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Synthesis and cytotoxic activity of substituted Luotonin A derivatives

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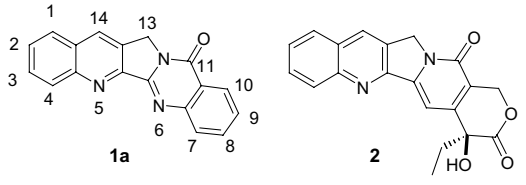
Abstract—Luotonin A is a cytotoxic alkaloid that has been shown to inhibit topoisomerase I via stabilization of the binary complex topoisomerase–DNA in the same fashion as camptothecin. The synthesis and the cytotoxic activity on the lung carcinoma cell line H460 of a series of derivatives of Luotonin A is reported. The compounds inhibit topoisomerase I but show weak cytotoxic activity, thus confirming the peculiarity of ring E of camptothecin for antitumor activity.

Luotonin A (**1a**, quino[2′,3′:3,4]pyrrolo[2,1-*b*]quinazolin-11(13H)-one) is an alkaloid isolated[1] from *Peganum nigellastrum* Bunge that has received increasing attention in the last few years, due to its structural similarity with the well-known cytotoxic alkaloid camptothecin (**2**) (CPT).[2] After the report of its isolation in 1997,[1] more than 10 syntheses of Luotonin A have been reported in the literature,[3] included one from our own group.[3h]



Notwithstanding the similarity with CPT, Luotonin A is far less active than CPT in tests of antitumor activity.[1] However, a recent report by Hecht and co-workers[4] has shown that Luotonin A is a topoisomerase I poison. Luotonin A induces the same sequence selectivity of DNA cleavage by topoisomerase I as CPT, thus indicating that, although less potently than CPT, it stabilizes in a similar way the binary DNA–topoisomerase cleavable complex. The formation of a stable ternary complex is deemed to be the reason for the cytotoxic activity of CPT. These results raise the question of which structural features of the ring E of these compounds are indeed necessary, or important, in stabilizing such a complex, and of the possible correlation between inhibition of topoisomerase I and cytotoxic activity.

The interest of synthesizing and testing analogues or derivatives of Luotonin A with substituents of different position and polarity in ring E to this purpose was apparent to us as soon as we synthesized the parent compound. A very recent publication by Hecht and co-workers[5] that reports the synthesis, stabilizing activity of the binary complex, and cytotoxicity of some ring E derivatives of Luotonin A prompts us to disclose our own results.

We report here the synthesis, the assessment of the cytotoxic activity on a tumor cell line and of the stabilizing activity of the DNA–topoisomerase I complex of a series of Luotonin A derivatives substituted in ring E but also in ring A. Except for one compound (**1b**) they differ from those reported recently by Hecht and co-workers[5] and therefore may complement and enlarge his results.

We explored some of the syntheses so far reported in the literature, but none of them seemed the optimal procedure for all derivatives, so that the ring E-substituted

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compounds **1a**–**g** (Table 1) were prepared from the tricyclic intermediate **3**[3h] by condensation with the appropriately substituted anthranilic acid[6] or by microwave reaction with the appropriate isatoic anhydride[3g] (Scheme 1). The two derivatives substituted at ring A **4a**,**b** were obtained by Friedländer condensation of the tricyclic compound **5**[3c,3d,7] with the appropriate 2-aminobenzaldehyde.[8] Compound **6**, with a reduced ring B, was obtained in the attempt to reduce the nitro group of **1c** to an amino group.

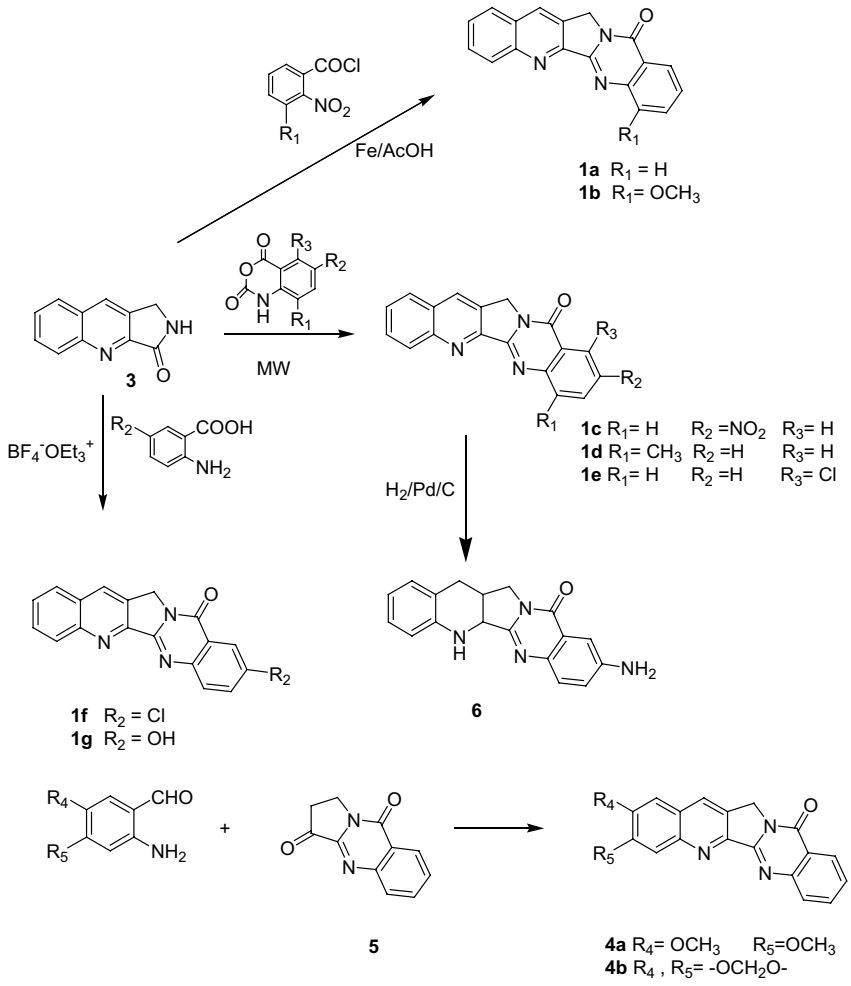
Table 1. In vitro cytotoxic (IC_{50} (μM)) activity of Luotonin A derivatives on H460 cell line

Compound	IC_{50} (μM) 1 h	IC_{50} (μM) 72 h
1a	67	7.7
1b	85	11
1c		23
1d	21	7
1e		13
1f	172	45
1g	40	3.8
4a		5.47
4b		81
6		86
SN-38	0.21	

The compounds prepared were evaluated for their cytotoxicity against the human non-small lung carcinoma cell line H460, using SN-38, that is the active principle of one of the two camptothecin drugs currently in clinical practice, Irinotecan, as a reference compound (Table 1).

This cell model was chosen for its sensitivity to topoisomerase I inhibitors, likely related to overexpression of the target enzyme.[9]

Moreover, stimulation of the topoisomerase I-mediated cleavage of DNA was studied for some compounds (**1a**,**d**,**e**,**g**, **6**) (Fig. 1). This experiment shows that indeed these Luotonin derivatives induce cleavage at the same sites as well as camptothecins, although in a lesser extent. Compounds **1d** and **1e** were the most potent as topoisomerase I poisons. From Table 1, it appears that all compounds are cytotoxic toward the H460 cell line.[10] However, their potency was substantially lower than that of SN-38. Optimal effects of topoisomerase I inhibitors require a persistent stabilization of the cleavable



Scheme 1. Synthesis of Luotonin A derivatives.

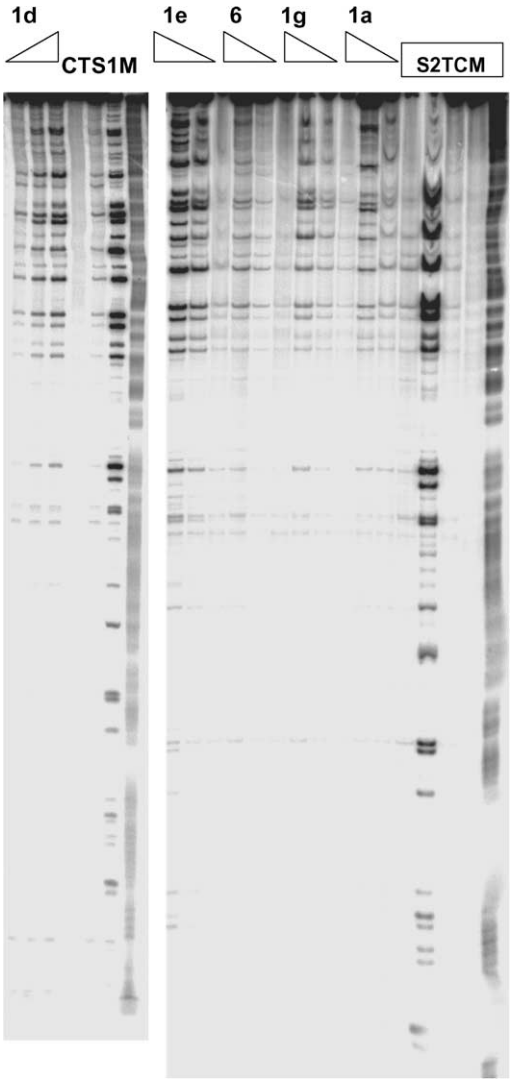


Figure 1. Dose dependent stimulation of topoisomerase I-mediated DNA cleavage in the presence of Luotonin A and derivatives. The samples were reacted for 30 min with 1, 10, and 50 μM compound. The triangle indicates the increase of drug concentration. (C) Control DNA (751 bp); (T) reaction without the compound; (M) purine markers; (S1) 10 μM SN-38; (S2) 50 μM SN-38. SN-38 is the reference drug 10-hydroxy-7-ethylcamptothecin.

complex to favor the formation of the lethal double-strand DNA lesions during DNA synthesis. Indeed topoisomerase I poisons are known to be S-phase specific. Since, as for camptothecins, the drug interaction with the DNA–enzyme complex is expected to be reversible, the drug potency is likely related to the drug affinity in the ternary complex. The cytotoxic effects of the compounds tested in the present study increased following a prolonged exposure (72 h). The low cytotoxic potency likely reflected a reduced affinity or increased reversibility of the complex. However, the appreciable stabilization of the cleavable complex observed in vitro with isolated topoisomerase I suggests an unfavorable behavior in cellular pharmacokinetics of compounds of this series, likely related to their physico-chemical properties (e.g., low solubility).

These data, together with the observation that one of the most active compound is the 2,3-dimethoxy analogue **4a**, lead us to suggest that the biological activity of Luotonin A derivatives is similar to that of other planar polycyclic heteroaromatic compounds, such as indenoisoquinolines,[11] benzo[*c*][1,7] and [1,8]phenanthrolines,[12] benzo[*i*]phenanthridines[13] and other analogues of protoberberine alkaloids, that show noticeable stabilization of the binary DNA–topoisomerase I complex, but cytotoxicity of the order of magnitude of Luotonin A derivatives, that is far less than that of camptothecins. The unique arrangement of functionalities in the E ring of camptothecins remains so far an unparalleled feature for antitumor activity.

Luotonin A (**1a**) was prepared following the procedure reported in our previous paper.[3h]

7-Methoxyluotonin A (**1b**): To a suspension of 84 mg (0.46 mmol) of **3** in 4 mL of dry THF, 20 mg (0.5 mmol) of NaH (60% mineral oil) were added with cooling in one portion. The mixture was heated at 60 °C until the evolution of gas ceased, then ice cooled and added with 108 mg (0.5 mmol) of 2-nitro-3-methoxybenzoylchloride. After heating at 60 °C for 3 h the solvent was evaporated to obtain a crude product, which was used without further purification. ^{1}H NMR ($CDCl_3$): δ 4.05 (s, 3H), 5.15 (s, 2H), 7.05 (d, J = 9.09 Hz, 1H), 7.45–7.55 (m, 1H), 7.55–7.75 (m, 2H), 7.83 (d, J = 9.09 Hz, 1H), 7.96 (d, J = 9.09 Hz, 1H), 8.35 (d, J = 9.09 Hz, 1H), 8.40 (s, 1H). The above compound (88 mg, 0.24 mmol) was suspended in 8 mL of AcOH/abs EtOH 1:1, added with 88 mg (1.58 mmol) of Fe, refluxed for 2 h, the iron was filtered and washed with AcOEt and chloroform. The organic layers were washed with satd aq $NaHCO_3$ and brine, then dried. Evaporation and purification by flash column chromatography (FC) (AcOEt) gave **1b** (26 mg, 17%), mp > 250 °C. ^{1}H NMR ($CDCl_3$): δ 4.10 (s, 3H), 5.37 (s, 2H), 7.31 (d, J = 8.82 Hz, 1H), 7.55 (dd, J = 8.82 and 8.82 Hz, 1H), 7.70 (dd, J = 8.82 and 8.82 Hz, 1H), 7.85 (dd, J = 8.82 and 8.82 Hz, 1H), 7.90–8.05 (m, 2H), 8.46 (s, 1H).

1. General procedure for the synthesis under MW irradiation

A suspension of 3-oxo-1*H*-pyrrolo[3,4-*b*]quinoline[3h] (**3**, 50 mg, 0.27 mmol) in CH_2Cl_2 was added to activated K-10 clay (50 mg) in a beaker and the solvent was evaporated. The appropriate isatoic anhydride (0.27 mmol) in CH_2Cl_2 was added, stirred for 5 min, and the solvent evaporated. The beaker was exposed to MW irradiation at 450 W for 7–15 min under solvent-free conditions. After conversion, the reaction mixture was added with CH_2Cl_2, the clay filtered, the organic layer concentrated in vacuo, and the crude product purified by FC on silica gel (Merck, 230–400 mesh).

9-Nitroluotonin A (**1c**): The reaction mixture with 5-nitroisatoic anhydride was subjected to MW irradiation for 7 min to obtain, after purification by FC (eluent: CH_2Cl_2/ethyl acetate 95:5), **1c** as a white solid (12 mg,

13%), mp > 300 °C dec. ^{1}H NMR (DMSO-d_6): δ 5.35 (s, 2H), 7.78 (dd, J = 7.72 and 7.72 Hz, 1H), 7.92 (dd, J = 7.72 and 7.72 Hz, 1H), 8.10–8.28 (m, 3H), 8.64 (dd, J = 9.19 and 2.57 Hz, 1H), 8.80 (s, 1H), 8.96 (d, J = 2.57 Hz, 1H).

7-Methylluotonin A (**1d**): The reaction mixture with 3-methylisatoic anhydride[14] was subjected to MW irradiation for 12 min to obtain, after purification by FC (eluent: from hexane/ethyl acetate 1:1 to ethyl acetate), **1d** (59 mg, yield 35%), mp 282–283 °C. ^{1}H NMR ($CDCl_3$): δ 2.75 (s, 3H), 5.32 (s, 2H), 7.45 (dd, J = 8.19 and 8.19 Hz, 1H), 7.70 (dd, J = 8.19 and 8.19 Hz, 2H), 7.80 (dd, J = 8.19 and 8.19 Hz, 1H), 7.90 (dd, J = 8.19 and 1.84 Hz, 1H), 8.26 (dd, J = 8.19 and 1.84 Hz, 1H), 8.45 (s, 1H), 8.48 (dd, J = 8.19 and 1.84 Hz, 1H).

10-Chloroluotonin A (**1e**): The reaction mixture with 6-chloroisatoic anhydride was subjected to MW irradiation for 12 min to obtain, after FC (eluent: from hexane/ethyl acetate 7:3 to ethyl acetate) and crystallization from Et_2O, **1e** (60 mg, 33%), mp 313–315 °C dec. ^{1}H NMR ($CDCl_3$): δ 5.30 (s, 2H), 7.54 (dd, J = 8.19 and 1.49 Hz, 1H), 7.68 (dd, J = 8.19 and 8.19 Hz, 2H), 7.84 (ddd, J = 8.19, 8.19, and 1.49 Hz, 1H), 7.94 (dd, J = 8.19 and 1.49 Hz, 1H), 8.02 (dd, J = 8.19 and 1.49 Hz, 1H), 8.44 (s, 1H), 8.46 (dd, J = 8.19 and 1.49 Hz, 1H).

2. General procedure for the condensation with substituted anthranilic acid[14]

To a solution of **3** (300 mg, 1.63 mmol) in 60 mL of anhydrous CH_2Cl_2 under N_2 were added 945 mg (4.89 mmol) of $BF_4^-Et_3O^+$ and the resulting mixture was stirred at room temperature for 48 h. After addition of NaOH 1 N (30 mL) the aqueous phase was extracted with CH_2Cl_2 (20 × 3 mL). The collected organic layers were washed with brine, dried, and evaporated to afford, after FC (eluent: from AcOEt to AcOEt/MeOH 97:3), 110 mg of 3-ethoxy-1*H*-pyrrolo[3,4-*b*]quinoline as a white solid, yield 32%, mp 138 °C. ^{1}H NMR ($CDCl_3$): δ 1.60 (t, J = 7 Hz, 3H), 4.68 (q, J = 7 Hz, 2H), 4.80 (s, 2H), 7.60 (m, 1H), 7.75 (m, 1H), 7.90 (dd, J = 8.09 and 1.48 Hz, 1H), 8.25 (s, 1H), 8.35 (dd, J = 8.09 and 1.48 Hz, 1H). The above compound (100 mg, 0.47 mmol) was dropped into an ice-cooled solution of the appropriate anthranilic acid (0.43 mmol) in acetone or dioxane. The resulting solution was refluxed for 1–2 days, then it was cooled with an ice bath and the precipitate was filtered and purified by FC.

9-Chloroluotonin A (**1f**): The solution was refluxed 24 h in acetone (3 mL) to afford after FC (eluent: EtOAc) **1f** (82 mg, 57%), mp > 300 °C. ^{1}H NMR ($CDCl_3$): δ 5.39 (s, 2H), 7.70–8.10 (m, 5H), 8.40 (d, J = 1.49, 1H), 8.51 (dd, J = 7.53 and 1.49 Hz, 1H), 8.60 (s, 1H).

9-Hydroxyluotonin A (**1g**): The solution was refluxed 48 h in dioxane (8 mL) to afford after FC (eluent: EtOAc/MeOH 95:5) **1g** (50 mg, 38%), mp > 300 °C. ^{1}H NMR ($CDCl_3$): δ 5.26 (s, 2H), 7.36 (d, J = 8.75 Hz, 1H), 7.55 (s, 1H), 7.72 (dd, J = 8.75 and 8.75 Hz, 1H), 7.80 (d, J = 8.75 Hz, 1H), 7.87 (dd, J = 8.75 and 8.75 Hz, 1H), 8.13 (d, J = 8.75 Hz, 1H), 8.22 (d, J = 8.75 Hz, 1H), 8.70 (s, 1H).

3. General procedure for the Friedländer cyclization[8]

To a solution of **5**[3d] (20 mg, 0.1 mmol) in toluene (3 mL) were added the appropriate aminobenzaldehyde (0.11 mmol) and *p*-toluenesulfonic acid (2 mg). The mixture was heated using a Dean–Stark trap for 2–4 h. The solvent was removed in vacuo and the residue purified by FC.

2,3-Dimethoxyluotonin A (**4a**): The mixture was heated for 4 h. Evaporation of the solvent and purification by FC (CH_2Cl_2/MeOH 97:3) gave **4a** (11 mg, 32%), mp > 270 °C. ^{1}H NMR ($CDCl_3$): δ 4.07 (s, 6H), 5.29 (s, 2H), 7.14 (s, 1H), 7.55 (dd, J = 8.19 and 8.19 Hz, 1H), 7.77 (s, 1H), 7.83 (dd, J = 8.19 and 8.19 Hz, 1H), 8.08 (d, J = 8.19 Hz, 1H), 8.26 (s, 1H), 8.42 (d, J = 8.19 Hz, 1H).

2,3-Methylenedioxyluotonin A (**4b**): The mixture was heated for 2 h. Evaporation and purification by FC (hexane/AcOEt 2:8) gave **4b** (12 mg, 36%), mp > 270 °C. ^{1}H NMR ($CDCl_3$): δ 5.26 (s, 2H), 6.18 (s, 2H), 7.16 (s, 1H), (dd, J = 8.19 and 1.49 Hz, 1H), 7.55 (ddd, J = 8.56, 8.19, and 1.49 Hz, 1H), 7.71 (s, 1H), 7.83 (ddd, J = 8.56, 8.19, and 1.49 Hz, 1H), 8.09 (dd, J = 8.19 and 1.49 Hz, 1H), 8.23 (s, 1H), 8.41 (dd, J = 8.19 and 1.49 Hz, 1H).

9-Amino-4a,5,13a,14-tetrahydroquino[2′,3′:3,4]pyrrolo[2,1-b]quinazolin-11(13H)-one (**6**): A suspension of **4c** (37 mg, 0.11 mmol) and Pd/C (7 mg) in 10 mL of AcOH was hydrogenated for 6 h: filtration and evaporation gave, after FC (eluent: AcOEt/hexane 9:1) **6** as a yellow solid (18 mg, 52%), mp > 250 °C. ^{1}H NMR (DMSO-d_6): δ 2.21 (dd, J = 9.93 and 15.44 Hz, 1H), 2.7 (m, 1H), 2.80 (dd, J = 5.14 and 15.44 Hz, 1H), 3.80 (dd, J = 2.57 and 11.77 Hz, 1H), 4.10 (dd, J = 6.25 and 11.77 Hz, 1H), 4.75 (d, J = 6.62 Hz, 1H), 6.48 (dd, J = 9.19 and 9.19 Hz, 1H), 6.69 (d, J = 9.19 Hz, 1H), 6.85–6.95 (m, 2H), 7.05 (dd, J = 9.19 and 1.49 Hz, 1H), 7.16 (d, J = 1.49 Hz, 1H), 7.35 (d, J = 9.19 Hz, 1H).

4. In vitro studies

A human lung large cell carcinoma cell line, H460 (ATCC HTB 177) was used in this study. Cells were cultured in RPMI-1640 containing 10% fetal calf serum. Cytotoxicity was assessed by growth inhibition assay after 1 and 72 h drug exposure. Cells in the logarithmic phase of growth were harvested and seeded in duplicates into six-well plates. Twenty-four hours after seeding, cells were exposed 1 or 72 h to the drug and, in the case of 1 h exposure, harvested 72 h before counting with a Coulter counter. IC_{50} is defined as the inhibitory drug concentration causing a 50% decrease of cell growth

over that of untreated control. All compounds were dissolved in DMSO prior to dilution into the biological assay.

Topoisomerase I-dependent DNA cleavage assay (see Refs. 15 and 16).

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