HETEROCYCLES, Vol. 65, No. 9, 2005, pp. 2203 - 2219
Received, 9th June, 2005, Accepted, 13th July, 2005, Published online, 15th July, 2005

LUOTONIN A: A LEAD TOWARD ANTI-CANCER AGENT DEVELOPMENT

Zhongze Ma,[a,*] Yoshio Hano,[b,*] and Taro Nomura[b,c]

[a]Bio-organic and Natural Products Laboratory, McLean Hospital, Harvard Medical School, 115 Mill Street, Belmont, MA 02478, USA. E-mail: zhongze_ma@hms.harvard.edu

[b]Faculty of Pharmaceutical Sciences, Toho University, 2-2-1 Miyama, Funabashi, Chiba, 274-8510, Japan. E-mail: hano@phar.toho-u.ac.jp

[c]Department of Kampo Pharmaceutical Sciences, Nihon Pharmaceutical University, 10281 Komuro, Kita-adachi, Saitama 362-0806, Japan

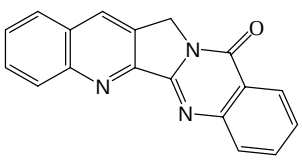
Abstract – Luotonin A, a novel pyrroloquinazolinoquinoline alkaloid isolated from the aerial parts of *Peganum nigellastrum* Bunge, showed a cytotoxic activity against mouse leukemia cells (P-388) and an inhibitory activity against topoisomerase I and II. This review covers the isolation, structural determination, synthesis and biological activity of luotonin A and its related derivatives. A water-soluble topoisomerase I inhibitor, 14-azacamptothecin analogous to luotonin A and camptothecin, was also discussed.

INTRODUCTION

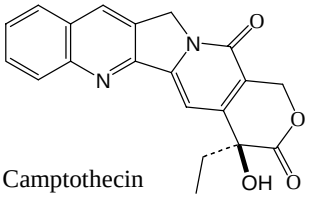
Plants and other natural resources used as traditional medicines have been widely explored in drug discovery. Bioassay-directed isolation followed by identification and characterization of bioactive compounds leads a development for new medicinal drugs. During the improvement stage of such lead compounds, rational drug design-based modification affords synthetic analogs with increased activity, decreased toxicity, or improved pharmacological availability. Thus, many modified analogs are examined and some ones are applied to clinical uses.

Alkaloids are one of attractive natural products leading to medicinal drug development. Indeed, many alkaloids and their synthetic derivatives are widely used as clinical medicines. On the other hand, we reported the first pyrroloquinazolinoquinoline type alkaloid luotonin A (**1**) from aerial parts of *Peganum nigellastrum* (Figure 1) [1] and later cytotoxic activity against mouse leukemia P-388 cells and

topoisomerase II inhibitory activity of **1**.[2] Recently, **1** has been found to stabilize the human DNA topoisomerase I-DNA covalent binary complex, affording the same manner of cleavage as a potent topoisomerase I inhibitor camptothecin (CPT).[3] In order to provide sufficient material for pharmacological profile, coupled with possibility of more potential anti-cancer activity of modified compounds, synthetic study of **1** is a considerable attention. The present review deals with synthetic study and biological activity of luotonin A (**1**) and further various synthetic approaches to its modified compounds in relation to biological activity.



1

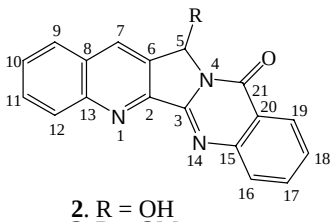


Camptothecin

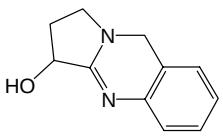
Figure 1

LUOTONIN A AND RELATED ALKALOIDS

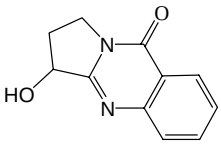
Luotonin A (**1**) is the first pyrroloquinazolinoquinoline alkaloid which has been isolated by our group from the aerial parts of *Peganum nigellastrum* (Zygophyllaceae).[1] This plant has been used as Chinese traditional medicine for treatment of various diseases, including rheumatism and inflammation.[1] The lipophilic alkaloid fraction of *P. nigellastrum* showed anti-tumor effect on mice implanted with ascetic hepatoma cells, involving an increase of *c*AMP through immune system and subsequent inhibition of DNA and protein syntheses in the hepatoma cells.[4]



2. R = OH
3. R = OMe



4



5

Figure 2

Further fractionation of *P. nigellastrum* gave two minor pyrroloquinazolinoquinoline alkaloids luotonin B (**2**) and luotonin E (**3**), along with the main quinazoline alkaloids, vasicine (**4**) and vasicinone (**5**) (Figure

2).[1,5] Hydroxyl group in **2** and methoxyl group in **3** both at C-5 were estimated by comparison of NMR spectral data of **1**, and consequently confirmed by chemical way from **1** to **2** and **3** (Figure 3).[5,6]

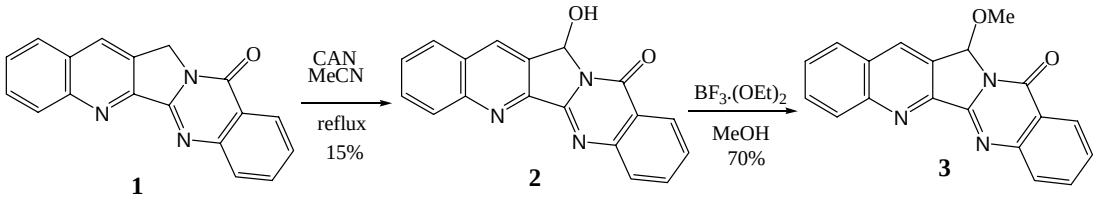


Figure 3

As luotonin A (**1**) and pyrroloquinazoline alkaloids, vasicine (**4**) and vasicinone (**5**), coexist in *P. nigellastrum*, **1** seems to be biosynthesized through reaction of keto amide (**6**) with anthranilic acid as shown in Figure 4. Previous biosynthetic studies in *P. harmala* showed that [carboxyl-^{14}C] anthranilic acid was incorporated into **4**, and ^{14}C-labeled ornithine, proline, and pterescine were effectively incorporated into a pyrrolidino-ring system.[7,8] These experiments suggested that anthranilic acid is a key precursor for the biosynthesis of *Peganum* alkaloids.

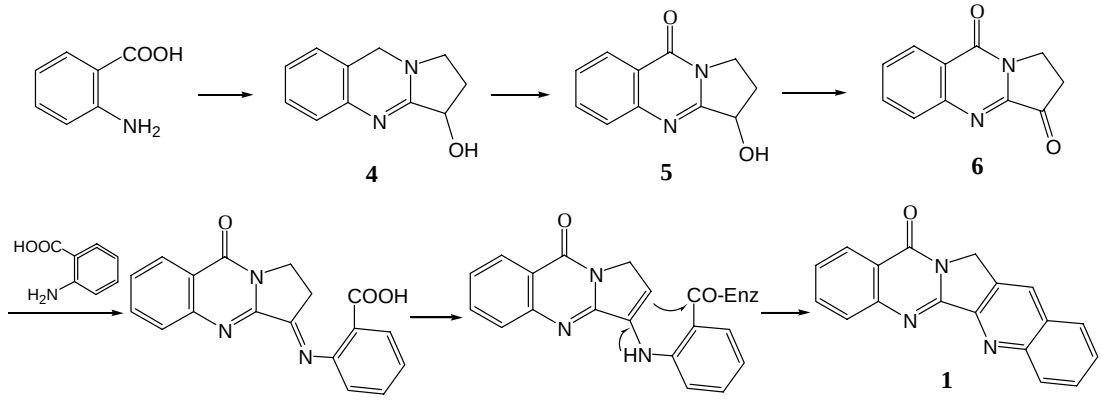


Figure 4 Hypothesis of the biosynthetic route to luotonin A (**1**) from anthranilic acid

TOTAL SYNTHESES OF LUOTONIN A

Over the past eight years, thirteen reports for the total synthesis of luotonin A (**1**) have been demonstrated by different laboratories with various strategies.[6,9-22] As shown in Figure 5, the thirteen strategies are roughly classified into 6 synthetic routes; route I (formation of B-ring by Friedländer condensation), route II (simultaneous formation of B- and C-rings by Povarov reaction), route III (formation of C-ring by Heck reaction), route IV (formation of C-ring by Mitsunobu cyclization), route V (simultaneous formation of C- and D rings by intramolecular aza-Diels-Alder reaction) and route VI (formation of D-ring by improved Kametani amide condensation).

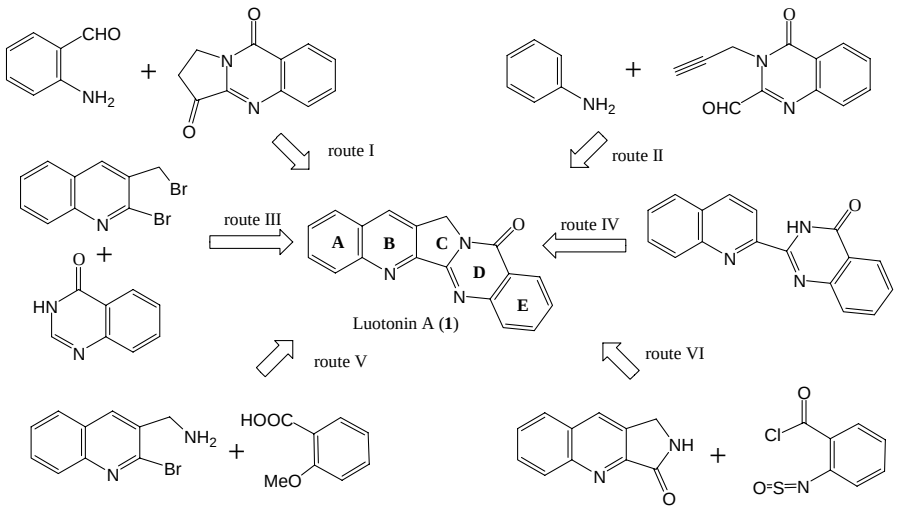


Figure 5 Strategies for total synthesis of luotonin A (**1**)

Route I: Friedländer Condensation (formation of B-ring)

Luotonin A (**1**) may be derived from vasicinone (**5**) *via* pyrrolo[2,1-*b*]quinazoline-3,9-dione (**6**) through a plausible biosynthetic pathway as depicted in Figure 4.[1] Actually, condensation reaction of anthranilate with keto amide (**6**) was applied. The keto amide (**6**) has been prepared by three ways, 1) oxidation reaction of deoxyvaciconone (**7**) with selenium dioxide, 2) ozonolysis of benzilidene derivative (**8**)[9] and 3) oxidation of **5** with Jones reagent.[10] Subsequently Friedländer condensation of **6** with 2-aminobenzaldehyde yielded luotonin A (**1**) in 36 % yield.[10] Tentatively, when a solution of **5** and *N*-(2-aminobenzylidene)-*p*-toluidine in xylene was refluxed under presence of toluene-*p*-sulfonic acid, the target alkaloid (**1**) was obtained in 30 % yield.[6] In this reaction, a keto-form tautomer (**9**) originated from an enamine form of vasicinone (**5**) was supposed to be a key intermediate for the formation of **1**.[6] Furthermore, the presence of *p*-benzoquinone as a hydrogen acceptor in this reaction increased the yield (46 %).[6]

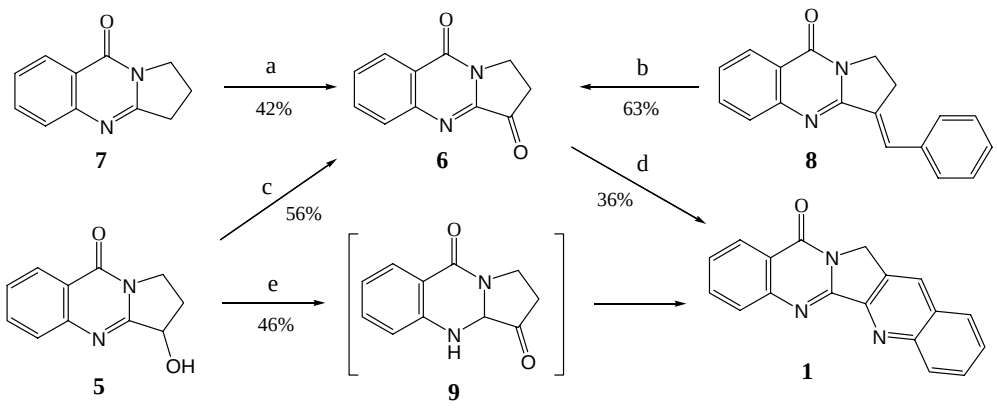


Figure 6 Reagents and conditions: a) SeO_2, dioxane-H_2O, reflux, 12 h; b) O_3, CH_2Cl_2, Me_2S, rt, 30 min; c) Jones reagent, acetone, 0 ˚C to rt, 5 h; d) *o*-aminobenzaldehyde, Triton B, EtOH, reflux, 2.5 h; e) *N*-(2-aminobenzylidene)-*p*-toluidine, *p*-benzoquinone, *p*-toluenesulfonic acid, xylene, reflux, 20 h.

Route II: Intramolecular Povarov Reaction (simultaneous formation of B- and C-ring)

Twin *et al*. demonstrated an intramolecular aza-Diels-Alder reaction (Povarov reaction) approach in a total synthesis of luotonin A, in which pyrrolo[3,4-*b*]quinoline core can be formed through inverse-electron demand hetero-Diels-Alder reaction.[11]

As shown in Figure 7, 2-aminobenzamide (**10**), prepared from ring-opening of isatoic anhydride with propargylamine, was converted into *N*-acetoxyacetate (**11**). Reaction of **11** with triphenylphosphine and iodine in the presence of Hünig's base afforded an imidate (**12**). Treatment of **12** with piperidine followed by silica gel occurs a rearrangement of a benzoxazine ring system to a quinazoline ring system (**13**). Base-catalysed hydrolysis of **13** for removal of acetyl group and subsequent Dess-Martin oxidation gave aldehyde (**14**). In the final step, the key intramolecular Povarov reaction between aldehyde (**14**) and aniline in the presence of 10 mol % dysprosium (III) triflate [$Dy(OTf)_3$)] furnished luotonin A (**1**) in 51 % yield through oxidation of the initially formed 1,2-dihydroquinoline intermediate *in situ*.

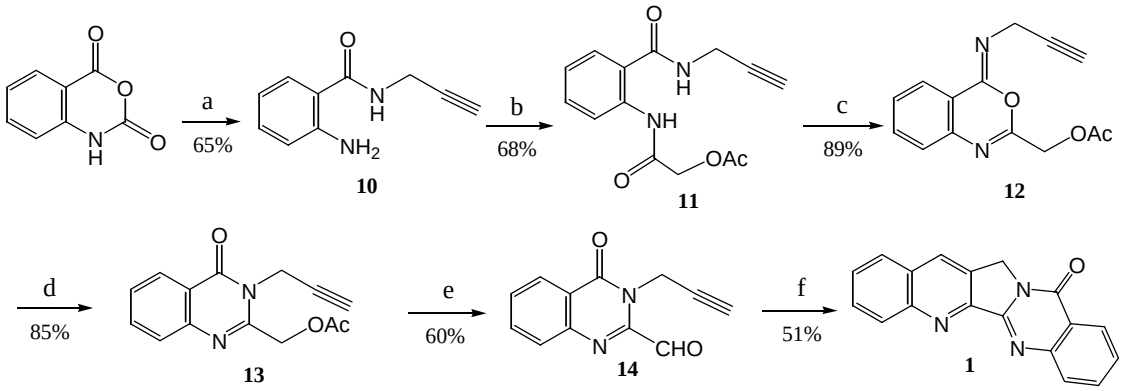


Figure 7 Reagents and conditions: a) propargylamine, DMF, 40-50 ˚C, 3 h; b) acetoxyacetyl chloride, Et_3N, benzene, 40 ˚C to rt, 16 h; c) PPh_3, I_2, EtN*i*-Pr_2, CH_2Cl_2, rt, 5 h; d) (i) 20% piperidine in EtOAc, rt, 1 h, (ii) silica gel, EtOAc, rt, 16 h; e) (i) 1M NaOH, THF-H_2O, rt, 2 h, (ii) Dess-Martin periodinane, pyridine, MeCN, rt, 1 h; f) aniline, 10 mol% $Dy(OTf)_3$, MeCN, rt, 24 h.

Route III: Heck Reaction (formation of C-ring)

Simple approach by Harayama *et al*.[12,13] involved *N*-alkylation of quinazolin-4(3*H*)-one with the quinolinyl bromide to give 3-[(2-bromoquinolin-3-yl)methyl]-4(3*H*)-quinazolinone (**15**) (Figure 8). The biaryl coupling reaction of **15** using electrophilic Pd reagent in DMF under reflux afforded luotonin A (**1**). At the same time, conversion of **1** to luotonin B (**2**) was also achieved by regioselective hydroxylation, involving NBS bromination in the presence of AIBN under irradiation with a tungsten lump and subsequent solvolysis with silver nitrate in aqueous acetone.

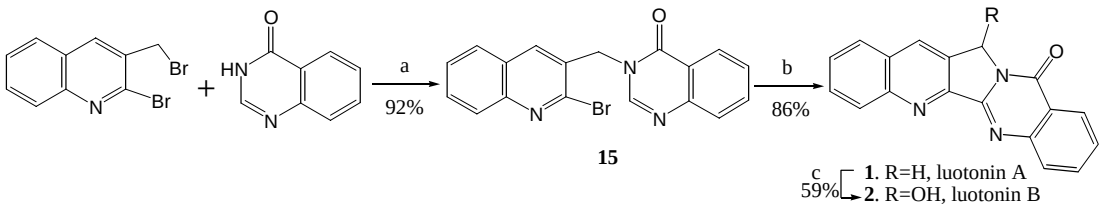


Figure 8 Reagents and conditions: a) *t*-BuOK, DMF, rt, 1.5 h; b) $Pd(OAc)_2$ (0.1 eq.), PCy_3 (2 eq.), KOAc (2 eq.), DMF, reflux, 30 min; c) (i) NBS, AIBN, CH_2Cl_2, tungsten lamp (300 W), reflux, 1 h; (ii) $AgNO_3$, aq. Me_2CO, reflux, 1 h.

Route IV: Mitsunobu cyclization (formation of C-ring)

Mhaske *et al.* demonstrated succesuful *ortho* lithiation of quinoline moiety on quinazolinone as a key step for the synthesis of luotonins A (**1**), B (**2**) and E (**3**) (Figure 9).[14] In the final step, Mitsunobu condensation was applied to a cyclization reaction leading to C-ring of **1**.[14]

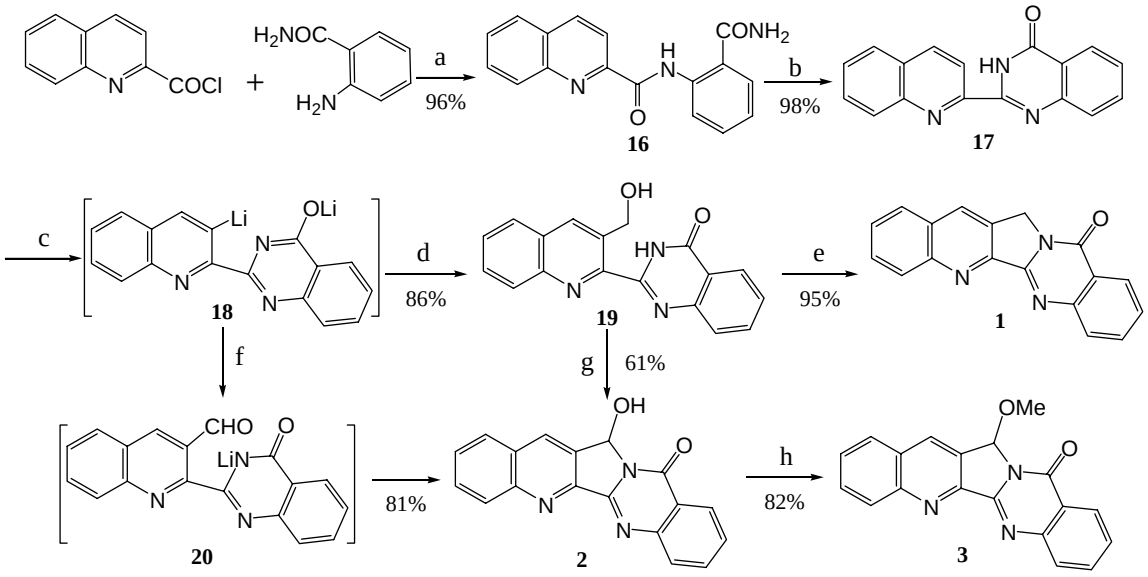


Figure 9 Reagents and conditions: a) Et_3N, THF, rt, 3 h; b) 5% aq KOH, EtOH, reflux, 5 min; c) mesityllithium, -78 ˚C, 30 min to -20 ˚C; d) THF solution of HCHO, - 30 ˚C, 20 min, saturated aq. solution of NH_4Cl; e) PPh_3, DEAD, THF, rt, 1 h; f) DMF, - 20 ˚C, 30 min, saturated aq. solution of NH_4Cl; g) PCC, powdered 4 Å molecular sieves, DCM, rt, 1 h; h) *p*-TSA, MeOH, reflux, 3 h.

N-Quinaldylanthranilamide (**16**) undergoes a base-catalyzed cyclization and dehydration to afford 2-quinolinoquinazolinone (**17**). Tandem lithiation of **17** with mesityllithium at -20 ˚C proceeds *in situ* to afford the desired dilithiated product (**18**), and coupling reaction of **18** with formaldehyde exclusively yielded *o*-hydroxymethylquinolinoquinazolinone (**19**). Subsequently, application of the Mitsunobu

cyclization to **19** resulted in luotonin A (**1**). Furthermore, the reaction of **18** with *N*,*N*-dimethylformamide spontaneously furnished luotonin B (**2**) *via* intermediate (**20**). Compound (**2**) can be also coverted by PCC oxidation of **19**. Further, **2** undergoes *O*-methylation under reflux condition in the presense of *p*-toluenesufonic acid to afford luotonin E (**3**).

On the other hand, Chavan *et al*.[15] also reported a similar fashion involving Mitsunobu reaction in the final step for the formation of C-ring of **1** as shown in Figure 10, in which a protective cyclic acetal compound was efficiently used. Condensation of acetal (**21**) with anthranilamide readily occurred under basic condition to afford the dihydroquinazolinone (**22**). Subsequently, oxidation of **22** with $KMnO_4$ gave quinazolinone (**23**). The deprotection of **23** followed by $NaBH_4$ reduction of resulted aldehyde (**24**) afforded **19**. Finally, alcohol (**19**) was converted into **1** by Mitsunobu reaction and otherwise on heating at 80 °C under acidic condition. Luotonins B (**2**) and E (**3**) both were derived from **23** under strong acidic conditions (Figure 11). [15]

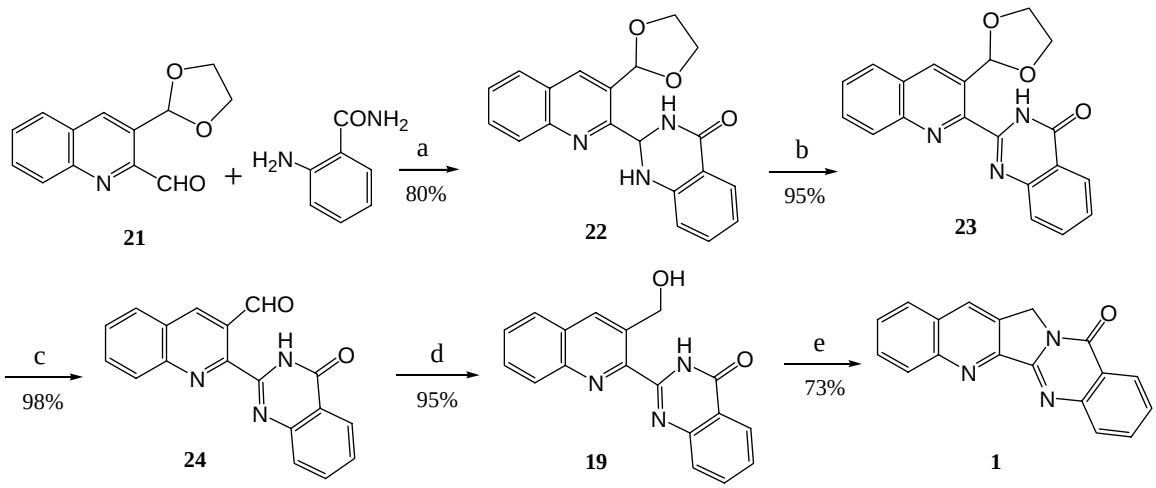


Figure 10 Reagents and conditions: a) 15% NaOH-EtOH solution, reflux, 5 h; or DMA, 80 ˚C, 3 h; b) $KMnO_4$, acetone, reflux, 3 h; c) 10% HCl-THF, 0.5 h; d) $NaBH_4$, MeOH; e) PPh_3, DEAD, THF; or 60% ethanolic H_2SO_4, reflux.

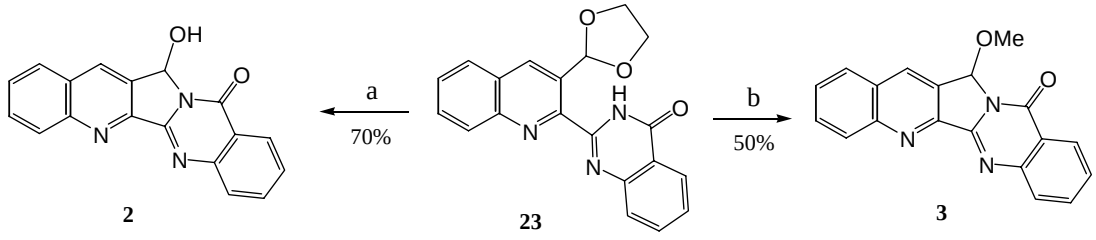


Figure 11 Reagents and conditions: a) 60% HCl, THF, reflux, 1 h; b) *conc.* HCl:MeOH (1:1), rt.

Route V: Intramolecular aza-Diels-Alder Reaction (formation of C- and D ring)

In an approach by Toyota *et al*.,[16,17] intramolecular aza-Diels-Alder reaction was employed as a key step, involving cyano group as a dienophile (Figure 12).

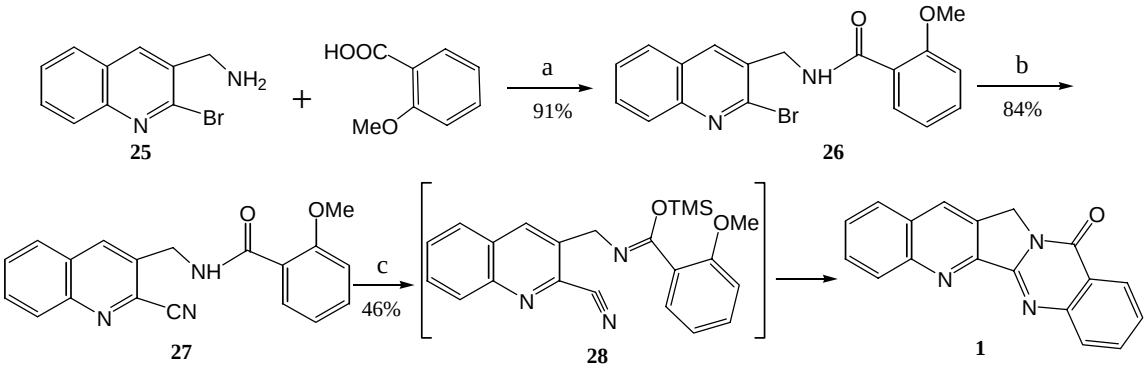


Figure 12 Reagents and conditions: a) bis(2-oxo-3-oxazolidinyl)phosphinic chloride, Et_3N, CH_2Cl_2, rt, 0.5 h; b) $Pd_2(dba)_3$, DPPF, CuCN, Et_4NCN, 1,4-dioxane, reflux, 1 h; c) TMSCl, $ZnCl_2$, Et_3N, PhMe, 150 °C in sealed tube.

Condensation of **25** with 2-methoxybenzoic acid in the presence of bis(2-oxo-3-oxazolidinyl)phosphinic chloride (BOP) and triethylamine provided the amide (**26**). Palladium-mediated cyanation of **26** with copper(I) cyanide afforded the quinoline-2-carbonitrile (**27**), which underwent an intramolecular hetero Diels–Alder addition in the presence of chlorotrimethylsilane and zinc chloride to furnish luotonin A (**1**) *via* the putative intermediate (**28**). The electron-donating methoxy group was crucial for activating the diene function.[17]

Route VI: Kametani Amide Condensation (formation of D-ring)

Kametani amide condensation is well-known reaction used to construct quinazolinones, starting from anthranilic acid.[23] Since the first isolation of luotonin A in 1997, thirteen syntheses have been reported so far. Five of these involved a coupling reaction of the pyrroloquinoline precursor (**29**) with anthranilates in the final step (Figure 13 and Table 1).[18-22] The first, Ganesan *et al*. (Entry 1)[18] employed 2-sulfinylaminobenzoyl chloride as a substrate in the presence of lithium bis(trimethylsilyl)amide to give luotonin A (**1**) in 85% yield. Dallavalle *et al*. (Entry 2)[19] demonstrated a one-pot sequence, three-step process for synthesis of **1**, which involved acylation of **29** with 2-nitrobenzoylchloride, reduction of the nitro group and subsequent ring closure. Yadav *et al*. (Entry 3) introduced solvent-free microwave-assisted reaction. Condensation reaction of **29** with isatoic anhydride in solvent-free under irradiation of microwave gave **1** in a high yield. Entry 4 by Jahng *et al*.[22] entailed the condensation of methyl anthranylate with imino chloride intermediate prepared from HCl salt of lactam (**29**) and

phosphoryl chloride.

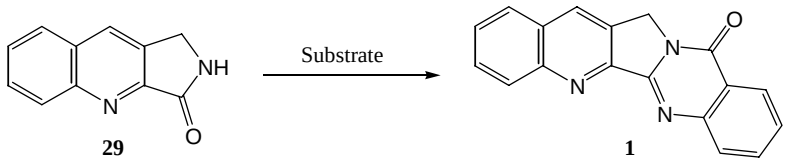
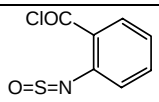
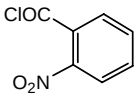
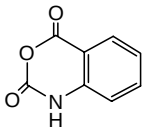
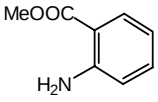


Figure 13 Reagents and conditions: see Table 1

Table 1 Synthetic conditions for luotonin A

Entry	Substrate	Condition	Yield (%)
1		excess $LiN(TMS)_2$, THF, rt, 2 h	85
2		i) NaH, THF, 60ºC, 1h; ii) substrate, 50ºC, 1 h; iii) Fe, AcOH/EtOH, reflux, 2h	38
3		Microwave irradiation, 450 watts, 7 min	85
4		i) dry HCl gas, $CHCl_3$; ii) $POCl_3$; iii) substrate, THF, 12 h, rt.	88

On the other hand, the pyrrolo[3,4-*b*]quinoline framework is a common structural feature in pharmacologically active alkaloids, for example camptothecin, nothapodytines A and B and mappicine.[14] Three convenient methodologies (Figures 14-16) for synthesis of 3-oxopyrrolo[3,4-*b*]quinoline (**29**) were developed in the access to luotonin A. As shown in Figure 14, Ganesan *et al*. prepared **29** by slightly modification of Danishefsky's method,[18] involving Friedländer condensation for construction of quinoline ring system.

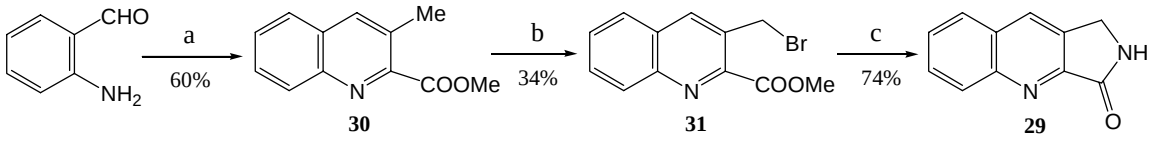


Figure 14 Reagents and conditions: a) (i) EtCOCOOH, NaOEt, MeOH, reflux, 16.5 h; (ii) H_2SO_4, MeOH, reflux, 24.5 h; b) NBS, AIBN, CCl_4, reflux, 7 h; c) NH_3, MeOH.

Dallavalle *et al.*[19] also employed Friedländer condensation for pyrroloquinoline system (**34**). The allylic protective group was easily isomerized with $PdCl_2$ to an enamide function and then quantitatively removed to give **29** through acid-catalyzed hydrolysis (Figure 15).

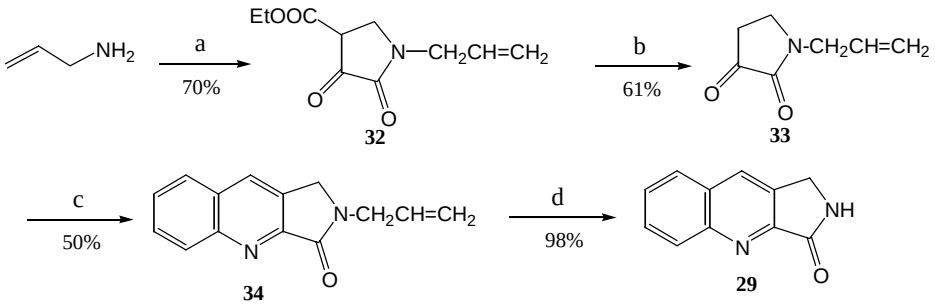


Figure 15 Reagents and conditions: a) (i) CH_2=CH-COOEt, EtOH, rt, 48 h; (ii) NaOEt, EtOOC-COOEt, reflux, 15 h; b) 10% HCl, reflux, 2 h; c) 2-aminobenzaldehyde, *p*-TsOH, PhMe, reflux; d) (i) $PdCl_2$, PPh_3, DMF–H_2O (4:1), reflux, 4 h; (ii) 6N HCl, reflux, 2 h.

Stevenson *et al.*[21] prepared **29** through Lewis acid-catalysed [4 + 2] cycloaddition reaction between an imine (**35**) and azetine (**36**) as a key step. Resulted quinoline (**37**) undergoes easily base-catalyzed cyclizatin to afford **29** (Figure 16).

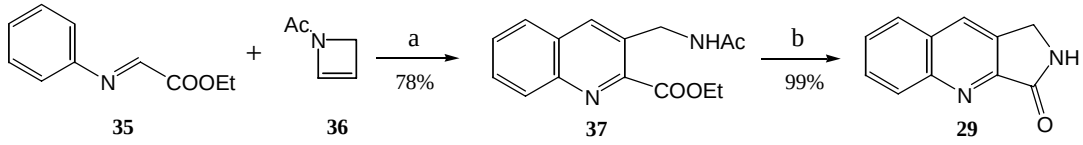


Figure 16 Reagents and conditions: a) (i) 3 mol% $Y(OTf)_3$, aniline, MeCN, rt, 12 h; (ii) HCl, MeCN, reflux, 1h; b) NaOEt, EtOH, 78°C.

STRUCTURE ACTIVITY RELATIONSHIPS

The fascination of luotonin A (**1**) as a synthetic target may be attributed to structural similarity to camptothecine (CPT) whose derivatives have been used as anticancer agents. CPT inhibits both the cleavage and religation of DNA by topoisomerase I through stabilization of topoisomarase I –DNA covalent complex intermediate. Luotonin A (**1**) was found to be less efficient than CPT in stabilizing the covalent complex between DNA and topoisomerase I and, therefore, less cytotoxic than CPT against a yeast cell lines expressing human topoisomerase I.[3]

Hecht *et al*. prepared over twenty luotonin A analogues (**1a** – **r** and **39a – j**) including luotonoin A (**1**) and isoluotonin A (**39**) (Figure 17, Table 2) for evaluation of the stabilization effect on human

topoisomerase I – DNA covalent complex and cytotoxicity against a transformed yeast strain expressing the human topoisomerase I.[24,25]

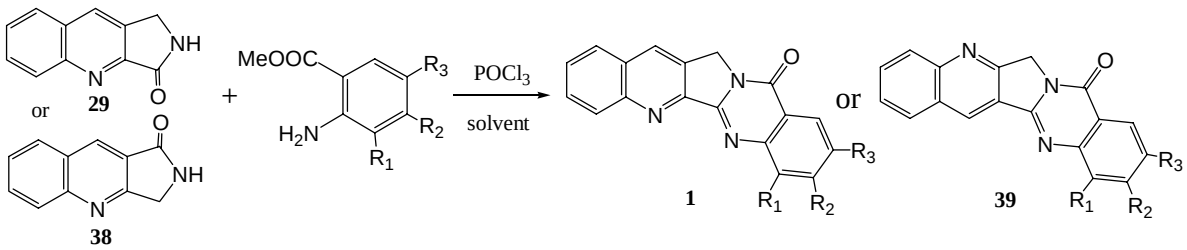


Figure 17

Table 2 Luotonin A analogues

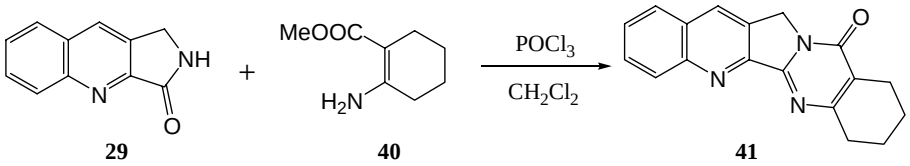
Compound	R_1	R_2	R_3	Yield of **1** (%)	Yield of **39** (%)
1a[a]	H	Cl	H	22	0
1b[a]	H	F	H	31	0
1c[a]	H	F	F	22	0
1d[a]	OMe	H	H	17	0
1e[a]	H	OMe	OMe	11	0
1f[a]	OMe	OMe	OMe	62	0
1g[a]	H	H	Me	12	0
1, **39**[b]	H	H	H	3~12	4~22
1a, **39a**[b]	H	Cl	H	37	9
1b, **39b**[b]	H	F	H	37	9
1h, **39c**[b]	H	COOMe	H	13	<1
1i, **39d**[b]	H	COOBn	H	24	15
1j, **39e**[b]	H	Ph	H	19	9
1k, **39f**[b]	H	CN	H	15	12
1l, **39g**[b]	H	H	Br	14	2
1m, **39h**[b]	H	NO_2	H	9	9
1n, **39i**[b]	CF_3	H	H	14	2
Other luotonin A analogues					
1o[c]	OH	H	H	10% from **1d**	
1p[d]	H	H	Ph	7% from **1l**	
1q[e]	H	NH_2	H	40% from **1m**	
1r[f]	H	CH_2OH	H	21% from **1h**	
39j[f]	H	CH_2OH	H	32% from **39b**	

Reagents and conditions: [a]CH_2Cl_2 as solvent; [b]THF as solvent; [c]HBr, reflux; [d]$Pd(OAc)_2$, $PhB(OH)_2$, 10% K_2CO_3, DMF, reflux, 4 h; [e]$SnCl_2$, HCl, MeOH, reflux, 2 h; [f]$NaBH_4$, THF, reflux, 8 h.

Luotonin A (**1**) and its several analogues **1a**, **1b**, **1k**, **1o**, **1q**, **1r**, **39**, **39a**, **39b** and **39f** were found to exhibit reasonable ability toward stabilization of the human topoisomerase I–DNA covalent complex in direct comparison with CPT at 50 μM concentration. Among them, **1b**, **1q**, **1r**, and **39b** that showed good ability have substituents at C-17 position roughly spatially similar to the position of C-20-OH of CPT. The results also exhibited that fused manner of quinoline ring does not significantly affect stabilization of topoisomerase I-mediated DNA cleavage.

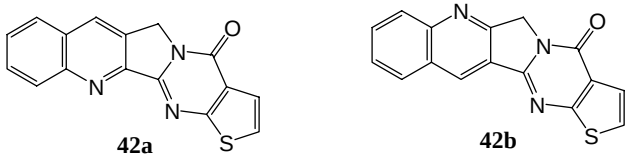
Those analogues were also examined for topoisomerase I-depend cytotoxicity in a transformed *Saccharomyces cerevisie* that lacks the homologous topoisomerase I, but expressed the enzyme by galactose promoter. **1**, **1b**, **1q** and **39** were found to exhibit reasonably strong topoisomerase I-dependent cytotoxicity toward yeast strain grown on garactose medium, and had IC_{50} values 9.58, 36.3, 15.7 and 11.8 μM, respectively.

Modified E-ring derivative, tetrahydroluotonin A (**41**) (Figure 18), showed less cytotoxicity toward the transformed yeast strain and less stabilization of the human topoisomerase I–DNA covalent complex than luotonin A (**1**).[24,25] The lesser activity of **41** exhibited that the aromatic E-ring of luotonin A contributes importantly to complex stabilization.

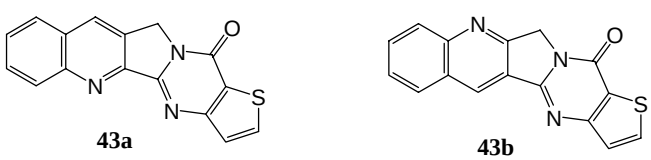


29 **40** **41**

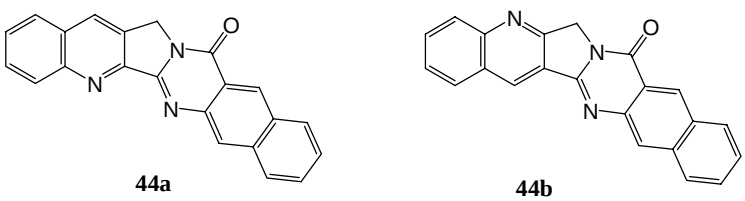
Figure 18



42a **42b**



43a **43b**



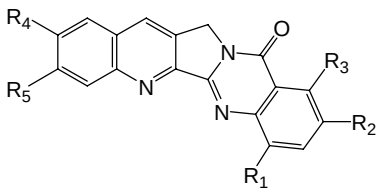
44a **44b**

Figure 19

As shown in Figure 19, the other luotonin A derivatives, having different E-ring systems - thiophene and naphthalene (**42a**–**44a** and **42b–44b**), were also tested in the same manner.[25] Only thiophene derivatives (**42a**, **43a** and **43b**) showed potent cytotoxicity against yeast cell lines without garactose promotor, but both **43a** and **43b** exhibited good activity in the stabilization of the enzyme–DNA covalent binary complex. [25]

Table 3 Luotonin A derivatives

Compd	R_1	R_2	R_3	R_4	R_5	IC_{50} (μM) 1 h	IC_{50}(μM) 72 h
1	H	H	H	H	H	67	7.7
1b	OMe	H	H	H	H	85	11
1aa	H	NO_2	H	H	H		23
1bb	Me	H	H	H	H	21	7
1cc	H	H	Cl	H	H		13
1dd	H	Cl	H	H	H	172	45
1ee	H	OH	H	H	H	40	3.8
1ff	H	H	H	OMe	OMe		5.5
1gg	H	H	H	-OCH_2O-			81
1hh	H	NH_2	H	H	H		86



Nine luotonin A derivatives synthesized by Dallavalle *et al*.[26] were evaluated for their cytotoxicity against the human lung carcinoma cell line H460 and the stabilizing activity of the DNA–topoisomerase I complex. From Table 3, it appears that all compounds are cytotoxic toward the H460 cell line, and **1bb**, **1ee** and **1ff** are more potent than luotonin A (**1**). On the other hand, in topoisomerase I- dependent DNA cleavage assay, **1bb** and **1cc** exhibited potent topoisomerase I poisons among these derivatives.[26]

SYNTHESIS AND BIOLOGICAL ACTIVITY OF 14-AZA CAMPTOTHECIN

The pyrroloquinazolinoquinoline alkaloid luotonin A (**1**) has been shown to be topoisomerase I poison.[3] Though less potent than CPT, luotonin A produced DNA strand breaks at the same sites and was also cytotoxic toward a yeast strain expressing human topoisomerase I . Ring D of luotonin A is nearly identical with that of CPT, differing only in the presence of a N atom instead of the CH at C-14 in CPT. Hecht *et al*. designed 14-azacamptothecin (**45**, 14-aza CPT, Figure 20) was designed for aqueous solubility.[27]

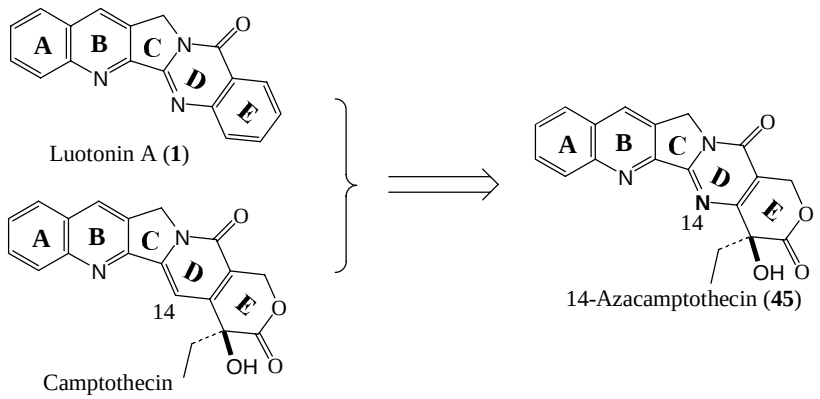


Figure 20

The synthesis of 14-aza CPT was accomplished as outlined in Figures 21 and 22. 4-Iodopyrimidine (**46**) was iodinated from commercially available 2-chloro-6-methoxypyrimidine. **46** was lithiated with *n*-BuLi, subsequently treated with ethyl formate to provide the aldehyde (**47**). Reductive etherification with crotyl alcohol afforded crotyl ether (**48**). When the ether (**48**) was heated in DMF in the presence of K_2CO_3, $Bu_4N^+Br^-$, and catalytic $Pd(OAc)_2$, cyclized olefin (**49**) was obtained. Treatment of **49** with K_2OsO_4 in the presence of the chiral ligand ($(DHQD)_2$-PYR) gave asymmetric diol (**50**). After I_2-mediated oxidation of the lactol, the corresponding lactone (**51**) was obtained. The lactone (**51**) was then dechlorinated to afford **52** by hydrogenolysis over 10% palladium-carbon and demethylated with 6 N HCl to afford pyrimidone (**53**). Pyrimidone (**53**) was condensed with 2-bromo-3-bromomethylquinoline to afford key intermediate (**54**), followed by radical mediated cyclization to give 14-aza CPT (**45**).

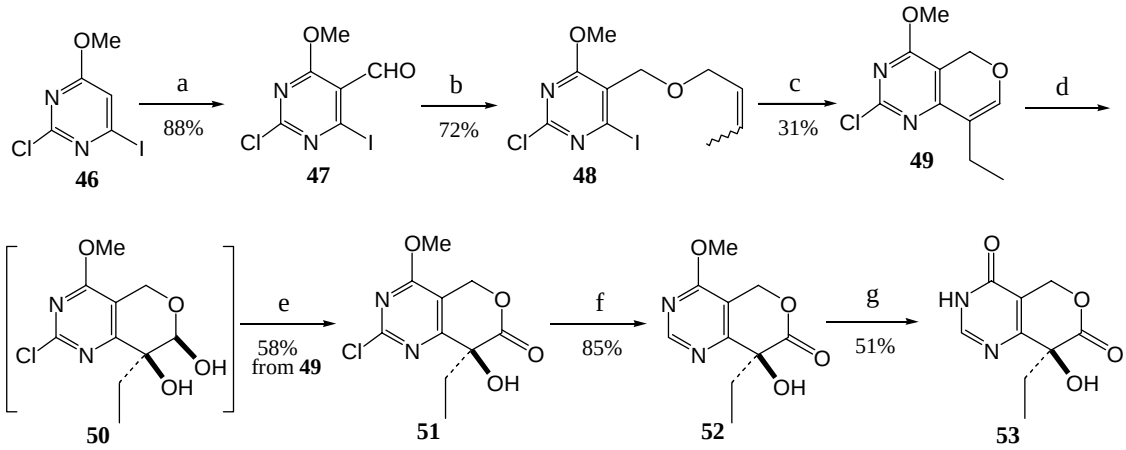


Figure 21 Reagents and conditions: a) (i) tetramethylpiperidine, *n*-BuLi, THF, -70 °C; (ii) HCOOEt, THF, -70 °C; (iii) aq. HCl, EtOH, THF; b) $MeCH{=}CHCH_2OH$, TFA, Et_3SiH, 25 °C, 16 h; c) $Pd(OAc)_2$, *n*-Bu_4NBr, K_2CO_3, DMF, 85 °C, 12 h; d) $(DHQD)_2$-Pyr, $K_3Fe(CN)_6$, K_2CO_3, $K_2OsO_4.2H_2O$, $MeSO_2NH_2$, *t*-BuOH-H_2O; e) I_2, $CaCO_3$; f) H_2, Pd/C, EtOH, Et_3N, 24 h; g) 6N HCl, MeOH, reflux, 3 h.

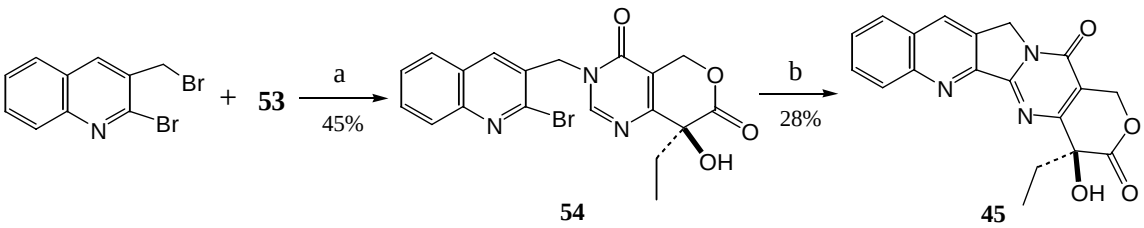


Figure 22 Reagents and conditions: a) *t*-BuOK, DME, reflux, 12 h; b) TTMSS, AIBN, benzene.

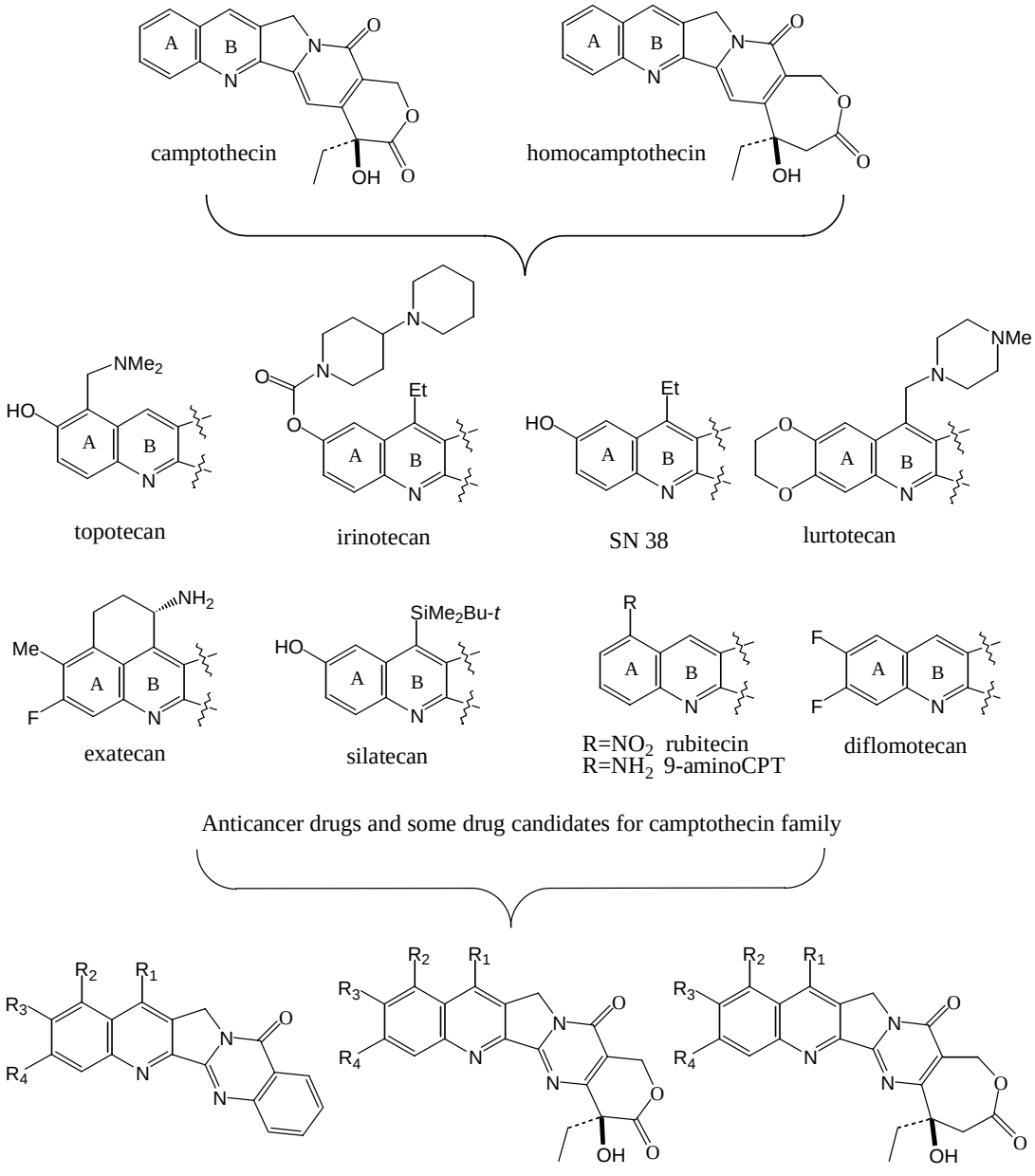


Figure 23

14-Aza CPT (**45**) was found to have much greater aqueous solubility than CPT, to inhibit topoisomerase I-mediated DNA relaxation more efficiently than CPT, and to stabilize the covalent binary complex to almost the same extent. 14-Aza CPT was found to be slightly less active (IC_{50} 2.2 μM) than CPT (IC_{50} 0.74-0.86 μM) in mediating cytotoxicity toward yeast expressing human topoisomerase I, and the cytotoxicity of 14-aza CPT was completely topoisomerase I-dependent. Thus, water-soluble 14-aza CPT (**45**) represents an attractive core structure for the elaboration of CPTs with improved properties.[27,28]

CONCLUSION AND PERSPECTIVES

The pyrroloquinazolinoquinoline alkaloid luotonin A (**1**) has been demonstrated as a pharmacophore for the development of a new class of topoisomerase I inhibitors. Thirteen convenient and efficient syntheses of luotonin A have been reported in high overall yields using a variety of elegant synthetic strategies. Though substitutions in the E-ring of luotonin A have been explored during the past years for their ability to effect stabilization of the covalent binary complex formed between human topoisomerase I and DNA, and for cytotoxicity toward a yeast strain expressing the human topoisomerase I, it is still necessary to synthesize more bioactive derivatives not only in E-ring but also in A- and B-rings to increase the biological activity and to improve the solubility in aqueous solution. As much effort has been spent on development of new anti-cancer drugs of CPT, it will provide extensive useful information to develop luotonin A derivatives as antineoplastic agents. As shown in Figure 23, several CPT derivatives modified in A- and B-rings have been used as anticancer drugs or drug candidates in clinical trials.[29,30] The same type of analogues can be easily applied to luotonin A, 14-aza CPT as well as azahomoCPT. The expectations in luotonin A, 14-aza CPT and azahomoCPT will remain high for their structure-activity relationship study, and the research should be straightforward.

REFERENCES

1. Z. Z. Ma, Y. Hano, T. Nomura, and Y. J. Chen, *Heterocycles*, 1997, **46**, 541.
2. Z. Z. Ma, Y. Hano, T. Nomura, and Y. J. Chen, *Bioorg. Med. Chem. Lett.*, 2004, **14**, 1193.
3. A. Cagir, S. H. Jones, R. Gao, B. M. Eisenhauer, and S. M. Hecht, *J. Am. Chem. Soc.*, 2003, **125**, 13628.
4. X. H. Xiao, G. L. Qou, H. L. Wang, L. S. Liu, Y. L. Zheng, Z. J. Jia, and Z. B. Deng, *Chinese J. Pharmacol. Toxicol.*, 1988, 232.
5. Z. Z. Ma, Y. Hano, T. Nomura, and Y. J. Chen, *Heterocycles*, 1999, **51**, 1883.
6. Z. Z. Ma, Y. Hano, T. Nomura, and Y. J. Chen, *Heterocycles*, 1999, **51**, 1593.
7. D. R. Liljegren, *Phytochemistry*, 1968, **7**, 1299.
8. D. R. Liljegren, *Phytochemistry*, 1971, **10**, 2661.

9. P. Molina, A. Tarraga, and A. Gonzalez-Tejero, *Synthesis*, 2000, 1523.
10. T. R. Kelly, S. Chamberland, and R. A. Silva, *Tetrahedron Lett.*, 1999, **40**, 2723.
11. H. Twin and R. A. Batey, *Org. Lett.*, 2004, **6**, 4913.
12. T. Harayama, Y. Morikami, Y. Shigeta, H. Abe, and Y. Takeuchi, *Synlett*, 2003, 847.
13. T. Harayama, A. Hori, G. Serban, Y. Morikami, T. Matsumoto, H. Abe, and Y. Takeuchi, *Tetrahedron*, 2004, **60**, 10645.
14. S. B. Mhaske and N. P. Argade, *J. Org. Chem.*, 2004, **69**, 4563.
15. S. P. Chavan and R. Sivappa, *Tetrahedron*, 2004, **60**, 9931.
16. M. Toyota, C. Komori, and M. Ihara, *Heterocycles*, 2002, **56**, 101.
17. M. Toyota, C. Komori, and M. Ihara, *ARKIVOC*, 2003, 15.
18. H. Wang and A. Ganesan, *Tetrahedron Lett.*, 1998, **39**, 9097.
19. S. Dallavalle and L. Merlini, *Tetrahedron Lett.*, 2002, **43**, 1835.
20. J. S. Yadav and B. V. S. Reddy, *Tetrahedron Lett.*, 2002, **43**, 1905.
21. D. Osborne and P. J. Stevenson, *Tetrahedron Lett.*, 2002, **43**, 5469.
22. E. S. Lee, J. G. Park, and Y. Jahng, *Tetrahedron Lett.*, 2003, **44**, 1883.
23. T. Kametani, C. V. Loc, T. Higa, M. Koizumi, M. Ihara, and K. Fukumoto, *J. Am. Chem. Soc.*, 1977, **99**, 2306.
24. A. Cagir, S. H. Jones, B. M. Eisenhauer, R. Gao, and S. M. Hecht, *Bioorg. Med. Chem. Lett.*, 2004, **14**, 2051.
25. A. Cagir, B. M. Eisenhauer, R. Gao, S. J. Thomas, and S. M. Hecht, *Bioorg. Med. Chem.*, 2004, **12**, 6287.
26. S. Dallavalle, L. Merlini, G. L. Beretta, S. Tinelli, and F. Zunino, *Bioorg. Med. Chem. Lett.*, 2004, **14**, 5757.
27. N. J. Rahier, K. Chen, R. Gao, B. M. Eisenhauer, and S. M. Hecht, *Org. Lett.*, 2005, **7**, 835.
28. K. Chen, N. J. Rahier, B. M. Eisenhauer, R. Gao, S. J. Thomas, and S. M. Hecht, *J. Am. Chem. Soc.*, 2005, **127**, 838.
29. C. J. Thomas, N. J. Rahier, and S. M. Hecht, *Bioorg. Med. Chem.*, 2004, **12**, 1585.
30. C. Bailly, *Crit. Rev. Oncol. Hematol.*, 2003, **45**, 91.