (s, 1H), 8.39 (d, 1H, J = 7.2 Hz), 8.69 (s, 1H), 8.73 (d, 1H, J = 7.8 Hz), HRMS *m/z* calcd for $C_{21}H_{14}N_2O_3Cl$ (MH^+) 377.06875, found 377.06892; (c) *Compound **13c:*** mp 267–268 °C, ^{1}H NMR ($CDCl_3$) δ 4.06 (s, 3H), 5.30 (s, 2H), 6.17 (s, 2H), 7.12 (s, 1H), 7.51 (s, 1H), 7.56 (t, 1H, J = 7.9 Hz), 8.12 (s, 1H), 8.38 (d, 1H, J = 7.5 Hz), 8.63 (s, 1H), 8.76 (d, 1H, J = 7.3 Hz); (d) *Compound **13d:*** mp 295–297 °C, ^{1}H NMR ($CDCl_3$) δ 4.03 (s, 3H), 4.06 (s, 3H), 4.10 (s, 3H), 5.31 (s, 2H), 7.06 (s, 1H), 7.55 (m, 2H), 8.14 (s, 1H), 8.36 (m, 1H), 8.58 (s, 1H), 8.74 (d, 1H, J = 7.8 Hz), HRMS *m/z* calcd for $C_{23}H_{18}N_2O_5Na$ (MNa^+) 425.11079, found 425.11154.
12. *Compound **14b:*** mp 297.5–299 °C, ^{1}H NMR ($CDCl_3$/MeOD 4/1) δ 5.13 (s, 2H), 5.38 (s, 2H), 7.58 (t, 1H, J = 7.6 Hz), 7.76 (m, 1H), 7.87 (d, 1H, J = 6.9 Hz), 7.92 (m, 1H), 7.97 (s, 1H), 8.13 (d, 1H, J = 9.1 Hz), 8.34 (s, 1H), 8.41 (d, 1H, J = 8.1 Hz), HRMS *m/z* calcd for $C_{20}H_{14}N_2O_2Cl$ (MH^+) 349.07383, found 349.07390.
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14. (a) *Compound **17a:*** mp 276–278.5 °C, ^{1}H NMR ($CDCl_3$) δ 2.73 (s, 3H), 3.98 (s, 3H), 4.05 (s, 3H), 5.31 (s, 2H), 7.45 (m, 2H), 7.56 (t, 1H, J = 7.7 Hz), 8.14 (d, 1H, J = 9.2 Hz),

8.37 (d, 1H, J = 7.7 Hz), 8.64 (s, 1H), 8.77 (d, 1H, J = 7.4 Hz), HRMS m/z calcd for $C_{23}H_{19}N_2O_4$ (MH^+) 387.13393, found 387.13398; (b) *Compound**17b:*** mp 236–237 °C, ^{1}H NMR ($CDCl_3$) δ 1.40 (t, 3H, J = 7.5 Hz), 3.11 (q, 2H, J = 7.5 Hz), 3.96 (s, 3H), 4.06 (s, 3H), 5.29 (s, 2H), 7.24 (s, 1H), 7.41 (m, 1H), 7.54 (t, 1H, J = 7.8 Hz), 8.11 (d, 1H, J = 9.2 Hz), 8.35 (d, 1H, J = 7.5 Hz), 8.59 (s, 1H), 8.73 (d, 1H, J = 7.8 Hz), HRMS m/z calcd for $C_{24}H_{21}N_2O_4$ (MH^+) 401.14958, found 401.14958; (c) *Compound **17c:*** mp 307–308 °C, ^{1}H NMR ($CDCl_3$) δ 1.39 (t, 3H, J = 7.7 Hz), 3.09 (q, 2H, J = 7.7 Hz), 4.06 (s, 3H), 4.43 (s, 4H), 5.29 (s, 2H), 7.48 (s, 1H), 7.56 (t, 1H, J = 7.7 Hz), 7.68 (s, 1H), 8.38 (m, 1H), 8.64 (s, 1H), 8.77 (d, 1H, J = 8.1 Hz), HRMS m/z calcd for $C_{25}H_{21}N_2O_5$ (MH^+) 429.14450, found 429.14351; (d) *Compound **17d:*** mp 324–326 °C, ^{1}H NMR (DMSO-d_6, 70°C) δ 4.01 (s, 3H), 4.48 (s, 4H), 5.34 (s, 2H), 5.42 (s, 2H), 7.68 (m, 2H), 7.76 (s, 1H), 8.38 (m, 2H), 8.65 (m, 1H); (e) *Compound **17e:*** mp 260–262 °C, ^{1}H NMR ($CDCl_3$) δ 1.42 (t, 3H, J = 7.7 Hz), 2.61 (s, 3H), 3.20 (q, 2H,J = 7.7 Hz), 4.07 (s, 3H), 5.33 (s, 2H), 7.59 (m, 2H), 7.85 (s, 1H), 8.14 (d, 1H, J = 8.6 Hz), 8.38 (d, 1H, J = 7.2 Hz), 8.68 (s, 1H), 8.78 (d, 1H, J = 8.6 Hz), HRMS m/z calcd for $C_{24}H_{21}N_2O_3$ (MH^+) 385.15467, found 385.15474; (f) *Compound **17f:*** mp 297–299.5 °C, ^{1}H NMR ($CDCl_3$) δ 2.64 (s, 3H), 4.07 (s, 3H), 5.05 (s, 2H), 5.45 (s, 2H), 7.62 (m, 2H), 7.91 (s, 1H), 8.18 (d, 1H, J = 8.6 Hz), 8.40 (d, 1H, J = 7.4 Hz), 8.72 (s, 1H), 8.78 (d, 1H, J = 8.3 Hz), HRMS m/z calcd for $C_{23}H_{18}N_2O_3Cl$ (MH^+) 405.10005, found 405.10017; (g) *Compound **17g:*** mp 296–298 °C, ^{1}H NMR ($CDCl_3$) δ 1.42 (t, 3H, J = 7.7 Hz), 3.15 (q, 2H, J = 7.7 Hz), 4.06 (s, 3H), 5.33 (s, 2H), 7.59 (t, 1H, J= 7.8 Hz), 7.70 (m, 1H), 8.04 (m, 1H), 8.17 (d, 1H, J = 9.0 Hz), 8.40 (m, 1H), 8.71 (s, 1H), 8.77 (d, 1H, J = 7.9 Hz), HRMS m/z calcd for $C_{23}H_{18}N_2O_3Cl$ (MH^+) 405.10005, found 405.10010.

15. (a) *Compound **18b:*** mp 280–282 °C, NMR ^{1} H (DMSO-d_6) δ 1.35 (t, 3H, J = 7.2 Hz), 3.23 (m, 2H), 3.99 (s, 3H), 4.94 (m, 2H), 5.37 (s, 2H), 5.46 (m, 1H), 7.53 (m, 3H), 7.63 (s, 1H), 7.80 (d, 1H, J = 6.2 Hz), 8.11 (d, 1H, J = 9.4 Hz), 8.30 (d, 1H, J = 8.3 Hz), HRMS m/z calcd for $C_{23}H_{21}N_2O_3$ (MH^+) 373.15467, found 373.15464; (b) *Compound **18e:*** mp 312–314 °C, NMR ^{1}H ($CDCl_3$/MeOD, 4/1) δ 1.36 (t, 3H, J = 7.7 Hz), 2.47 (s, 3H), 3.15 (q, 2H, J = 7.7 Hz), 5.04 (s, 2H), 5.27 (s, 2H), 7.50 (t, 1H, J = 7.7 Hz), 7.58 (d, 1H, J = 8.7 Hz), 7.82 (m, 3H), 8.00 (d, 1H, J = 8.6 Hz), 8.35 (d, 1H, J = 7.9 Hz), HRMS m/z calcd for $C_{23}H_{21}N_2O_2$ (MH^+) 357.16049, found 357.15975; (c) *Compound **18g:*** mp 301–302 °C, NMR ^{1} H (DMSO-d_6) δ 1.33 (t, 3H, J = 7.5 Hz), 3.21 (q, 2H, J = 7.5 Hz), 4.94 (m, 2H), 5.38 (s, 2H), 5.47 (t, 1H, J = 5.4 Hz), 7.57 (t, 1H, J = 7.7 Hz), 7.69 (s, 1H), 7.84 (m, 2H), 8.19 (d, 1H, J = 9.0 Hz), 8.29 (m, 2H), HRMS m/z calcd for $C_{22}H_{18}N_2O_2Cl$ (MH^+) 377.10574, found 377.10513.
16. Since no significant improvement of activity relative to **4** on the three tumor cell lines was observed, tests on normal cell lines were not performed.
17. Although 22-hydroxyacuminatine does not inhibit topoisomerase I,[8b] several non-lactonic derivatives of camptothecin do show inhibitory activity (Hautefaye, P.; Cimetière, B.; Pierré, A.; Léonce, S.; Hickman, J.; Laine, W.; Bailly, C.; Lavielle, G. *Bioorg. Med. Chem. Lett.* **2000**, *13*, 2731). Since the new compounds reported in this paper can also be viewed as non-lactonic derivatives of camptothecin, they were screened as well for inhibition of topoisomerase I.
18. Supercoiled pBR322 plasmid DNA (130 ng) was incubated with 4 U of human topoisomerase I (TopoGen) at 37 °C for 45 min in 20 μL of relaxation buffer (50 mM tris(hydroxymethyl)aminomethane (pH 7.8), 50 mM KCl, 10 mM $MgCl_2$, 1 mM dithiothreitol, 1 mM EDTA and 1 mM ATP) in the presence of the tested compounds and DMSO (5%). The reactions were terminated by addition of SDS to 0.25% and proteinase K to 250 μg/mL and incubation at 50 °C for a further 30 min. Three microliters of the electrophoresis dye mixture was added to the DNA samples, which were then separated by electrophoresis in a 1% agarose gel containing ethidium bromide (1 μg/mL). Gels were run at room temperature for 2 h at 120 V and scanned with a Typhoon 9410 imager.