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SYNTHESIS AND PROPERTIES OF LUOTONIN A HOMOLOGUES AND THEIR AZA-ANALOGUES

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Abstract – A series of new 3,2'-polymethylene-2-(quinol-2-yl)-4(3*H*)-quinazolinones and their aza-analogues were prepared as a homologous series of luotonin A. Their inhibitory activities against topoisomerase and cytotoxicities against selected cancer cell lines were evaluated to show that luotonin A and its aza-analogue were shown significant cytotoxicity. Conformational study revealed that trimethylene-bridged systems were rigid at room temperature with activation energies for interconversion of 15.7 and 14.6 kcal/mol for 3,2'-trimethylene-2-(quinol-2-yl)-4(3*H*)-quinazolinone and its aza-anlogue, respectively.

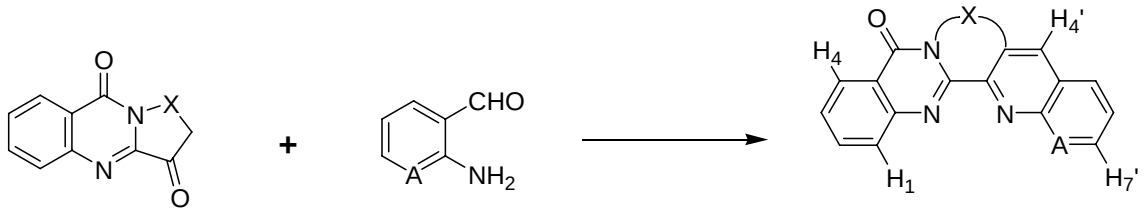
INTRODUCTION

Luotonin A (**3b**) is a pyrroloquinazolinoquinoline alkaloid isolated from *Peganum nigellastrum* (Zygophyllaceae) which has named "Luo-Tuo-Hao" in China and been used as a traditional medicine for the treatment of rheumatism, abscess, and inflammation.[1] The basic fractions of *P. nigellastrum* showed anti-tumor activtity,[2] and the origin of such an activity was recently revealed by identifying its constituent luotonin A which inhibited the growth of leukemia P-388 cells (IC_{50} = 1.8 μg/mL).[3] Recent findings on the topoisomerase I-dependent cytotoxicity of luotonin A[4] supported the mechanism of cytotoxic action. Such intriguing properties of luotonin A led developments of several efficient methods for total synthesis[5] and derivatizations of the parent molecule.[6,7]

Our interests[5i,8] in the conformational effects on biological activity as well as the search for anti-tumor agents spurred us to design a series of luotonin A-related compounds in which the dihedral angles between planar 4(3*H*)-quinazolinone and aromatic (*i.e.* quinoline or 1,8-naphthyridine) rings could be controlled in a regular fashion by a methylene bridge connecting N3 of 4(3*H*)-quinazolinone and C2 of quinoline. We reasoned that conformational aspects of the molecules designed might affect the stability of topoisomerase-DNA binary complex, which then influenced cytotoxicity.

RESULTS AND DISCUSSION

Chemistry: Synthesis of the designed compounds (**3**) was straightforward as shown. Friedländer condensation of ketones (**1b-d**) with 2-aminobenzaldehyde (**2a**)[9] and 2-aminonicotinaldehyde (**2b**)[10] in the presence of KOH afforded **3** and **4** in good yields. The prerequisite 2-acetyl-4(3*H*)-quinazolinone (**1a**)[11] and related ketones (**1b-d**)[8] were prepared by employing previously reported method.



1 + **2a** A = CH, **b** A = N → **3** A = CH, **4** A = N

a X = H, H (non-bridged system)
b X = -CH_2- (A = CH, luotonin A)
c X = -$(CH_2)_2$-
d X = -$(CH_2)_3$-

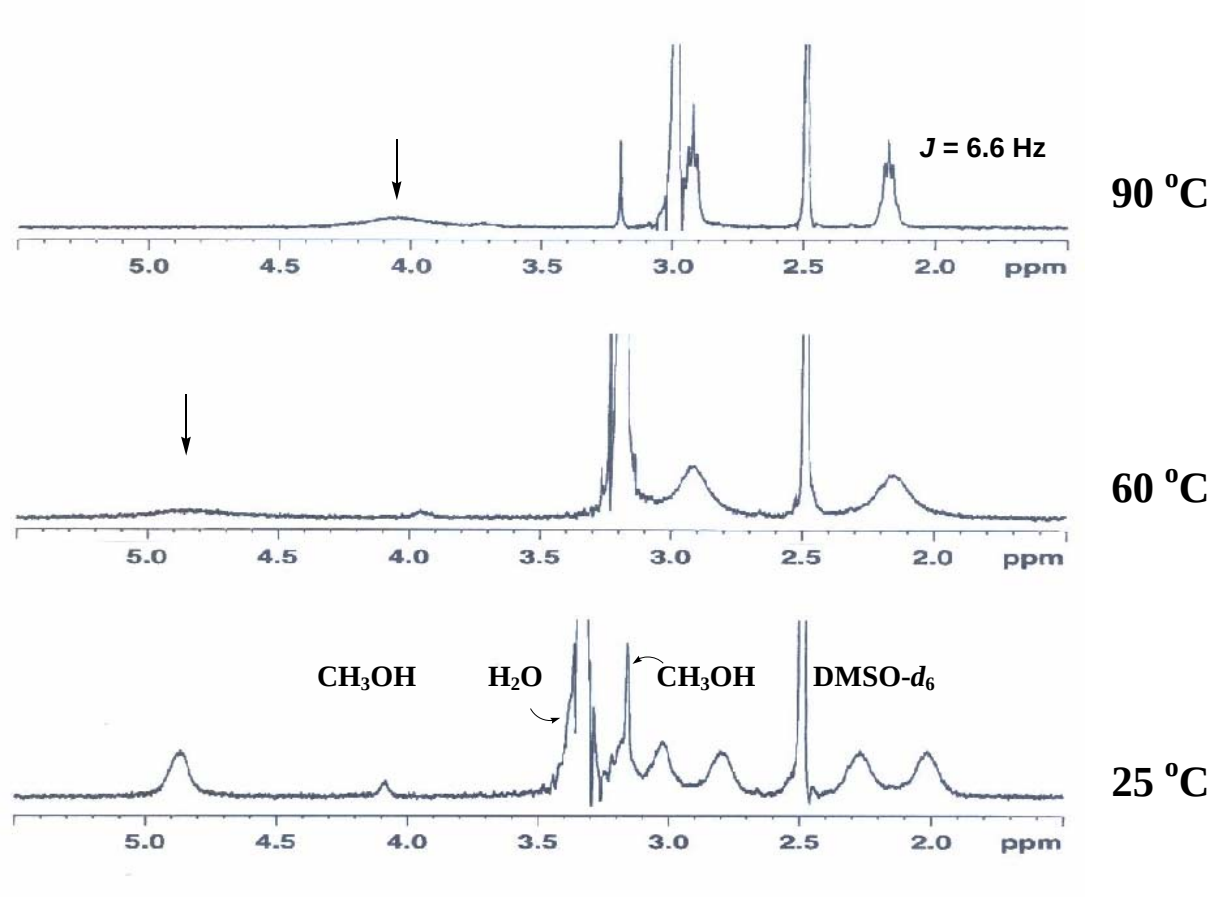
It is worthy noting that attempts to condense **1a** with **2a** and **2b** for the preparation of non-bridged analogues (**3a** and **4a**) were not successful even under harsh reaction condition such as heating 200 ^{o}C in ethylene glycol. However, **3a** and **4a** could be prepared in 4 steps from quinoline-2-carboxylic acid and 1,8-naphthyidine-2-carboxylic acid, respectively, by employing previously reported method.[12]

The products were readily characterized by their ^{1}H NMR spectra. Chemical shifts of H1, H4' and H8' (for quinoline derivatives) are very sensitive to the conformations of the compounds in which such a proton resonance is affected by neighboring aromatic ring. The resonance of H8' in the most planar **3b** is most downfield shifted due to the deshielding ring current of neighboring and the deshielding effect of lone pairs of N1 of 4(3*H*)-quinazolinone moiety to appear at δ 8.46 which is comparable to δ 8.34 for **3c** the next planar system. Such deshielding effects diminished for with the increase of the length of the methylene bridge. In **3d** and **4d**, H4' is the most deshielded one due to the dihedral angle between the two planar rings that are distorted enough to out of range to affect the chemical shifts of H1 and H8'.

In annulated bi-aryls,[8,13] ^{1}H NMR spectra, specially the aliphatic regions, afforded information on the conformations of compounds which were highly dependent on the length of the bridge. The aliphatic regions of ^{1}NMR spectra of **3d** and **4d** showed six different proton resonances, respectively, in the range of δ 4.88-2.07 indicating that all the six protons were not magnetically equivalent. However, ^{13}C NMR spectra showed three sp^3-hybridged carbon resonances at δ 48.7, 27.8, and 27.4 for **3d** and at δ 40.6, 27.6, and 27.2 for **4d**, respectively. The trimethylene bridges on **3d** and **4d** are, thus, rigid at room temperature in the NMR time scale while the trimethylene-bridges in most of annulated biaryls are flexible.[10,12] One

hydrogen (Hα) of the two H's on the carbon adjacent to N3 of quinazolinone ring lies the deshielding region of the oxygen of C=O, thus is deshielded to δ 4.88 in one enantiomer while the geminal hydrogen (Hβ) experiences the similar deshielding effect in the other enantiomer. Temperature variation experiment of the aliphatic region of **3d** led coalition of two sets of resonances at 60 °C which then resolved each resonance at 90 °C (Figure 1). The proton resonance at δ 4.88 disappeared at 60 °C and started to grow at δ 4.05 at the temperature of 90 °C. Such a time-dependent corresponding to coalescence could be explained that the rate of interconversion of the two conformational isomers would become comparable with the frequency difference between Hα and Hβ.[14] Similar phenomenon of the two H's in [18]-annulene was previously reported.[15]

Figure 1. Temperature Variation Experiment of **3d** (Aliphatic region only)



On the other hand, the resonances at δ 3.04 and δ 2.80 as well as δ 2.28 and δ 2.02 reached coalescence at 60 °C and finally resolved as a triplet (J = 6.6 Hz) δ 2.92 and a quartet (J = 6.6 Hz) at δ 2.18, respectively. Two geminal hydrogens at each carbon are equivalent to confirm flexibility of the bridge of **3d** at 90 °C. Based on these observations, the activation energy of the interconversion was estimated by previously

employed equation[16] to give 15.1 kcal/mol. Similarly the activation energy for **4d** was estimated to give 14.6 kcal/mol. These values are comparable to the estimated value (13 kcal/mole) of flexible 2,2'-trimethylenebiphenyl.[17] In addition, the difference of estimated energy barrier between **3d** and **3d** is not large enough to differentiate the interactions between C1-H *vs* either C8'-H or N8' by twisting about C2-C2' bond. Such small difference can be explained by the fact that the two moieties are too far in distance to affect interactions.

DNA topoisomerase inhibitory activity: The inhibitory activities of the compounds prepared on DNA topoisomerase I and II were evaluated by the procedure described previously.[18] Although luotonin A and its aza-analogue (**4b**) showed somewhat noticeable inhibitory activities on Topo I and II, other annulated luotonin A derivatives did not show any significant inhibitory activity up to 100 μM. These results may imply that the planarity of the compound is important for the inhibitory activity.

Cytotoxicity: Cytotoxicities of compounds prepared were screened by previously reported method[19] against selected human cancer cell lines: A-549 (lung carcinoma), HCT-15 (colon adenocarcinoma), SK-OV-3 (ovary adenocarcinoma), SK-MEL-2 (malignant melanoma) and L1210 (leukemia) in an MTT assay. Only luotonin A and its aza-analogue showed promising cytotoxic activity especially against L1210 leukemia cell line as shown in Table 1. Surprisingly compounds with non-, di- and trimethylene-bridged system did not show any significant cytotoxicity up to 50 μM.

Table 1. Cytotyoxic Activity of **3b** and **4b** Using a Cell Proliferation Assay (IC_{50} in μM)[a]

Compounds	cell lines				
	A549	HCT-15	SK-OV-3	SK-MEL-2	L1210
3b	42.2	>20	6.8	14.5	0.09
4b	36.3	>20	5.6	7.6	0.13
Doxorubicin	0.023	0.031	0.042	0.002	0.007

[a]Values are an average of duplicate experiments.

In conclusion, a series of 3,2'-polymethylene-2-(quinol-2-yl)-4(3*H*)-quinazolinones and their aza-analogues were prepared as homologues of luotonin A by employing Friedländer condensation of corresponding ketones with 2-aminobenzaldehyde and 2-aminonicotinaldehyde, respectively. Inhibitory activities on topoisomerase and cytotoxicity against selected human cancer cell lines to show that only monomethylene-bridged sytems show promising cytotoxic activity against selected human cancer lines except HCT-15. Conformational study revealed that trimethylene- bridged systems were rigid at room temperature with activation energies of 15.1 kcal/mol and 14.6 kcal/mol for **3d** and **4d**, respectively.

EXPERIMENTAL

Melting points were determined using a Fischer-Jones melting points apparatus and are not corrected. IR spectra were obtained using a Perkin-Elmer 1330 spectrophotometer. NMR spectra were obtained using a Bruker-250 spectrometer 250 MHz or 400 MHz for ^{1}H NMR and 62.5 MHz or 100 MHz for ^{13}C NMR and are reported as parts per million (ppm) from the internal standard tetramethylsilane (TMS). Chemicals and solvents were commercial reagent grade and used without further purification. Elemental analyses were taken on a Hewlett-Packard Model 185B elemental analyzer.

3,2'-Dimethylene-2-(quinol-2-yl)-4(3*H*)-quinazolinone (**3c**) **General Procedure**: To a solution of **1c** (2.14 g, 0.01 mol) and 2-aminobenzaldehyde (1.33g, 0.011 mol) in 20 mL of dry EtOH was added 2 mL of saturated KOH (1.3 mL, 0.2 mmol) in dry EtOH. Resulting mixture was refluxed stirred for 8 h and evaporated *in vacuo* to give a solid which was dissolved in CH_2Cl_2 and washed with water. The organic layer was dried over $MgSO_4$. Evaporation of the solvent gave 2.8 g (94%) of pale yellow solid, which was either recrystallized from CH_3OH or chromatographed on silica gel eluting with CH_3OH:CH_2Cl_2 (1:19) gave colorless plates: mp > 260 ^{o}C. ^{1}H NMR ($CDCl_3$, 250 MHz) δ 8.62 (d, *J* = 8.5 Hz, H4), 8.34 (dd, *J* = 8.0, 1.3 Hz, H8'), 8.11 (s, H4'), 8.10 (d, *J* = 7.8 Hz, H1), 7.83 (dd, *J* = 8.0, 0.9 Hz, H5), 7.80 (td, *J* = 8.4, 1.2 Hz, H2), 7.77 (td, *J* = 8.8, 1.5 Hz, H7'), 7.62 (ddd, *J* = 8.0, 7.6, 1.0 Hz, H6). 7.53 (ddd, *J* = 7.9, 7.5, 1.0 Hz, H3), 4.54 (t, *J* = 6.4 Hz, 2H), 3.33 (t, *J* = 6.0 Hz, 2H). ^{13}C NMR ($CDCl_3$, 62.5 MHz) δ 161.36, 148.24, 148.06, 147.39, 146.87, 134.95, 134.34, 131.01, 129.90, 129.59, 129.06, 128.72, 128.60, 127.62, 126.99, 126.71, 121.05, 39.45, 27.32. *Anal*. Calcd for $C_{19}H_{13}N_3O{\cdot}0.5H_2O$: C, 74.01; H, 4.58; N, 13.63. Found: C, 74.06; H, 4.62; N, 13.70.

3,2'-Trimethylene-2-(quinol-2-yl)-4(3*H*)-quinazolinone (**3d**)

Colorless plates (98%): mp > 260 ^{o}C. ^{1}H NMR (DMSO-d_6, 250 MHz) δ 8.36 (s, H4'), 8.24 (d, *J* = 7.8 Hz, H4), 8.17 (d, *J* = 8.3 Hz, H8'), 8.04 (d, *J* = 7.5 Hz, H1), 7.93-7.80 (m, 3H, H2, H5', and H7'), 7.71 (ddd, *J* = 7.8, 7.5, 1.2 Hz, H6), 7.62 (ddd, *J* = 7.8, 7.5, 1.3 Hz, H3), 4.86 (br s, 1H), 3.15 (br s, 1H), 3.01 (br s,

1H), 2.82 (br s, 1H), 2.63 (br s, 1H), 2.07 (br s, 1H). ^{13}C NMR (DMSO-d_6, 62.5 MHz) δ 160.44, 154.05, 152.63, 147.63, 146.57, 135.81, 134.81, 131.32, 130.03, 129.56, 128.71, 128.20, 128.17, 127.74, 127.67, 126.64, 48.71, 27.77, 27.47. *Anal*. Calcd for $C_{20}H_{15}N_3O{\cdot}H_2O$: C, 72.49; H, 5.17; N, 12.68. Found: C, 72.46; H, 5.14; N, 13.00.

3,2'-Monomethylene-2-(1,8-naphthyrid-2-yl)-4(3*H*)-quinazolinone (**4b**)

Colorless plates (97%): mp > 260 °C. ^{1}H NMR ($CDCl_3$, 250 MHz) δ 9.25 (dd, *J* = 4.1, 1.9 Hz, H7'), 8.49 (s, H4'), 8.68 (dd, *J* = 8.2, 1.9 Hz, H4), 8.31 (d, *J* = 7.8 Hz, H1), 7.97-7.95 (m, 2H, H2 & H5'), 7.79 (dd, *J* = 8.2, 4.1 Hz, H6'), 7.66 (ddd, *J* = 8.8, 5.5, 2.6 Hz, H3), 5.35 (s, 2H). *Anal*. Calcd for $C_{19}H_{17}N_4O{\cdot}H_2O$: C, 67.10; H, 3.94; N, 18.41. Found: C, 67.06; H, 3.94; N, 18.37.

3,2'-Dimethylene-2-(1,8-naphthyrid-2-yl)-4(3*H*)-quinazolinone (**4c**)

Colorless plates (57%): mp > 260 °C. ^{1}H NMR ($CDCl_3$, 250 MHz) δ 9.17 (dd, *J* = 4.1, 2.0 Hz, H7'), 8.49 (dd, *J* = 8.1, 1.9 Hz, H4), 8.48 (s, H4'), 8.21 (d, *J* = 7.8 Hz, H1), 7.94-7.87 (m, 2H, H2 & H5'), 7.70 (dd, *J* = 8.2, 4.1 Hz, H6'), 7.61 (ddd, *J* = 8.1, 5.5, 1.9 Hz, H3), 4.43 (t, *J* = 6.2 Hz, 2H), 3.37 (t, *J* = 6.2 Hz, 2H). ^{13}C NMR ($CDCl_3$, 62.5 MHz) δ 160.7, 155.0, 154.6, 150.0, 148.3, 147.5, 137.2, 136.6, 134.8, 132.0, 128.4, 127.8, 126.6, 123.8, 123.6, 121.2, 39.2, 26.3. *Anal*. Calcd for $C_{18}H_{12}N_4O$: C, 71.99; H, 4.03; N, 18.66. Found: C, 72.06; H, 4.06; N, 18.70.

3,2'-Trimethylene-2-(1,8-naphthyrid-2-yl)-4(3*H*)-quinazolinone (**4d**)

Colorless plates (98%): mp > 260 °C. ^{1}H NMR (DMSO-d_6, 250 MHz) δ 9.17 (dd, *J* = 4.2, 2.0 Hz, H7'), 8.54 (dd, *J* = 8.3, 2.0 Hz, H4), 8.48 (s, H4'), 8.26 (dd, *J* = 7.9, 1.0 Hz, H1), 7.91 (ddd, *J* = 8.3, 7.8, 1.8 Hz, H2), 7.82 (d, *J* = 7.3 Hz, H5'), 7.73 (dd, *J* = 8.3, 3.8 Hz, H6'), 7.64 (ddd, *J* = 8.2, 7.5, 1.2 Hz, H3), 4.86 (br s, 1H), 3.15 (br s, 1H), 3.05 (br s, 1H), 2.86 (br s, 1H), 2.28 (br s, 1H), 2.04 (br s, 1H). ^{13}C NMR (DMSO-d_6, 62.5 MHz) δ 160.4, 155.2, 154.6, 144.2, 153.6, 147.6, 137.5, 137.3, 134.8, 132.4, 128.2, 127.8, 126.6, 123.7, 123.6, 121.0, 40.6, 27.6, 27.2. *Anal*. Calcd for $C_{19}H_{14}N_4O{\cdot}H_2O$: C, 68.66; H, 4.85; N, 16.86. Found: C, 68.46; H, 4.85; N, 16.92.

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