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Novel quinazoline–quinoline alkaloids with cytotoxic and DNA topoisomerase II inhibitory activities

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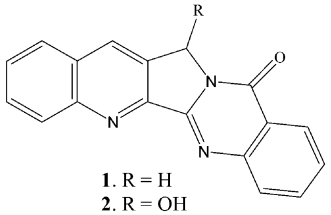
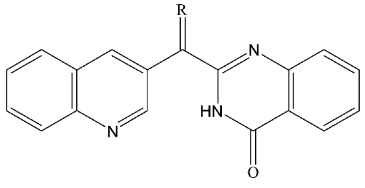
Abstract—Two new synthetic analogues of luotonins A and F, 7-acetylaminoluotonin A (**6**) and 3-[3*H*(quinazolino-4-one)]quinoline (**7**) were synthesized. The new analogues, along with four natural quinazoline–quinoline alkaloids, luotonins A (**1**), B (**2**), E (**3**), F (**4**) and a synthetic deoxoluotonin F (**5**), showed cytotoxic activity (IC_{50} 1.8–40.0 μg/mL) and DNA topoisomerase II inhibition at a concentration of 25 μM.

Luotonins A (**1**), B (**2**), E (**3**) and F (**4**) are novel quinazoline–quinoline alkaloids that were isolated from the aerial parts of *Peganum nigellastrum* Bunge.[1,2] Luotonin A (**1**) has unique pyrroloquinazolinoquinoline ring system and showed cytotoxic activity against mouse leukemia P-388 cells in vitro at a concentration of 1.8 μg/mL.[1] The structure of luotonin A (**1**) is strikingly reminiscent of the cytotoxic alkaloid camptothecin, whose derivatives are clinically useful anti-cancer agents.[3] This similarity has stimulated much activity directed toward the synthesis of this compound by several groups.[4] The syntheses of luotonins B[4c,j] (**2**), E[2] (**3**) and F[2,5] (**4**) have also been achieved, but no biological data was reported. In a recent paper, Hecht et al.[6] demonstrated that luotonin A (**1**) stabilized the human DNA topoisomerase I–DNA covalent binary complex, affording the same pattern of cleavage as the structurally related topoisomerase I inhibitor camptothecin. Further it was shown that luotonin A (**1**) also mediated topoisomerase I-dependent cytotoxicity toward *Saccharyomyces cerevisiae* lacking yeast topoisomerase I, but harboring a plasmid having the human topoisomerase I gene under the control of a galactose promoter.[6] The above findings make the synthesis of luotonin A analogues an attractive target to evaluate their biological activities. In this communication, we report the syntheses of 7-acetylaminoluotonin A (**6**) and a luotonin F analogue, 3-[3*H*(quinazolino-4-one)]quinoline (**7**), and compare their cytotoxic and topoisomerase II inhibitory activities with natural alkaloids, luotonins A (**1**), B (**2**), E (**3**), F (**4**) and synthetic deoxoluotonin F[2] (**5**).



1. R = H
2. R = OH
3. R = OMe
6. R = NHAc



4. R = O
5. R = H_2

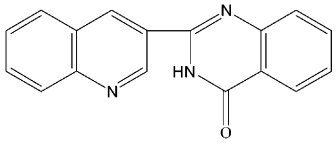


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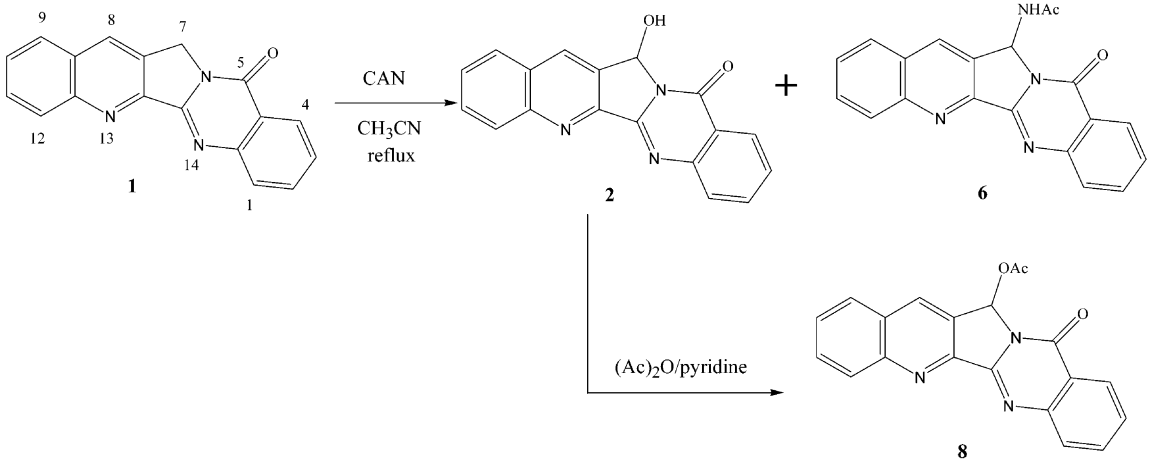
Table 1. NMR data of **2**, **6** and **8** at C-7 position

Compd	^{1}H (δ)	^{13}C (δ)
2	7.14 s	80.9
6	7.16 s	63.8
8	8.06 s	78.3

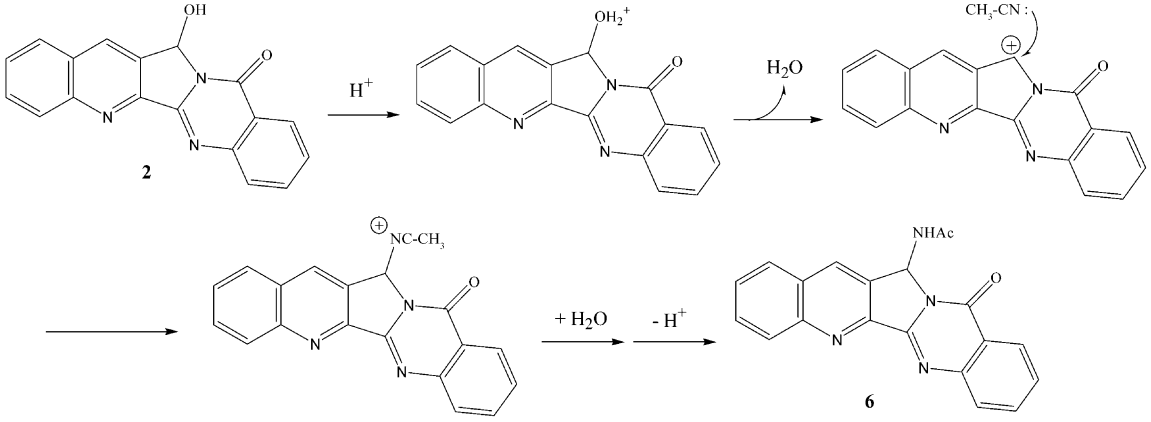
Luotonin B (**2**) was synthesized[4c] by CAN oxidation of luotonin A (**1**) in acetonitrile under reflux conditions (Scheme 1). After 6 h reaction, the desired luotonin B (**2**) was isolated in 15% yield along with compound **6** in 25% yield. The structure of **6** was elucidated by comparison of ^{1}H and ^{13}C NMR data (see Table 1) of luotonin B (**2**) and 7-acetoxyluotonin A[7] (**8**). The chemical shift value of the methine carbon (δ 63.8) in **6** is at higher-field than those of **2** (δ 80.9) and **8** (δ 78.3), while the chemical shift value of the methine proton (δ 8.06) in **8** is at lower-field than those of **2** (δ 7.14) and **6** (δ 7.16). The NMR data suggested that nitrogen atom should be adjacent to C-7 position. The structure of compound **6** was further confirmed by its molecular weight obtained by FAB-MS spectrum.[8] The molecular weight of **6** (MW: 342) is one unit less than that of **8** (MW: 343) which suggests the presence of an additional nitrogen in compound **6**. From the above evidence, the structure of **6** was determined as 7-acetylaminoluotonin A. The formation of **6** could be explained by Ritter reaction[9] as shown in Scheme 2.

As shown in Scheme 3, hydrolysis of 3-quinolinenitrile (**9**) with concentrated sulfuric acid yielded 3-quinolinecarboxamide (**10**) in 95% yield. Subsequent reaction of the amide (**10**) with isatoic anhydride[10] at 200–210 °C for 2 h afforded 3-[3*H*(quinazolino-4-one)]quinoline[11] (**7**) in 39% yield. Compounds **7** and **4** have similar structural features and contain quinoline and quinazolinone moieties. In compound **7**, the C-3 of quinoline moiety is directly linked to the C-2 of quinazolinone moiety whereas in **4** the two moieties are linked through a junction of the carbonyl group.

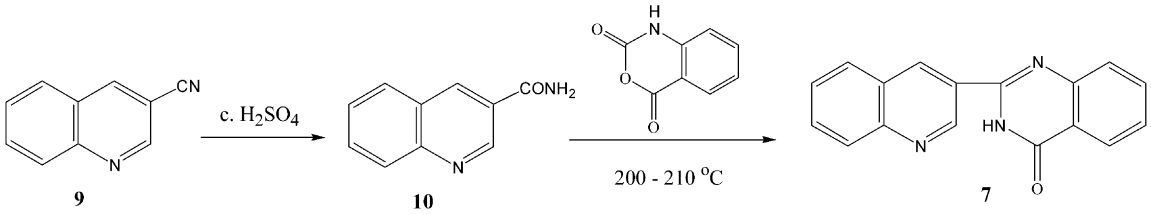
Luotonins A (**1**), B (**2**), E (**3**) and F (**4**) are less polar alkaloids isolated from the hexane, benzene and chloroform extracts of *Peganum nigellastrum*.[1,2] It has been reported[12] that the lipophilic alkaloid fraction of *P. nigellastrum* showed anti-tumor effect on mice implanted with ascetic hepatoma cells, and inhibited



Scheme 1.



Scheme 2.



Scheme 3.

Table 2. Cytotoxic activity against mouse leukemia P-388 cells

Compd	1	2	3	4	5	6	7
IC_{50} (μg/mL)	1.8	5.0	9.0	20.0	2.3	33.0	40.0

DNA and protein syntheses in the hepatoma cells. In order to evaluate the anti-tumor activity of these novel alkaloids (**1**–**4**) and their synthetic derivatives (**5**–**7**), we carried out biological assays for the cytotoxic activity against P 388 mouse leukemia cells[13] and the inhibitory activity against human topoisomerase II.[14] The cytotoxic effects of compounds **1**–**7** were tested and the results were summarized in Table 2. Among them, luotonin A (**1**) and deoxoluotonin F (**5**) showed cytotoxic activity at lower concentrations, IC_{50} 1.8 μg/mL and IC_{50} 2.3 μg/mL respectively. Comparison of the activities of **1**, **2**, **3** and **6**, having the same pyrroloquinazolinoquinoline skeleton, revealed that the substituents containing oxygen or nitrogen atom at C-7 position have lower cytotoxicity. These data suggested that the methylene group is very important for the activity. The same tendency was also observed for the analogues **4**, **5** and **7**. On the other hand, DNA unknotting assays with human topoisomerase II on knotted P4 phage DNA was examined to detect the topoisomerase II inhibitory activity. All seven compounds (**1**–**7**) had potent inhibitory activity against human topoisomerase II at the concentration of 25 μM, and all of them showed the similar inhibitory intensity. In another experiment, luotonin A exhibited IC_{50} value of 28.5 μM, which was comparable to ICRF-193,[15] a DNA topoisomerase II inhibitor (IC_{50} 13.9 μM).

In conclusion, the present study demonstrated that luotonin A (**1**) and its analogues (**2**–**7**) exhibited cytotoxic activity through inhibition of DNA topoisomerase II. Considering the new finding by Hecht's group,[6] luotonin A was suggested as a potent inhibitor for both topoisomerases I and II. Luotonin A (**1**) may have utility as an antineoplastic agent because of its novel mechanism of cytotoxicity.

Acknowledgements

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7. Compound **8**: mp 282–285 °C. UV λmax (MeOH) nm (log ε): 213 (4.70), 250 (4.75), 300 (sh, 4.18), 322 (sh, 4.31), 336 (4.38), 352 (4.28). ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 2.22 (s, 3H), 7.60 (dt, J = 1.5 and 8.0 Hz, 1H), 7.73 (dt, J = 1.5 and 8.0 Hz, 1H), 7.86 (dt, J = 1.5 and 8.0 Hz, 1H), 7.90 (dt, J = 1.5 and 8.5 Hz, 1H), 7.99 (d, J = 8.0 Hz, 1H), 8.06 (s, 1H), 8.10 (dd, J = 1.5 and 8.0 Hz, 1H), 8.41 (dd, J = 1.5 and 8.0 Hz, 1H), 8.48 (d, J = 8.5 Hz, 1H), 8.60 (s, 1H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 20.4, 78.3, 121.7, 126.6, 127.8, 128.5, 128.6, 128.8, 129.4, 130.5, 130.6, 131.3, 133.8, 134.8, 148.6, 150.0, 150.1, 150.9, 160.4, 169.9. FAB-MS: m/z 344 $(M+H)^+$.
8. Compound **6**: mp >300 °C. UV λmax (MeOH) nm (log ε): 207 (4.79), 248 (4.67), 297 (4.20), 325 (4.23), 338 (4.25), 354 (4.14). ^{1}H NMR ($CDCl_3$–CD_3OD: 3:1, 400 MHz) δ: 1.95 (s, 3H), 7.16 (s, 1H), 7.41 (t, J = 8.0 Hz, 1H), 7.55 (t, J = 8.0 Hz, 1H), 7.70 (t, J = 8.0 Hz, 1H), 7.72 (t, J = 8.0 Hz, 1H), 7.82 (d, J = 8.0 Hz, 1H), 7.86 (d, J = 8.0 Hz, 1H), 8.16 (d, J = 8.0 Hz, 1H), 8.21 (d, J = 8.0 Hz, 1H), 8.35 (s, 1H). ^{13}C NMR ($CDCl_3$–CD_3OD: 3:1, 100 MHz) δ: 22.7, 63.8, 121.7, 126.2, 127.6, 128.2, 128.3, 128.5, 128.9, 129.9, 130.9, 131.7, 132.0, 134.7, 148.5, 149.4, 149.7, 151.4, 160.1, 171.4. FAB-MS: m/z 343 $(M+H)^+$.
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11. Compound **7**: mp >300 °C. IR νmax (KBr) cm^{-1}: 3403, 1669, 1620, 1572, 1498, 1470, 1307, 1149, 930, 772. ^{1}H NMR (DMSO-d_6, 400 MHz), δ: 7.57 (dt, J = 1.2 and 8.1 Hz, 1H), 7.73 (t, J = 8.0 Hz, 1H), 7.82 (d, J = 8.1 Hz, 1H), 7.88 (dt, J = 1.4 and 8.4 Hz, 1H), 7.90 (dt, J = 1.2 and 8.2 Hz, 1H), 8.12 (d, J = 8.3 Hz, 2H), 8.20 (dd, J = 1.2 and 8.1

Hz, 1H), 9.15 (d, $J = 2.2$ Hz, 1H), 9.60 (d, $J = 2.2$ Hz, 1H), 12.82 (brs, 1H). EI–MS: m/z 273 (M^+, 78), 119 (100). HR-EIMS: m/z 273.0904 (M^+, $C_{17}H_{11}N_3O$, requires 273.0902).

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