Synthesis and Biological Properties of Benzo-Annulated Rutaecarpines

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A series of benzo-annulated rutaecarpines were prepared from anthranilic acid and 3-aminonaphthalene-2-carboxylic acid by Fischer indole synthesis as key reaction. Cytotoxicity was somewhat increased by the introduction of benzo-annulation, which was not directly related to the inhibitory activity against topoisomerases (topo) I and II. Benzo-annulation on ring A led to significant increase of inhibitory activity against topo II while annulations on ring E increased inhibitory activity against topo I.

Key words rutaecarpine; benzorutaecarpine; Fischer indole synthesis; topoisomerase; cytotoxicity

Rutaecarpine[1)] is an indoloquinazolinone alkaloid of Rutaceous plants such as *Evodia rutaecarpa* that has long been employed for the treatment of inflammation-related disorders in traditional oriental medicinal practice[2)] and has shown a variety of important biological properties such as selective inhibitory activity against cyclooxygenase-2 (COX-2),[3)] and vasorelaxing,[4—6)] antiplatelet,[7,8)] antianoxic,[9)] antiobesity,[10,11)] and cytotoxic activities,[12—14)] which were summarized very well as a review.[15)] Such a variety of intriguing biological properties have led not only to the development of synthetic methods[16—20)] but also to the systematic examination of its metabolites.[21—23)] Although the action mechanisms of antiinflammatory,[3)] vasorelaxing,[6)] antiplatelet,[8)] and antiobesity[11)] effect of rutaecarpine have been studied, those of cytotoxicity have not been pursued as yet.

Continuing interest in rutaecarpine[17,18,21,22,24,25)] and in the effect of benzoannulation on chemical[26)] and/or biological properties[27)] spurred us to prepare a series of benzo-annulated rutaecarpines, especially on rings A and E, and to examine their biological properties focusing on the inhibitory activities in selected human cancer cell lines.

MATERIALS AND METHODS

Melting points were determined on a Fischer–Jones melting points apparatus and are not corrected. IR spectra were recorded on a Perkin-Elmer 1330 spectrophotometer. NMR spectra were recorded on a Bruker-250 spectrometer 250 MHz for ^{1}H-NMR and 62.5 MHz for ^{13}C-NMR and are reported as ppm from the internal standard tetramethylsilane (TMS). Chemicals and solvents were commercial reagent grade and used without further purification. The starting 3-aminonaphthalene-2-carboxylic acid was prepared by employing previously reported method.[28)] Elemental analyses were taken on a Hewlett-Packard Model 185B elemental analyzer.

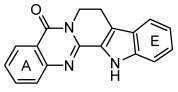

Fig. 1. Structure of Rutaecarpine

3,4-Dihydro-1*H*-benzo[*g*]pyrido[2,1-*b*]quinazolin-12(2*H*)-one (10) A solution of 3-aminonaphthalene-2-carboxylic acid (2.27 g, 0.01 mol), 2-piperidone (1.19 g, 0.012 mol), and $SOCl_2$ (2 ml, *ca.* 2 eq) in dry pyridine (20 ml) was refluxed for 4 h. The reaction mixture was poured to ice-water and made basic with NH_4OH (100 ml), and the precipitate was collected and chromatographed on silica gel eluting with CH_2Cl_2 and CH_2Cl_2 : EtOAc (1 : 1). The early fractions of mixed solvent afforded pale yellow needles (1.80 g, 72%): mp 163 °C. IR (KBr) ν 3100, 2850 cm^{-1}. ^{1}H-NMR ($CDCl_3$, 250 MHz) δ: 8.74 (s, 1H, H11), 8.52 (s, 1H, H6), 7.90 (d, 1H, J=8.0 Hz, H10), 7.88 (d, 1H, J=8.0 Hz, H7), 7.63 (td, 1H, J=8.0, 1.0 Hz, H9), 7.54 (td, 1H, J=8.0, 1.0 Hz, H8), 4.20 (t, 2H, J=6.3 Hz), 3.68 (t, 2H, J=6.3 Hz), 2.16—2.07 (m, 4H). ^{13}C-NMR (62.5 MHz, $CDCl_3$) δ: 163.1, 154.4, 143.2, 137.1, 131.7, 129.7, 128.6, 128.5, 128.3, 126.3, 124.2, 120.1, 42.6, 32.5, 22.6, 19.8. MS (electrospray ionization (ESI)) Calcd for $C_{16}H_{15}N_2O^+$ $[M+H]^+$: 251. Found: 251.

(*E*)-4-Benzylidene-3,4-dihydro-1*H*-benzo[*g*]pyrido[2,1-*b*]quinazolin-12(2*H*)-one (11) A mixture of **10** (2.50 g, 10 mmol) and benzaldehyde (3.18 g, 30 mmol) in acetic anhydride (20 ml) was refluxed for 48 h. Excess benzaldehyde and acetic anhydride were removed under reduced pressure. To the residue was added water (100 ml). The resulting mixture was made basic with 50% aqueous NaOH and extracted with CH_2Cl_2 (50 ml×3). The organic layers were combined, washed with water, and dried over anhydrous $MgSO_4$. Evaporation of the solvent afforded an oily material (3.22 g), which was chromatographed on silica gel eluting with CH_2Cl_2. The latter fractions gave 2.81 g (83%) of white needles after crystallization from the eluent: mp 169—170 °C. IR (KBr) ν 1664, 1606, 1530, 1470, 1390, 1340, 1306, 773, 758, 689 cm^{-1}. ^{1}H-NMR (250 MHz, $CDCl_3$) δ: 8.88 (s, 1H, H11), 8.25 (s, 1H, benzylidene H), 8.21 (s, 1H, H6), 8.04 (d, J=8.0 Hz, 1H), 7.97 (d, J=8.5 Hz, 1H), 7.57 (t, J=7.5 Hz, 1H), 7.52—7.30 (m, 6H), 4.19 (t, J=5.8 Hz, 2H), 2.97 (t, J=5.8 Hz, 2H), 2.04 (quintet, J=7.2 Hz, 2H). ^{13}C-NMR (62.5 MHz, $CDCl_3$) δ: 162.7, 151.0, 142.9, 136.9, 136.4, 135.3, 131.4, 130.4, 130.2 (two C's), 129.4, 128.4, 128.2, 128.01, 127.95, 126.0, 124.9, 119.4, 42.2, 25.9, 22.2. MS (ESI) Calcd for $C_{23}H_{19}N_2O^+$ $[M+H]^+$: 339. Found: 339.

2,3-Dihydro-1*H*-benzo[*g*]pyrido[2,1-*b*]quinazoline-4,12-dione (12) A solution of **11** (900 mg, 3.12 mmol) in

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CH_2Cl_2 (50 ml) was cooled in acetone-dry ice bath, and ozone was bubbled through the solution until the solution became blue. Excess ozone was purged by bubbling oxygen, and $(CH_3)_2S$ (10 ml) was added into the mixture. Evaporation of the solvent afforded semi-solid (650 mg), which was chromatographed on silica gel eluting with CH_2Cl_2. The latter fractions [*Rf*=0.4 (CH_2Cl_2 : EtOAc)] gave pale yellow needles (560 mg, 83%) after crystallization from the eluent: mp 224—226 °C. IR (KBr) ν 1720, 1670, 1607, 1580, 1470, 1338, 1310, 1240, 1196, 1154, 913, 778, 694 cm^{-1}. ^{1}H-NMR (250 MHz, $CDCl_3$) δ: 8.92 (s, 1H, H11), 8.52 (s, 1H, H6), 8.07 (dd, *J*=8.1, 1.4 Hz, 1H, H10), 8.03 (d, *J*=8.8 Hz, H7), 7.67—7.57 (m, 2H, H8, H9), 4.36 (t, *J*=6.8 Hz, 2H), 2.96 (t, *J*=6.8 Hz, 2H), 2.38 (quintet, *J*=6.8 Hz, 2H). ^{13}C-NMR (62.5 MHz, $CDCl_3$) δ: 190.5, 162.0, 143.6, 141.3, 136.6, 132.7, 129.3, 128.8, 128.5 (two C's), 128.2, 127.5, 119.9, 42.2, 37.7, 20.6. MS (ESI) Calcd for $C_{16}H_{13}N_2O_2^+$ $[M+H]^+$: 265. Found: 265. *Anal.* Calcd for $C_{16}H_{12}N_2O_2$: C, 72.72; H, 4.58; N, 10.60. Found: C, 72.68; H, 4.65; N, 10.81.

Benzo[*a*]rutaecarpine (8,15-Dihydrobenz[6′,7′]indolo-[2′,3′:3,4]pyrido[2,1-*b*]quinazolin-5(7*H*)-one, 3a) A solution of 7,8-dihydropyrido[2,1-*b*]quinazoline-6,11-dione (**1**, 110 mg, 0.5 mmol) and 1-hydrazino-naphthalene·HCl (95 mg, 0.6 mmol) in 95% EtOH was refluxed for 12 h to yield corresponding hydrazone as a yellow precipitate [56%, mp 227—232 °C: IR (KBr) ν 3066, 1704 (C=N), 1626, 1534, 1512 cm^{-1}. ^{1}H-NMR ($CDCl_3$, 250 MHz) δ: 15.29 (s, 1H, NH), 8.30 (d, 1H, *J*=8.0 Hz), 8.16 (d, 1H, *J*=8.5 Hz), 7.86 (d, 1H, *J*=8.5 Hz), 7.81—7.80 (m, 2H), 7.71 (t, 1H, *J*=7.5 Hz), 7.60 (ddd, 1H, *J*=8.0, 7.5, 1.0 Hz), 7.53—7.46 (m, 4H), 4.13 (t, 2H, *J*=5.7 Hz), 2.94 (t, 2H, *J*=5.7 Hz), 2.187 (m, 2H)], which was then treated with polyphosphoric acid (PPA) (10 ml) at 130 °C for 45 min. The resulting mixture was poured to ice-water to yield yellow needles (92 mg, 53%): mp 250—252 °C. ^{1}H-NMR ($CDCl_3$, 600 MHz) δ: 12.78 (s, 1H, NH), 8.87 (d, 1H, *J*=8.4 Hz), 8.18 (dd, 1H, *J*=8.4, 1.2 Hz), 7.96 (d, 1H, *J*=8.4 Hz), 7.83 (td, 1H, *J*=8.4, 1.2 Hz), 7.77 (d, 1H, *J*=8.4 Hz), 7.73 (d, 1H, *J*=9.0 Hz), 7.57 (td, 1H, *J*=8.4, 1.2 Hz), 7.54 (d, 1H, *J*=8.4 Hz), 7.51 (td, 1H, *J*=8.4, 1.2 Hz), 7.48 (td, 1H, *J*=8.4, 1.2 Hz), 4.50 (t, 2H, *J*=7.2 Hz), 3.26 (t, 2H, *J*=7.2 Hz). MS (ESI) Calcd for $C_{22}H_{16}N_3O$ $[M+H]^+$: 338, Found: 338. *Anal.* Calcd for $C_{22}H_{16}N_3O \cdot 1.0H_2O$: C, 74.35; H, 4.82; N, 11.82. Found: C, 74.50; H, 4.85; N, 11.75.

Benzo[*c*]rutaecarpine (8,15-Dihydrobenz[4′,5′]indolo-[2′,3′:3,4]pyrido[2,1-*b*]quinazolin-5(7*H*)-one, 3b) A solution of **1** (1.06 g, 5.0 mmol) and 2-hydrazinonaphthalene·HCl (95 mg, 6.0 mmol) in 95% EtOH was refluxed for 12 h to yield corresponding hydrazone as a yellow precipitate [mp 219—220 °C: IR (KBr) ν 3066, 1704 (C=N), 1626, 1534, 1512 cm^{-1}. ^{1}H-NMR (DMSO-d_6, 250 MHz) δ: 11.51 (s, 1H, NH), 8.22 (overlapped d, 2H, *J*=9.1 Hz), 8.21 (s, 1H), 8.11—7.96 (m, 3H), 7.90—7.81 (m, 2H), 7.68 (t, 1H, *J*=7.5 Hz), 7.52 (t, 1H, *J*=7.5 Hz), 7.41 (t, 1H, *J*=7.5 Hz), 4.16 (t, 2H, *J*=5.7 Hz), 2.89 (t, 2H, *J*=5.7 Hz), 2.18 (m, 2H)], which was then treated with PPA (10 ml) at 180 °C for 45 min. The resulting mixture was poured to ice-water to yield needles (98%): mp >300 °C (lit.[29] mp 298 °C. ^{1}H-NMR (DMSO-d_6, 600 MHz) δ: 12.45 (s, 1H, NH), 8.29 (d, 1H, *J*=8.4 Hz), 8.17 (dd, 1H, *J*=7.8, 1.2 Hz), 7.97 (d, 1H, *J*=8.4 Hz), 7.82 (td, 1H, *J*=7.8, 1.5 Hz), 7.74 (d, 1H, *J*=9.0 Hz), 7.70 (d, 1H, *J*=7.8 Hz), 7.66 (d, 1H, *J*=9.0 Hz), 7.60 (t, 1H, *J*=7.8 Hz), 7.47 (t, 1H, *J*=7.8 Hz), 7.46 (t, 1H, *J*=7.8 Hz), 4.52 (t, 2H, *J*=7.2 Hz), 3.60 (t, *J*=2H, 7.2 Hz). ^{13}C-NMR (DMSO-d_6, 150 MHz) δ: 160.66, 147.68, 145.39, 136.23, 134.54, 129.30, 128.98, 128.81, 126.69, 126.57, 126.38, 126.19, 125.79, 125.50, 123.69, 123.03, 120.62, 119.56, 118.45, 21.50 (two C's).

Benzo[*m*]rutaecarpine (3d) Method A: To a solution of ketone **12** (246 mg, 1.0 mmol)) in 95% EtOH (20 ml) was slowly added freshly distilled phenylhydrazine (**2a**, 140 mg, 1.3 mmol). The resulting precipitate was collected and mixed with polyphosphoric acid (5 g) in a heavy-walled beaker. The mixture was heated at 130 °C for 1.5 h. After cooling, the mixture was made basic with 10% NaOH and extracted with CH_2Cl_2 (3×50 ml). The combined organic layers were washed with water and dried over anhydrous $MgSO_4$. Evaporation of the solvent gave a solid material, which was recrystallized from EtOAc to provide the desired product as pale yellow needles (219 mg, 65%): mp 327 °C. IR (KBr) ν 3340 (N–H), 1655 (C=O) cm^{-1}. ^{1}H-NMR (250 MHz, DMSO-d_6) δ: 11.93 (s, 1H), 8.89 (s, 1H), 8.22 (d, *J*=8.3 Hz, 1H), 8.21 (s, 1H), 8.13 (d, *J*=8.3 Hz, 1H), 7.69—7.66 (m, 2H), 7.60—7.49 (m, 2H), 7.28 (t, *J*=8.2 Hz, 1H), 7.11 (t, *J*=8.3 Hz, 1H), 4.50 (t, *J*=7.0 Hz, 2H), 3.21 (t, *J*=7.0 Hz, 2H). MS (ESI) Calcd for $C_{22}H_{16}N_3O$ $[M+H]^+$: 338, Found 338. *Anal.* Calcd for $C_{22}H_{15}N_3O \cdot H_2O$: C, 74.35; H, 4.82; N, 11.82. Found: C, 74.26; H, 4.89; N, 11.87.

Method B: A mixture of ketone **12** (246 mg, 1.0 mmol) and phenylhydrazine **2a** (140 mg, 1.3 mmol) in glacial acetic acid (15 ml) was refluxed for 1.5 h. The resulting mixture was cooled to room temperature and poured to a mixture of ice : water (1 : 1) to give precipitate, which was recrystallized from EtOAc to give the desired product (45%) of which the spectral data were identical to those from Method A.

Benzo[*a*]benzo[*m*]rutaecarpine (3e) A solution of **12** (130 mg, 50 mmol) and 1-hydrazinonaphthalene·HCl (95 mg, 60 mmol) in 95% EtOH was refluxed for 12 h to yield a yellow precipitate (190 g, 96%) [mp >300 °C, ^{1}H-NMR (DMSO-d_6, 250 MHz) δ: 11.54 (s, 1H, NH), 9.00 (s, 1H), 8.68 (s, 1H), 8.33 (d, 1H, *J*=7.8 Hz), 8.26 (d, 1H, *J*=2.1 Hz), 8.21 (d, 1H, *J*=7.8 Hz), 8.10 (dd, 1H, *J*=9.0, 2.1 Hz), 7.99 (d, 1H, *J*=9.0 Hz), 7.90 (overlapped d, 2H, *J*=8.5 Hz), 7.81 (t, 1H, *J*=7.8 Hz), 7.70 (td, 1H, *J*=8.5, 1.0 Hz), 7.53 (td, 1H, *J*=8.5, 1.0 Hz), 7.42 (d, 1H, *J*=8.5 Hz), 4.20 (t, 2H, *J*=6.0 Hz), 2.91 (t, 2H, *J*=6.0 Hz), 2.21 (quintet, 2H, *J*=6.0 Hz)], which was then treated with PPA (10 ml) at 150 °C for 45 min. The resulting mixture was poured to ice-water to yield needles: mp >300 °C, which is not soluble in any of organic solvent for ^{1}H-NMR.

Benzo[*c*]benzo[*m*]rutaecarpine (6f) A solution of **12** (1.32 g, 5 mmol) and 2-hydrazinonaphthalene·HCl (0.95 g, 6 mmol) in 95% EtOH was refluxed for 12 h to yield yellow precipitate, which was then treated with PPA (10 ml) at 150 °C for 45 min. The resulting mixture was poured to ice-water to yield needles (1.14 g, 59%): mp 234—236 °C. ^{1}H-NMR ($CDCl_3$, 250 MHz) δ: 15.02 (s, 1H, NH), 8.70 (s, 1H), 8.20 (s, 1H), 8.04 (overlapped d, 2H, *J*=7.8 Hz), 7.84 (d, 1H, *J*=8.5 Hz), 7.80 (d, 1H, *J*=8.5 Hz), 7.77 (d, 1H, *J*=8.5 Hz), 7.77—7.54 (m, 4H), 7.44 (t, 1H, *J*=7.8 Hz), 7.32 (t, 1H, *J*=7.8 Hz), 4.17 (t, 2H, *J*=6.0 Hz), 2.95 (t, 2H, *J*=6.0 Hz). MS (ESI) Calcd for $C_{26}H_{18}N_3O$ $[M+H]^+$: 388, Found 388. *Anal.* Calcd for $C_{25}H_{17}N_3O \cdot 1.5H_2O$: C, 75.35; H, 4.86; N, 10.14.

Found: C, 75.47; H, 4.96; N, 10.15.

DNA Relaxation Assay of Topoisomerase I The test compounds were dissolved in dimethyl sulfoxide (DMSO) at 10 mM as stock solution. The activity of DNA topoisomerase I was determined by assessing the relaxation of supercoiled DNA pBR322. The mixture of 100 ng of plasmid pBR322 DNA and 0.4 units of recombinant human DNA topoisomerase I (TopoGEN INC., U.S.A.) was incubated without and with the prepared compounds at 37 °C for 30 min in the relaxation buffer (10 mM Tris–HCl (pH 7.9), 150 mM NaCl, 0.1% bovine serum albumin, 1 mM spermidine, 5% glycerol). The reaction in the final volume of 10 μl was terminated by adding 2.5 μl of the stop solution containing 5% sarcosyl, 0.0025% bromophenol blue, and 25% glycerol. DNA samples were then electrophoresed on a 1% agarose gel at 15 V for 7 h with a running buffer of TAE. Gels were stained for 30 min in an aqueous solution of ethidium bromide (0.5 μg/ml). DNA bands were visualized by transillumination with UV light and were quantitated using AlphaImager™ (Alpha Innotech Corp., U.S.A.).

DNA Relaxation Assay of Topoisomerase II The mixture of 100 ng of supercoiled pBR322 plasmid DNA and 0.2 units of human DNA topoisomerase IIa (Amersham, U.S.A.) was incubated without and with the prepared compounds in the assay buffer (10 mM Tris–HCl (pH 7.9) containing 50 mM NaCl, 5 mM $MgCl_2$, 1 mM ethylenediaminetetraacetic acid (EDTA), 1 mM ATP, and 15 μg/ml bovine serum albumin) at 37 °C for 30 min. The reaction in a final volume of 10 μl was terminated by addition of 3 μl of 7 mM EDTA. Reaction products were analyzed on a 1% agarose gel at 25 V for 4 h with a running buffer of TAE. Gels were stained for 30 min in an aqueous solution of ethidium bromide (0.5 μg/ml). DNA bands were visualized by transillumination with UV light and supercoiled DNA was quantitated using AlphaImager™ (Alpha Innotech Corp., U.S.A.).

RESULTS AND DISCUSSION

Among a variety of synthetic methods for rutaecarpine,[16—20,24] the Fischer indole synthesis from the corresponding hydrazone of 6,7,8,9-tetrahydropyrido[2,1-*b*]quinazoline-6,11-dione (**1**)[24] and phenylhydrazine (**2a**) has been used as a simple and practical method and employed for preparing derivatives of rutaecarpines on ring E.[25] Benzo-annulation on ring E can, thus, be achieved by employing the ketone **1** and commercially available 1- and 2-hydrazinonaphthalenes.

Reactions of **1** with 1- and 2-hydrazinonaphthalene-HCl (**2b**, **c**) afforded the corresponding hydrazones, which were then subjected to Fischer indole synthesis condition to give benzo[*a*]rutaecarpine (**3a**) and benzo[*c*]rutaecarpine (**3b**) in 54 and 62% yield, respectively. Although the product of a one-step or two-step reaction of **1** with **2b** unambiguously is benzo[*a*]rutaecarpine (**3a**) as a sole product, the structure of the product from reaction of **1** with **2c** needs further investigation.

It should be noted that the previous Fischer indole products from **2c** are in controversy. The early Fischer indole synthesis of **2c** with cyclohexanone[30] afforded an angular 3*H*-benz[*e*]indole derivative, 8,9,10,11-tetrahydro-7*H*-benzo[*c*]-carbazole (**5**), instead of a linear 1*H*-benz[*f*]indole derivative, 2,3,4,5-tetrahydro-1*H*-benzo[*b*]carbazole (**6**).[31] The same reactions from cyclopentanone[32] and naltrexone,[33] a cyclohexanone derivative, with **2c**, however, afforded linear 1*H*-benz[*f*]indole derivatives **7** and **8** as a sole product, respectively.

Although the previous reaction of 6,6-dibromo-6,7,8,9-tetrahydropyrido[2,1-*b*]quinazoline-11-one and 2-naphthyldiazonium chloride, followed by Fischer indole synthesis was claimed to afford an angular benzo[*e*]indole derivative **3b** (mp 298 °C) as a sole product, the ^{1}H-NMR spectral data given did not afford any structural information.[29] The ^{1}H-

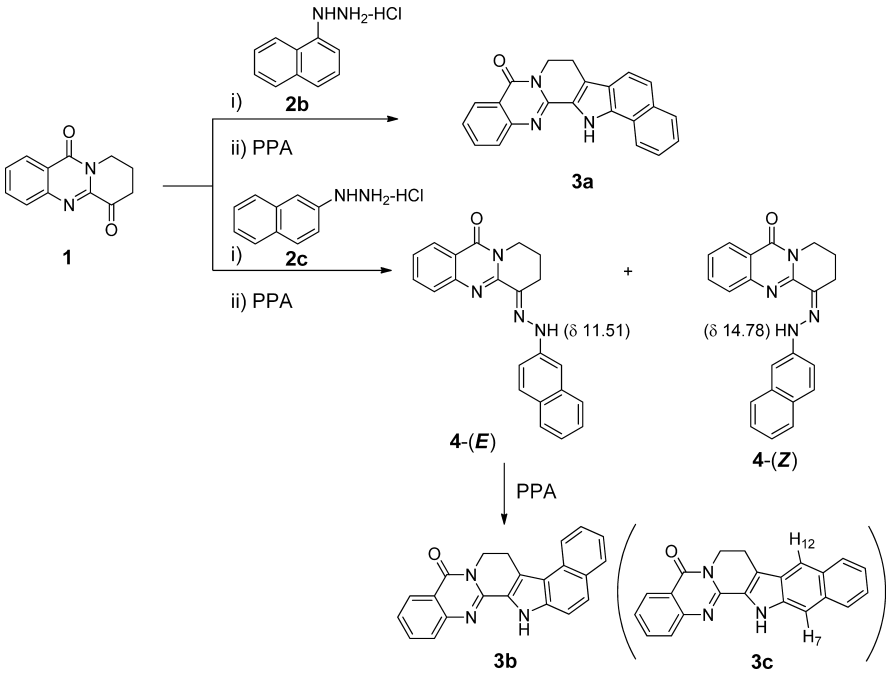


Chart 1

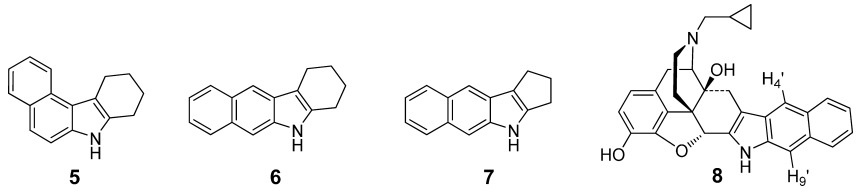


Fig. 2. Products of Fischer Indole Synthesis from 2-Hydrazinonaphthalene

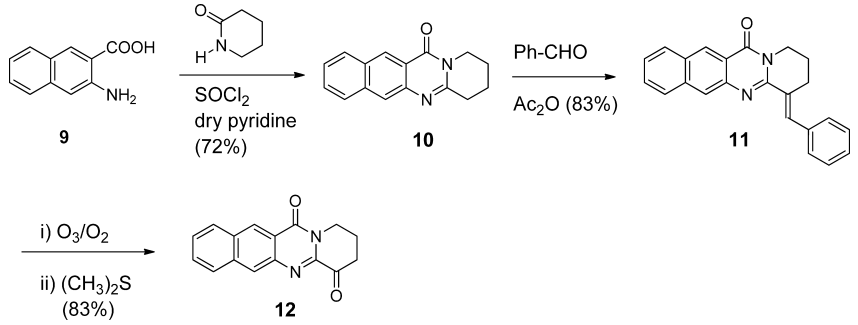
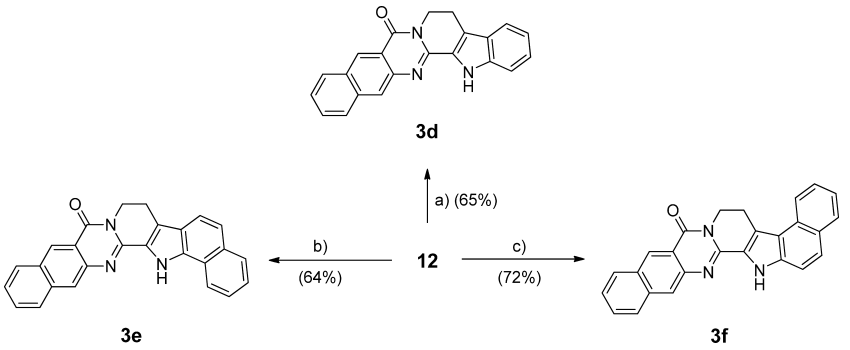


Chart 2



Key: a) i) phenylhydrazine (**2a**), ii) PPA; b) i) 1-hydrazinonaphthalene HCI (**2b**), ii) PPA; c) i) 2-hydrazinonaphthalene HCI (**2c**), ii) PPA

Chart 3

NMR (600 MHz) spectrum of **3b** showed 6 doublets and 4 triplets in the aromatic region, which were enough to eliminate a linear structure **3c**, in which two singlets for H7 and H12 should be apparent. These results are consistent with the reactivity of C1 position of the naphthalene ring. Since the frontier electron populations on C1 is 1.6 times high compared to those of C2,[34,35] the 3,3-sigmatropic rearrangement of the hydrazones would lead to C1 attack instead of C3 to result in **3b** instead of their linear congener **3c**.

To introduce a benzo-annulation on ring A, a benzo-fused derivative of **1** such as 2,3-dihydro-1*H*-benzo[*g*]-pyrido[2,1-*b*]quinazoline-4,12-dione (**12**) can be a starting material. Prerequisite starting compound **10** was, thus, prepared in 72% yield from 3-amino-2-naphthoic acid (**9**) and 2-piperidone in $SOCl_2$ by employing one-pot synthesis method for various 2,3-polymethylene-4(3*H*)-quinazolinones from anthranilic acid and lactams.[17] Reaction of **10** with benzaldehyde in the presence of acetic anhydride[24] or NaOAc in HOAc[36,37] afforded the corresponding benzylidene-derivative **11**, which was subjected to ozonolysis and followed by reductive work up with $(CH_3)_2S$ to yield **12** in 83% yield.

The reaction of **12** with phenylhydrazine (**2a**) afforded the corresponding hydrazone in quantitative yield, which was not fully characterized but instead subjected to Fischer indole synthetic condition to give benzo[*m*]rutaecarpine (**3d**) in 65% yield. In the same manner, reactions of **12** with **2b** and **2c** resulted in two regioisomers benzo[*a*]benzo[*m*]rutaecarpine (**3e**) and benzo[*c*]benzo[*m*]rutaecarpine (**3f**) in 64 and 72% yield, respectively.

It should be additionally noted that reactions of ketones (**1**, **12)** with hydrazines (**2**) in refluxing 95% EtOH afforded the corresponding hydrazones in good yields, of which ^{1}H-NMR spectra showed the presence of two regioisomers such as **4**-(*E*) and **4**-(*Z*) in a ratio of 4—6 : 1. The mixture of two isomeric hydrazones was not separated, but instead directly subjected to Fischer indole synthesis in PPA to afford the desired products.

Cytotoxicity Cytotoxicities of compounds **3a**—**d** and **3f** were screened by the method[38] previously described against selected human cancer cell lines such as human breast adenocarcinoma cell line (MCF7), human prostate tumor (DU-145), human colorectal adenocarcinoma on tumor (HCT15), and human chronic myelogenous leukemia cell line (HL60). Cell cytotoxocities of **3a**—**d** and **3f** on HEK293 cells (embryonic kidney cell line) were also evaluated for comparison.

IC_{50} values of compound **3** ranged from 9.94 to 36.55 μM

Table 1. Cytotoxicities of Rutaecarpine and Benzorutaecarpines

Compounds	Cell lines (IC_{50}, μM)[a]				
	HEK293	MCF7	DU-145	HCT-116	K562
Rutaecarpine	>50	19.57±1.29	31.65±2.23	33.89±0.75	25.77±0.56
3a	36.57±2.64	11.42±0.54	27.54±0.96	9.94±0.96	25.38±1.60
3b	>50	16.94±4.11	36.55±0.89	24.16±1.59	31.42±0.54
3d	>50	12.25±1.86	30.14±0.36	15.78±2.90	25.63±0.68
3e[b]	—	—	—	—	—
3f	>50	12.06±1.67	29.64±0.31	25.36±2.82	28.39±0.34
Adriamycin	3.09±0.20	3.25±0.13	1.44±0.10	1.34±0.29	1.11±0.01
Etoposide	4.09±0.15	3.02±0.15	3.75±0.79	1.88±0.14	2.23±0.28
Camptothecin	5.16±0.02	4.22±0.06	3.22±0.18	0.38±0.02	1.05±0.04

a) Each data point represents mean±standard deviation, from three different experiments performed in triplicate. Cell lines used are described in the text. *b*) Not soluble enough to produce meaningful values.

Table 2. Inhibition of Camptothecin (CPT), Etoposide, Rutaecarpine, and **3** against Topoisomerases I and II

	Reference	Rutaecarpine	**3a**	**3b**	**3d**	**3f**
Topo I inhibition at 20 μM[a]	29.46	NA[c]	NA	8.12	NA	21.47
Topo I inhibition at 100 μM[a]	52.52	1.20	NA	48.09	0.46	28.11
Topo II inhibition at 20 μM[b]	39.68	NA	8.34	5.88	NA	33.59
Topo II inhibition at 100 μM[b]	63.41	1.25	45.38	3.52	45.92	52.74

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.

as shown in Table 1 while IC_{50} values on HEK293 cells were >50 μM except **3a** (36.57 μM). Although a meaningful selectivity between the cell lines was not observed, benzo-annulation on ring A of rutaecarpine led to slight increase of cytotoxicity (rutaecarpine *vs.* **3d**, **3f**) and benzo-annulation on ring E led to 2-fold increase of activity (rutaecarpine *vs.* **3a**) for HCT15 cell line.

DNA Topoisomerase Inhibitory Activity Topoisomerases I and II (topo I and II) inhibitory activities of the compounds prepared were measured by assessing the relaxation of supercoiled pBR 322 plasmid DNA employing the method previously described.[39]

Although rutaecarpine did not show any inhibitory activity against topo I and II, benzo-annulation on ring A increased inhibitory activity against topo II up to that of etoposide while benzo-annulation on ring E increased activity against topo I similar to that of CPT. Inhibitory activities of rutaecarpine and its benzo-analogs against DNA topoisomerases are not directly related to the corresponding cytotoxicities, which imply that additional targets can be involved in their cytotoxic mechanisms of action.

In conclusion, a series of benzo-annulated rutaecarpines were prepared from anthranilic acid and 3-aminonaphthalene-2-carboxylic acid by Fischer indole synthesis as key reaction. Cytotoxicity was somewhat increased by the introduction of benzo-annulation, which was not directly related to the inhibitory activity against topo I and II. Benzo-annulation on ring A led to significant increase of inhibitory activity against topo II while annulations on ring E increased inhibitory activity against topo I.

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