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New Topoisomerases Inhibitors: Synthesis of Rutaecarpine Derivatives and Their Inhibitory Activity against Topoisomerases

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A series of rutaecarpine derivatives were prepared by employing previously reported methods and their inhibitory activities against topoisomerase I and II were evaluated. Among them, strongly cytotoxic 10-bromorutaecarpine and 3-chlororutaecarpine showed strong inhibitory activities against topo I and II.

Key words: Rutaecarpine, Topoisomerase I, Topoisomerase II, Indoloquinazoline alkaloid

INTRODUCTION

Rutaecarpine (**1a**) is one of well studied indoloquinazoline alkaloids isolated from Rutaceous plants (Asahina and Kashiwaki, 1915) such as *Evodia rutaecarpa* and *Evodia officinalis*, which have long been used for the treatment of inflammation-related symptoms in the traditional oriental medicinal practice (Chu, 1951). Continuous studies on rutaecarpine revealed a variety of biological properties such as an anti-inflammatory (Moon et al., 1999), vasorelaxing (Chiou et al., 1994; Chen et al., 2009), analgesic (Kong et al., 1976), anti-platelet (Sheen et al., 1996; Sheu et al., 1996), anti-anoxic (Yamahara et al., 1989), cytotoxic (Yang et al., 1995; Xu et al., 2006), and anti-obesity activities (Yokoo et al., 1997; Kim et al., 2009) as well as selective inhibitory activity on human cytochrome P450 1B1 (Ueng et al., 2002). Although the action mechanisms of anti-inflammatory, vasorelaxing, anti-platelet, and anti-obesity effect of rutaecarpine have been studied, those of cytotoxicity have not been systematically studied as yet.

Recently Xu et al. revealed that rutaecarpine itself did not show any significant activity against topoisomerase (topo) I and topo II (Xu et al., 2006) in spite of significant cytotoxicity of rutaecarpine on several cancer cell lines. On the other hand, early studies on the rutaecarpine derivatives revealed that 10-bromorutaecarpine and 3-chlororutaecarpine showed strong cytotoxicity against selected human cancer cell lines (GI_{50} = 3 μM for SKOV3 and DU145, Baruah et al., 2004; IC_{50} for HT-29 = 12 μM, Jahng et al., 2004).

As a part of our ongoing interest in finding natural product-based cytotoxic agents and our results of benzo-annulated rutaecarpines against topoisomerases (Hong et al., 2010) spurred us to pursue systematic study on the inhibitory activities of rutaecarpine derivatives against topoisomerases.

MATERIALS AND METHODS

Melting points (mp) were determined using a Fischer-Jones melting points apparatus and are not corrected. NMR spectra were obtained using a Bruker-250 spectrometer 250 MHz for ^{1}H-NMR and 62.5 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.

2-Chloro-6,7,8,9-tetrahydro-11*H*-pyrido[2,1-*b*]quinazolin-11-one (4)

A solution of 2-amino-5-chlorobenzoic acid (13.7 g, 0.10 mol), piperidin-2-one (12.0 g, 0.12 mol) and $SOCl_2$ (20 mL, *ca.* 2 eq) in dry pyridine (100 mL) was refluxed for 4 h. The reaction mixture was poured to ice-

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water and made basic with NH_4OH (100 mL), and the precipitate was collected as a crude product which was recrystallized from EtOH to give **4** as white needles: mp 106-107°C [mp 107°C (Jahng et al., 2008)]. ^{1}H-NMR (250 MHz, DMSO-d_6) δ 8.11 (d, 1H, J = 2.3 Hz, H1), 7.95 (dd, 1H, J = 8.8, 2.3 Hz, H3), 7.85 (d, 1H, J = 8.8 Hz, H4), 3.94 (t, 2H, J = 6.2 Hz), 3.13 (t, 2H, J = 6.2 Hz), 1.95 (quintet, 2H, J = 6.2 Hz), 1.85 (quintet, 2H, J = 6.2 Hz).

6-Benzylidene-2-chloro-6,7,8,9-tetrahydro-11H-pyrido[2,1-b]quinazolinon-11-one (**5**)

A mixture of **4** (930 mg, 3.96 mmol) and benzaldehyde (10 mL) in Ac_2O (20 mL) was refluxed for 48 h. Excess benzaldehyde and Ac_2O was removed under reduced pressure. To the residue was added water (100 mL). Mixture was made basic with 50% aq. NaOH and extracted with CH_2Cl_2 (50 mL × 3). The organic layers were washed with water, and dried over $MgSO_4$. Evaporation of the solvent afforded an oily material which was chromatographed on silica gel, eluting with CH_2Cl_2 to give the desired product (0.94 g, 75%) which was recrystallized from EtOH to give white needles: mp 148°C. ^{1}H-NMR (250 MHz, $CDCl_3$) δ 8.24 (br s, 1H), 8.17 (d, 1H, J = 8.6 Hz), 7.70 (d, 1H, J = 1.9 Hz, H3), 7.50-7.43 (m, 4H), 7.39-7.32 (m, 1H), 4.13 (t, 2H, J = 5.9 Hz), 2.95 (t, 2H, J = 6.4 Hz), 2.05 (quintet, 2H, J = 6.2 Hz). ^{13}C-NMR (62.5 MHz, DMSO-d_6) δ 161.45, 152.70, 148.27, 140.03, 135.96, 135.85, 129.88, 129.48, 128.29, 127.96, 126.52, 118.34, 42.22, 25.47, 21.73.

2-Chloro-6,7,8,9-tetrahydro-11*H*-pyrido[2,1-*b*]quinazoline-6, 11-dione (6)

A solution of **5** (500 mg, 2.13 mmol) in CH_2Cl_2 (50 mL) was cooled in acetone-dry ice bath and ozone was bubbled through the solution until the solution turns blue. Excess ozone was purged out and $(CH_3)_2S$ (10 mL) was added into the mixture. Evaporation of the solvent afforded semisolid (0.65 g) which was chromatographed on silica gel, eluting with CH_2Cl_2. The early eluent gave the desired product as semisolid (536 mg, 78%). ^{1}H-NMR (250 MHz, $CDCl_3$) δ 8.16 (dd, 1H, J = 8.6 Hz, H-1), 7.91 (d, 1H, J = 2.0 Hz, H-4), 7.67 (dd, 1H, J = 8.5, 2.0 Hz, H-2), 4.13 (t, 2H, J = 5.9 Hz), 2.80 (t, 2H, J = 6.4 Hz), 2.25 (quintet, 2H, J = 6.2 Hz). ^{13}C-NMR (62.5 MHz, DMSO-d_6) δ 190.25, 160.36, 147.45, 146.51, 139.45, 129.00, 128.47, 120.54, 42.13, 36.98, 19.78. *Anal.* Calcd for $C_{12}H_9ClN_2O_2$: C, 57.96; H, 3.65; N, 11.27. Found: C, 58.05; H, 3.70; N, 11.31.

6-Phenylhydrazono-2-chloro-6,7,8,9-tetrahydro-11*H*-pyrido-[2,1-*b*]quinazoline-6,11-dione (7)

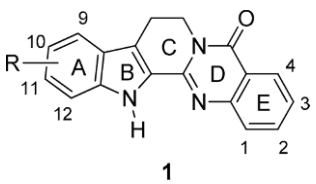
To a solution of **6** (100 mg, 0.47 mmol) in 95% EtOH (20 mL) was added phenylhydrazine-HCl (102 mg, 0.71 mmol, 1.5 equiv.) to yield white solids (105 mg, 66%), whose ^{1}H-NMR showed a set of proton resonances for two isomers (99%): ^{1}H-NMR (250 MHz, $CDCl_3$) (*E*-isomer, major) δ 11.03 (s, NH), 8.18 (d, 1H, J = 8.6 Hz, H-1), 7.91 (d, 1H, J = 1.8 Hz, H-4), 7.69-7.62 (m, 3H), 7.46-7.28 (m, 2H), 7.06 (t, 1H, J = 7.3 Hz, H-4'), 4.22 (t, 2H, J = 5.9 Hz), 2.79 (t, 2H, J = 6.4 Hz), 2.12 (m, 2H). (*Z*-isomer, minor) δ 14.25 (s, NH), 8.25 (d, 1H, J = 1.9 Hz, H-4), 8.13 (d, 1H, J = 8.6 Hz, H-2), 7.63-7.54 (m, 2H), 7.45-7.26 (m, 3H), 6.94 (t, 1H, J = 7.3 Hz, H-4'), 3.96 (t, 2H, J = 5.9 Hz), 2.79 (t, 2H, J = 6.4 Hz), 2.35 (m, 2H).

3-Chlororutaecapine (**8**)

The mixture of (*E*)- and (*Z*)-isomers of **7** (60 mg, 0.18 mmol) was dissolved in PPA (5 mL) and heated at 180°C for 1.5 h to yield white needles (50 mg, 88%): mp 321-322°C [lit. mp 314-316°C (Bergman and Bergman, 1985); lit. mp 320-322°C (Chen et al., 2009)]. ^{1}H-NMR (250 MHz, DMSO-d_6) δ 11.88 (s, NH), 8.14 (d, 1H, J = 8.6 Hz, H-4), 7.65 (d, 1H, J = 8.6 Hz, H-12), 8.63 (d, 1H, J = 1.8 Hz, H-4), 7.52-7.47 (m, 2H, H-2 & H-9), 7.27 (td, 1H, J = 7.3, 0.8 Hz, H-10), 7.09 (t, 2H, J = 7.3 Hz, H-11), 4.43 (t, 2H, J = 6.8 Hz), 3.18 (t, 2H, J = 6.8 Hz). ^{13}C NMR (62.5 MHz, DMSO-d_6) δ 160.32, 148.79, 146.82, 139.15, 139.04, 128.96, 126.99, 126.35, 125.55, 125.29, 125.03, 120.35, 120.09, 119.76, 118.86, 112.87, 41.13, 19.09.

RESULTS AND DISCUSSION

Rutaecarpine (**1a** R = H) (Lee et al., 2001) and its derivatives with a substituent on ring A (**1b** R = 9-F; **1c** R = 10-F; **1d** R = 11-F; **1e** R = 12-F; **1f** R = 9-Cl; **1g** R = 10-Cl; **1h** R = 11-Cl; **1i** R = 12-Cl; **1j** R = 9-Br; **1k** R = 10-Br; **1l** R = 11-Br; **1m** R = 12-Br; **1n** R = 9-CH_3; **1o** R = 10-CH_3; **1p** R = 11-CH_3; **1q** R = 12-CH_3) (Lee et al., 2005) were prepared by employing previously reported methods in which Fischer indole synthesis was employed as a key reaction.



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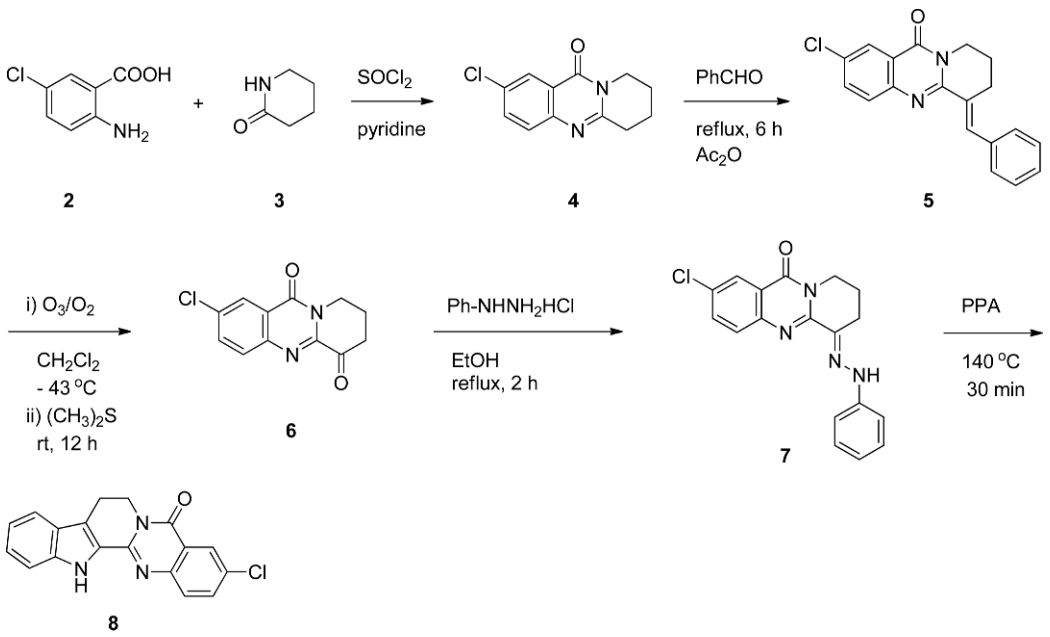
Although a couple of synthetic procedures for the preparation of 3-chlororutaecarpine have been reported a few cases in the literature (Bergman and Bergman, 1985; Hamid et al., 2006; Chen et al., 2009; Lee et al., 2009), a detailed synthetic procedure for 3-chlororutae-



Scheme 1. Synthesis of 3-chloroutaecarpine

carpine has been not reported as yet. The same synthetic method described above was applied to substituted anthranilic acids to give a series of rutaecarpine derivatives on the ring E.

One-pot reaction of 2-amino-5-chlorobenzoic acid (**2**) and piperidin-2-one (**3**) in the presence of $SOCl_2$ afforded 2-chloro-6,7,8,9-tetrahydro-11*H*-pyrido[2,1-*b*]quinazolin-11-one (**4**) in 86% yield (Jahng et al., 2008). Subsequent acetic anhydride-mediated condensation of **4** with benzaldehyde gave the corresponding 6-benzylidene compound **5** in 75% yield, which was then subjected to ozonolysis to yield the corresponding ketone **6** in 78% yield. Fischer indole synthesis was applied to the ketone **7** with phenylhydrazine-HCl yielded the desired 3-chlororutaecarpine (**8**) in 88% yield. The structure was confirmed by spectroscopic methods.

The conversion of supercoiled pBR 322 plasmid DNA to relaxed DNA by topo I and II was evaluated to investigate the inhibitory activity of rutaecarpine derivatives (**1b-q** and **8**) against topo I and II by employing previously reported methods (Fukuda et al., 1996). As illustrated in Figs. 1 and 2, compounds **1k** and **8**, showed strong inhibitory activity on both topo I and II and data are summarized in Table I. Although **1k** and **8** did not show any selectivity on topo I and II, their inhibitory activities on topo I and II were somewhat stronger than that of CPT and etoposide, respectively.

Although no obvious correlation between the cytotoxicity of rutaecarpine and any inherent activity on DNA relaxation and decatenation by DNA topo I and II was observed, cytotoxicities of 10-bromorutaecarpine (Baruah et al., 2004) and 3-chlororutaecarpine (Jahng et al., 2004) were closely correlated to their inhibitory activities against topo I and II.

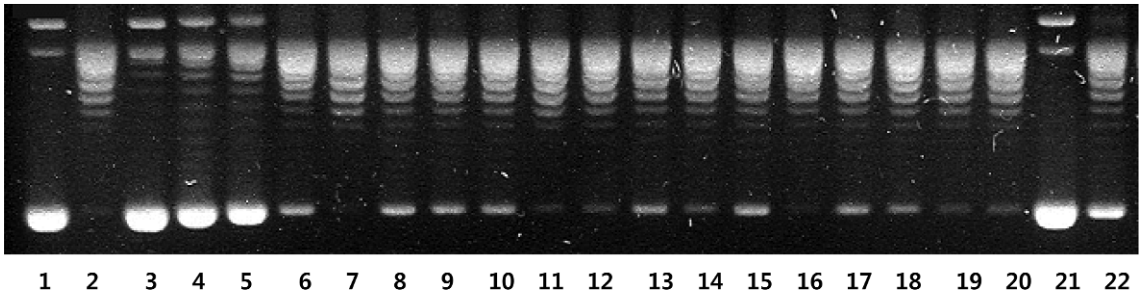


Fig. 1. Inhibitory activities of rutaecarpines **1a-q** and **8** against DNA topoisomerase I. Lane 1: DNA; Lane 2: DNA + topo I; Lane 3: DNA + topo I + CPT (20 μM); Lane 4: DNA + topo I + CPT (100 μM); Lane 5: DNA + topo I + 10-bromorutaecarpine (**1k**) (20 μM); Lane 6: DNA + topo I + rutaecarpine (**1a**) (20 μM) … Lane 21: DNA + topo I + 3-chlororutaecarpine (**8**) (20 μM); Lane 22: DNA + topo I + 12-methylrutaecarpine (**1q**) (20 μM)

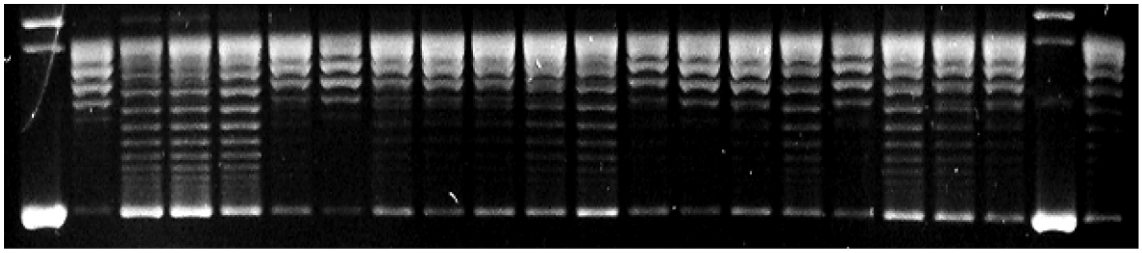


Fig. 2. Inhibitory activities of rutaecarpines **1a-q** and **8** against DNA topoisomerase II. Lane 1: DNA; Lane 2: DNA + topo II; Lane 3: DNA + topo II + Etoposide (20 μM); Lane 4: DNA + topo II + Etoposide (100 μM); Lane 5: DNA + topo I + **1k** (20 μM); Lane 6: DNA + topo I + rutaecarpine (**1a**) (20 μM) … Lane 21: DNA + topo I + 3-chlororutaecarpine (**8**) (20 μM); Lane 22: DNA + topo I + 12-methylrutaecarpine (**1q**) (20 μM).

Table I. Inhibition of camptothecin (CPT), etoposide, rutaecarpine, **1k** and **8** against topoisomerases I and II

	References	Rutaecarpine	1k	8
Topo I inhibition at 20 μM[a]	47.45	NA[c]	43.75	63.65
Topo I inhibition at 100 μM[a]	82.62	2.08	79.54	84.36
Topo II inhibition at 20 μM[b]	39.68	NA	23.76	45.76
Topo II inhibition at 100 μM[b]	63.41	1.25	35.43	69.51

[a]Data were taken with 0.2 unit of topo I and reference (CPT); [b]Data were taken with 0.2 unit of topo II and reference (etoposide) or compounds prepared and shown as % of inhibition; [c]not active.

In conclusion, inhibitory activities of the derivatives of rutaecarpine against topoisomerases I and II were evaluated. Among the compounds tested, 10-bromorutaecarpine (**1k**) and 3-chlororutaecarpine (**8**) were shown strong inhibitory activities against topo I and II. Studies on the synthesis of additional derivatives of rutaecarpine and evaluation of their biological properties are in progress, which will be due in the near future.

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