Bioorganic & Medicinal Chemistry 20 (2012) 4962–4967

Contents lists available at SciVerse ScienceDirect

Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc

Synthesis of benzo-annulated tryptanthrins and their biological properties

Jing Lu Liang [a], So-Eun Park [b], Youngjoo Kwon [b], Yurngdong Jahng [a,*]

[a] College of Pharmacy, Yeungnam University, Gyeongsan 712-749, Republic of Korea
[b] College of Pharmacy & Division of Life & Pharmaceutical Sciences, Ewha Womans University, Seoul 120-750, Republic of Korea

ARTICLE INFO

Article history:
Received 30 May 2012
Accepted 19 June 2012
Available online 2 July 2012

Keywords:
Tryptanthrin
Benzotryptanthrin
Topoisomerase I
Topoisomerase II
Isatin
3-Amino-2-naphthoic acid
Isatoic anhydride
Cytotoxicity

ABSTRACT

A series of benzo-annulated derivatives of tryptanthrin were prepared and their optical and redox properties were studied. Tryptanthrin and its benzo-annulated derivatives showed selective inhibitory activity on topo I with an increase of activity on topo II by benzo-annulation on quinazolin-4(3*H*)-one moiety. Although the benzo-annulation on quinazolin-4(3*H*)-one ring did not affect significantly on the inhibitory activities against topo I and II, the benzoannulation on indolin-3-one ring affected the inhibitory activity very much especially by linear annulation. Cytotoxicities were not significantly changed upon benzoannulation, which were not directly related either to the inhibitory activities against topo I and II or to the reduction potentials.

1. Introduction

Tryptanthrin (**1a**) was first reported as a sublimation product of indigo under reduced pressure[1] and later was isolated as an indoloquinazoline alkaloid from the culture of the yeast *Candida lipolytica*[2] and continuously from petroleum extract of dried and powdered fruit of *Couroupita guaianensis* Abul.,[3] and other higher plant sources.[4] Higher plants include Chinese traditional medicine 'Qing Dai' (*Isatis indigotica*) which has long been used as an anti-inflammatory agent. Isolated tryptanthrin showed various biological properties such as antifungal (MIC = 5 μg/mL),[5] antimicrobial (MIC's of 3.1–6.3 μg/mL),[6] tuberculostatic (MIC = 10 μg/mL),[7] COX-2 inhibitory (IC_{50} = 64 nM),[8] 5-LOX inhibitory (IC_{50} = 0.15 μM),[9] NO synthase inhibitory,[10] cytotoxic (10 μM for HT-1376),[11] and antimalarial[12] activities, and apoptosis by downregulation of glutathione *S*-transferase(GST)π-expression.[13]

In addition, tryptanthrin has two characteristic reduction potentials (−0.75 and −1.40 V vs SCE) for the reduction of two carbonyl groups,[14] thus having potentials for photoelectronic photoreceptor.[15] The more facile electron transfer to the oxygen atom of the five-membered ring could be a plausible path in the mechanism of action indicating that electron accepting ability of the carbonyl atoms is crucial for the antileishmanial activity of tryptanthrin.[14] Electron accepting ability also plays an important role in the antitumor activity for mitomycin C[16] and adriamycin.[17]

Such a variety of intriguing physicochemical and biological properties have led continuous efforts not only to find other plant sources[18] but also to trigger the development of new methods for the total synthesis[19] as well as cultivation methods.[20]

Although a series of tryptanthrin derivatives were reported, the introduction of substituents was limited only to the two external benzene rings.[21] Our interest in derivatization of simple alkaloids as well as in the effect of benzoannulation on the chemical[22] and/or biological properties[23] spurred us to prepare a series of benzotryptanthrins and examine their physicochemical as well as biological properties focusing on the relationships between the reduction potentials, inhibitory activities on topoisomerases I and II and cytotoxicities against selected human cancer cell lines.

2. Results and discussion

2.1. Chemistry

A series of benzotryptanthrins were prepared by employing previously reported method[19j] shown below. One-pot reaction of isatin (**2a**) and its benzo-analogues (**2b–d**)[24–26] with anthranilic acid (**3a**) and 3-amino-2-naphthoic acid (**3b**) in the presence of $SOCl_2$, respectively, afforded the corresponding tryptanthrin (**1a**) and benzotryptanthrins (**1b–h**) in 51–84% yields.

* Corresponding author.
E-mail addresses: ydjahng@yumail.ac.kr, ydjahng@yu.ac.kr (Y. Jahng).

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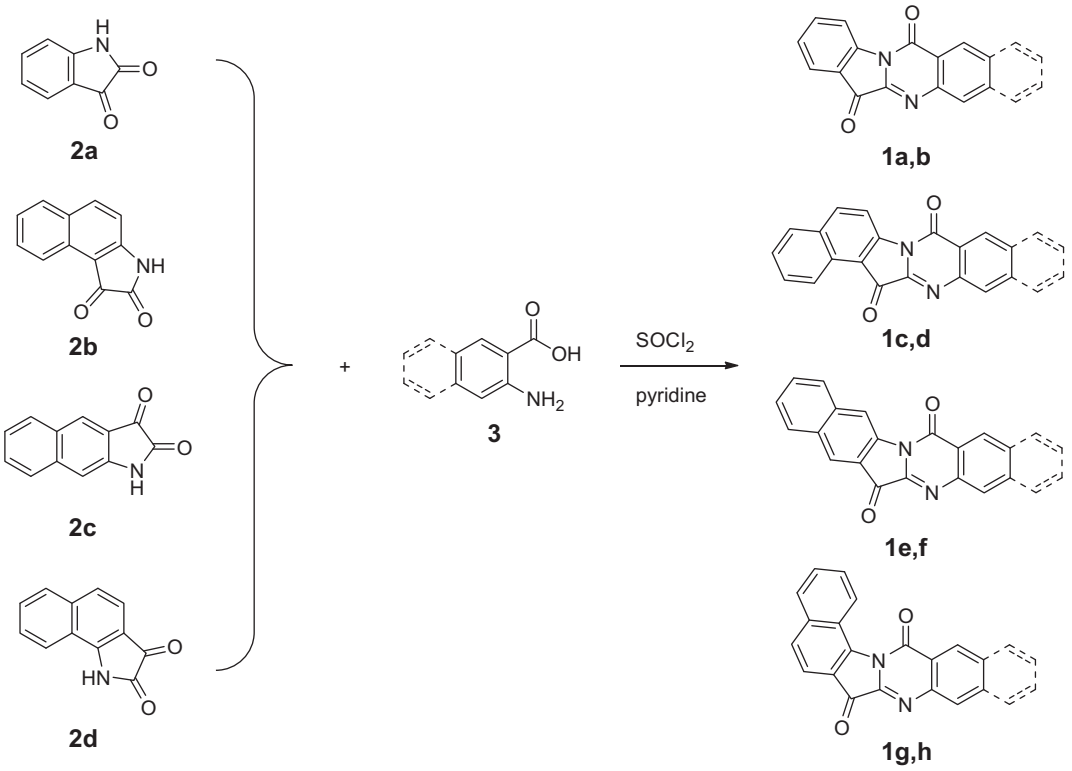
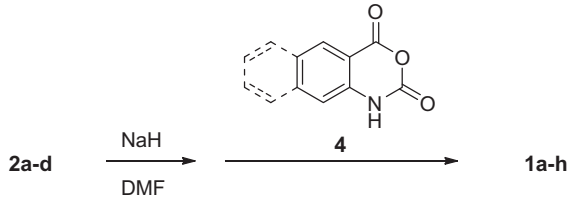
http://dx.doi.org/10.1016/j.bmc.2012.06.034



Alternatively, anions generated from isatin (**2a**) and its benzo-annalogues (**2b–d**) were condensed with isatoic anhydride (**4a**) and its benzo-annalogue (**4b**),[27] respectively, to afford the corresponding tryptanthrin (**1a**) and benzotryptanthrins (**1b–h**) in somewhat lower yields. The prerequisite **4b** were readily prepared by reaction of 3-amino-2-napthoic acid with bis(trichloromethyl) carbonate (triphosgene).



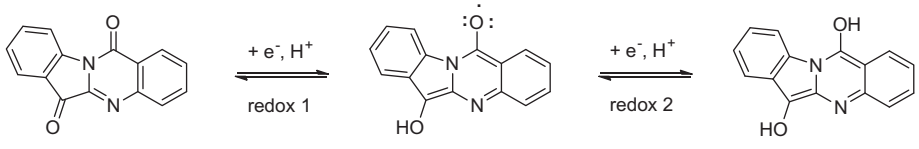
2.2. Properties

2.2.1. UV absorption

UV absorption spectra (Fig. 1) were obtained from EtOH (2.5×10^{-5} M) and the absorption maxima and extinction coefficients are summarized in Table 1. Four distinct absorption bands are observed in the region of 225–230, 245–255, 280–375, and 375–420 nm which correspond quite closely to the π–π^* absorptions. The intensity of this band increases considerably for the more linear compound where only a weak shoulder can be observed at 500 nm. These data are consistent with an electronic transition state in which the energy of the receptor π^* orbital is lowered by increasing delocalization which would be found for the more conjugated and linear systems. The electronic spectrum of the most linear **1f** showed a π–π^* absorptions band at 293 nm (ε 41,000) which is red-shifted from the parent tryptanthrin by 42 nm with little effect on the intensity of the absorption from the parent **1a**. It should be noted that benzoannulation led an additional absorption band in the region of 320–400 nm, of which **1d** showed the most intense and bathochromic shifted absorption due to the additional benzene ring which is comparable to those of **1f** and **1h** although **1f** is the most linear one.

2.2.2. Cyclic voltammetry

Cyclic voltammetry has been one of the efficient and powerful electrochemical methods to provide reliable quantitative assessment of electron transfer ability of the molecules.[28] Reduction potentials of the compounds prepared were measured using CV-50 W Voltammetric Analyzer by previously reported methods and are summarized in Table 2. In every case, two reversible reduction waves in the region of −0.64 to −0.76 V and −1.29 to −1.46 V versus SCE were observed. Camptothecin (CPT) also showed two cathodic reduction peaks at −0.73 and −0.93 V for the ester carbonyl and lactam carbonyl, respectively, which are well matched to those in the literature,[29] while etoposide (ETO) did not show any reduction potentials. The first reduction potentials covering the carbonyl in the five-membered ring were less negative by 0.07–0.11 V compared to that of tryptanthrin while the second for the carbonyl in the six-membered ring by 0.04–120 mV. Such a shift indicates that annulation of the benzene ring results in easier reduction compared to the parent tryptanthrin. The most planar linear **1f** showed the lowest reduction potentials −0.64 and −1.18 V, respectively.



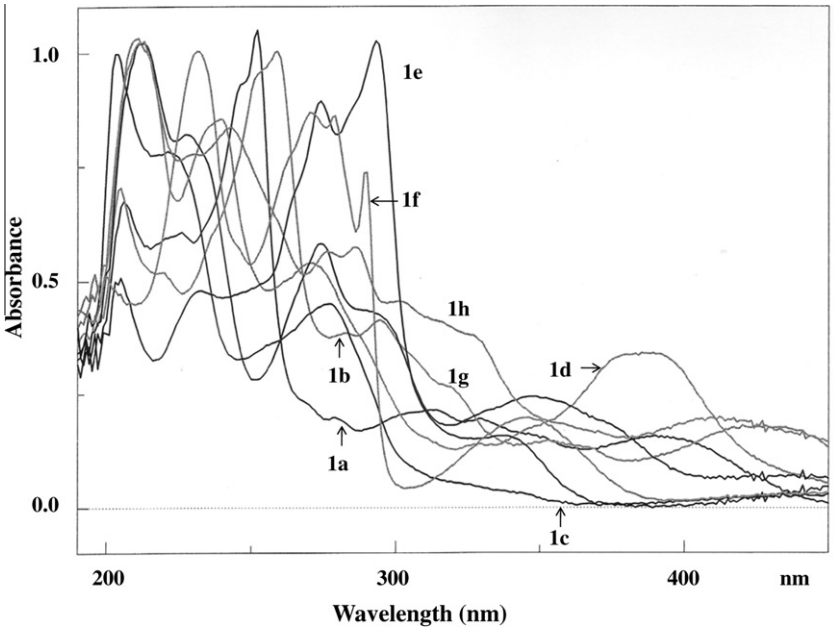


Figure 1.

Table 1
UV absorption data for tryptanthrin and its benzo-annulated derivatives

Compd	λ_{max} (ε, $cm^{-1}\,M^{-1}$) (2.5×10^{-5} mol in EtOH)
1a	225 (31,900), 251 (47900), 279 (7,900), 310 (13,900), 330 (8900), 398 (7200)
1b	220 (28,200), 259 (40,100), 284 (15,400), 294 (16,600), 353 (5900), 425 (7100)
1c	222 (31,200), 277 (18,000), 465 (1300)
1d	239 (34,200), 271 (21,600), 327 (5600), 389 (13,600)
1e	231 (40,100), 270 (34,500), 290 (29,500), 346 (7900), 469 (1500)
1f	232 (20,300), 232 (19,200), 274 (35,700), 293 (41,000), 339 (6300), 485 (2600)
1g	227 (32,800), 274 (23,200), 291 (17,300), 346 (9700), 440 (2700)
1h	230 (31,100), 243 (33,500), 276 (22,600), 286 (22,900), 302 (18,200), 328 (15,000), 412 (8000)

Table 2
Reduction potentials for tryptanthrin and its benzoannulated-derivatives in CH_3CN (0.1 M TBAP) at 25 °C

Compd	$E_{1/2}$ V (vs SSCE)		Compound	$E_{1/2}$ V (vs SSCE)	
	Reduction 1	Reduction 2		Reduction 1	Reduction 2
1a	−0.75 (75)[a]	−1.40 (73)	**1f**	−0.64 (74)	−1.18 (75)[b]
1b	−0.74 (73)	−1.36 (75)	**1g**	−0.64 (76)	−1.42 (73)
1c	−0.68 (75)	−1.40 (76)	**1h**	−0.64 (76)	−1.34 (75)
1d	−0.67 (73)	−1.35 (73)	Camptothecin	−0.73[b]	−0.93[b]
1e	−0.64 (74)	−1.21 (74)	Etoposide	Not observed	

[a] Numbers in parentheses are the differences between the cathodic and anodic potentials and reported in mV.
[b] Potentials for cathodic peak, anodic peak was not observed in CV.

Although redox properties of tryptanthrin and its benzo-annulated derivatives were not directly related to the biological properties, the more readily reducible **1e** and **1f** showed somewhat stronger cytotoxicity against selected cancer cell lines.

2.2.3. Topoisomerase-inhibitory activity

Inhibitory activities of the compounds **1** against topoisomerases I (topo I) and II (topo II) were evaluated as compared to topo I selective inhibitor, camptothecin and topo II selective inhibitor, etoposide by assessing the relaxation of supercoiled pBR 322 plasmid DNA employing previously described method,[30] and results are summarized in Table 3. The parent tryptanthrin showed strong selectivity on topo I of which the activity is comparable to that of CPT at the 100 μM level. Although the benzo-annulation did not change the inhibitory activities on topo I, the activities on topo II were significantly increased especially benzo-annulation on the indolin-3-one ring (**1d**, **e**, and **h**).

2.2.4. Cytotoxicities

Cytotoxicities of the compounds prepared were screened by the method[31] previously described against selected human cancer cell lines such as ductal breast epithelial tumor cell line (T47D), colorectal adrenocarcinoma on tumor (HCT15), prostate tumor (DU145), and embryonic kidney 293 cells (HEK293) as compared

Table 3
Inhibitory activity of **1** against topoisomerases I and II and their cytotoxicities against selected human cancer cell lines

Compd	Inhibitory activity (%)				Cytotoxicity [IC_{50} (μM)]			
	Topo I		Topo II		T47D	HCT15	DU145	HEK293
	100 μM	20 μM	100 μM	20 μM				
1a	68.44	4.09	4.54	N/T	14.29 ± 0.17	8.49 ± 0.02	6.22 ± 0.04	4.11 ± 0.11
1b	18.79	N/T	15.96	N/T	23.08 ± 0.87	25.76 ± 0.03	26.02 ± 0.01	31.52 ± 0.66
1c	47.79	4.90	8.13	N/T	31.31 ± 0.52	24.69 ± 0.09	27.67 ± 0.09	25.73 ± 0.02
1d	39.10	3.80	71.26	5.83	25.22 ± 0.46	32.78 ± 0.14	34.03 ± 0.35	24.22 ± 0.88
1e	69.56	5.40	75.93	5.95	21.50 ± 0.50	6.15 ± 0.23	17.75 ± 0.21	10.69 ± 0.03
1f	41.85	3.60	12.60	N/T	25.68 ± 0.01	26.21 ± 0.01	26.09 ± 0.01	2.31 ± 0.24
1g	53.41	4.10	14.04	N/T	22.75 ± 0.12	12.63 ± 0.13	21.39 ± 0.27	22.14 ± 0.72
1h	84.13	3.50	53.45	19.05	34.93 ± 0.77	23.96 ± 0.17	32.28 ± 0.04	20.32 ± 0.61
CPT	63.10	26.30			9.17 ± 1.12	9.92 ± 0.76	9.29 ± 0.71	7.94 ± 2.02
ETO			78.91	36.08	1.21 ± 0.13	0.28 ± 0.03	1.42 ± 0.19	0.41 ± 0.11
ADR					1.82 ± 0.68	1.76 ± 0.15	1.57 ± 0.22	1.00 ± 0.05

to those of adriamycin (ADR), and the results are summarized in Table 3. The parent tryptanthrin (**1a**) showed strong cytotoxicities against all the cancer cell lines while a benzo-annulation did not lead any significant increase of activity except **1e** and **1f** against especially HCT15 and HEK293, respectively.

3. Conclusions

In conclusion, a series of benzotryptanthrins were prepared by either one-pot reaction of isatin and benzoisatins with anthranilic acid and 3-amino-2-naphthoic acid or condensation of isatin and benzoisatins with isatoic anhydride and benzoisatoic anhydride in 34–84% yields. Tryptanthrin and its benzo-annulated derivatives showed somewhat selective inhibitory activity on topo I with an increase of activity on topo II by benzo-annulation on quinazolin-4(3*H*)-one moiety. Although the benzo-annulation on quinazolin-4(3*H*)-one ring did not affect significantly on the inhibitory activities against topo I and II, the benzoannulation on indolin-3-one ring affected the inhibitory activity very much especially by linear annulation. Cytotoxicities were not significantly changed upon benzoannulation, which were not directly related either to the inhibitory activities against topo I and II or to the reduction potentials.

4. Experimental

Melting points were determined using a Fischer-Jones melting points apparatus and are not corrected. UV absorption spectra were recorded on a JASCO-V550 spectrophotometer. IR spectra were obtained using a Perkin–Elmer 1330 spectrophotometer. NMR spectra were obtained using a Bruker-250 spectrometer 250 MHz or 600 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. The solvent (CH_3CN) used for cyclic voltammetry was dried and distilled over P_2O_5. Elemental analyses were taken on a Hewlett–Packard Model 185B elemental analyzer. Reduction potentials were measured using on a CV-50 W Voltammetric Analyzer with a C2 cell stand (Bioanalytical Systems, West Lafayette, IN, USA). The starting 1*H*-benzo[*e*]indole-1,2(3*H*)-dione (**2b**),[24] 1*H*-benzo[*g*]indole-2,3-dione (**2c**),[25] and 1-acetylbenz[5,6]isatin 2-oxime[26] were prepared by employing previously reported methods.

4.1. Chemistry

4.1.1. 1*H*-Benzo[*f*]indole-2,3-dione (2d)

A mixture of 1-acetylbenz[5,6]isatin 2-oxime (300 mg.1.18 mmol) in acetone (20 mL) and HCl (6 mL 1:1) was refluxed for 2 h, then evaporated in vacuo. The resulting residue was treated with H_2O, gives the desired product (165 mg, 71%) as red cubes or orange leaflets: mp 299–300 °C (lit.[26] mp 299–300 °C). Unreported spectral data were as follows: ^{1}H NMR (250 MHz, DMSO-d_6) δ 10.43 (br s, 1H, NH), 8.28 (s, 1H, H4), 7.98 (d, J = 8.0 Hz, 1H, H5), 7.80 (d, J = 8.0 Hz, 1H, H8), 7.53 (td, J = 8.0, 1.2 Hz, 1H, H6), 7.33 (td, J = 8.0, 1.0 Hz, 1H, H7), 7.19 (s, 1H, H9), 2.32 (s, 3H). ^{13}C NMR (62.5 MHz, DMSO-d_6) δ 183.93, 145.25, 145.03, 138.49, 130.96, 129.61, 128.07, 126.94, 126.12, 123.92, 122.14, 105.41.

4.1.2. 2*H*-Naphtho[2,3-*d*][1,3]oxazine-2,4(1*H*)-dione (4b)

To a stirred solution of 3-amino-2-naphthoic acid (1.12 g, 6.0 mmol) in CH_3CN (100 mL) at 50–55 °C were added dropwise pyridine (0.95 g, 12.0 mmol) and a solution of triphosgene (0.59 g, 2.0 mmol) in CH_2Cl_2 (20 mL) at the same time. After completion of the addition, the temperature of the reaction mixture was maintained at 50–55 °C for an additional 4 h. The solvent was removed under reduced pressure and water (50 mL) was added to the residue. The precipitated solid collected by filtration was washed with water followed by chilled dichloromethane, and dried in a vacuum dryer to yield 1.19 g (93%) of **2** as orange powder: mp 396 °C. (lit.[27] mp >290 °C). Unreported spectral data are as follows: ^{1}H NMR ($CDCl_3$, 250 MHz) δ 11.81 (s, 1H, NH), 8.74 (s, 1H), 8.13 (d, 1H, J = 8.3 Hz), 7.95 (d, 1H, J = 8.3 Hz), 7.66 (ddd, 1H, J = 8.3, 8.0, 1.3 Hz), 7.50 (ddd, 1H, J = 8.3, 8.0, 1.3 Hz), 7.49 (s, 1H), ^{13}C NMR (62.5 MHz, $CDCl_3$) δ 159.87, 146.84, 136.93, 135.90, 131.67, 130.11, 129.62, 128.81, 126.82, 125.41, 110.96, 110.37.

4.1.3. Benzo[*g*]indolo[2,1-*b*]quinazoline-6,14-dione (1b, benzo[*k*]tryptanthrin)

4.1.3.1. Method A. To a solution of 3-amino-2-naphthoic acid (0.10 g, 0.53 mmol) and **2a** (74 mg, 0.50 mmol) in dry pyridine (20 mL) was added $SOCl_2$ (0.2 mL). The resulting mixture was refluxed for 1 h and then poured to ice-water. Reaction mixture was made basic with saturated NH_4OH and the precipitate formed was collected to give the desired compound (125 mg, 84%): mp 317 °C. (lit.[32] mp >300 °C). Unreported spectral data are as follows: ^{1}H NMR ($CDCl_3$, 250 MHz) δ 9.00 (s, 1H), 8.65 (d, 1H, J = 8.3 Hz), 8.53 (s, 1H), 8.12–8.04 (m, 2H), 7.92 (dd, 1H, J = 7.5, 1.0 Hz), 7.80 (td, 1H, J = 8.3, 1.3 Hz), 7.73–7.64 (m, 2H), 7.41 (td, 1H, J = 8.3, 0.8 Hz). MS (ESI) calcd for $C_{19}H_{11}N_2O_2$ $[M+H]^+$ 299, found 299.

4.1.3.2. Method B. A solution of isatin (**2a**, 0.10 g, 0.68 mmol) in dry DMF (5 mL) was slowly added to a mixture of NaH (20 mg, 0.69 mmol) in dry DMF (7 mL). To the resulting purple solution was added compound **3b** (0.15 g, 0.70 mmol). The reaction mixture was stirred for 12 h and heated at 50 °C for 30 min. The yellow

precipitate formed was collected and washed with CH_3OH to give the desired product (0.11 g). Concentration of the filtrate afforded an additional product (0.05 g, 81% overall): mp 317 °C. Spectral data are identical to those described above in Method A.

4.1.4. Benzo[4,5]indolo[2,1-*b*]quinazoline-8,14-dione (1c, benzo[*c*]tryptanthrin)

4.1.4.1. Method A. Yellow needles (57%): mp 298 °C. ^{1}H NMR ($CDCl_3$, 250 MHz) δ 8.96 (d, 1H, *J* = 8.8 Hz), 8.80 (d, 1H, *J* = 8.8 Hz), 8.43 (dd, 1H, *J* = 8.0, 1.5 Hz), 8.27 (d, 1H, *J* = 8.8 Hz), 8.04 (d, 1H, *J* = 7.5 Hz), 7.90 (d, 1H, *J* = 8.3 Hz), 7.83 (td, 1H, *J* = 8.0, 1.0 Hz), 7.74 (td, 1H, *J* = 8.3, 1.3 Hz), 7.65 (td, 1H, *J* = 8.0, 1.3 Hz), 7.56 (td, 1H, J = 8.0, 1.3 Hz). ^{13}C NMR ($CDCl_3$, 62.5 MHz) δ 182.32, 157.94, 148.90, 146.67, 144.05, 140.04, 135.15, 131.86, 131.19, 130.76, 130.20, 128.86 (two C's), 127.66, 127.18, 124.29, 123.78, 116.00, 114.98. MS (ESI) calcd for $C_{19}H_{11}N_2O_2$ $[M + H]^+$ 299, found 299. Anal. calcd for $C_{19}H_{10}N_2O_2$ C, 76.50; H, 3.38; N, 9.39. Found C, 76.38; H, 3.41; N, 9.39.

4.1.5. Benzo[*g*]benzo[4,5]indolo[2,1-*b*]quinazoline-8,16-dione (1d, dibenzo[*c,k*]tryptanthrin)

4.1.5.1. Method A. Yellow powder (59%): mp >400 °C. ^{1}H NMR ($CDCl_3$, 250 MHz) δ 9.06 (s, 1H), 8.87 (dd, 1H, *J* = 7.5, 1.3 Hz), 8.78 (d, 1H, *J* = 7.5 Hz), 8.61 (s, 1H), 8.54 (d, 1H, *J* = 7.5 Hz), 8.35 (dd, 1H, *J* = 7.5, 1.3 Hz), 8.24 (dd, 1H, *J* = 7.5,1.3 Hz), 8.15 (d, 1H, *J* = 9.0 Hz), 7.86 (td, 1H, *J* = 7.5, 1.0 Hz), 7.77 (td, 2H, *J* = 8.3, 1.3 Hz), 7.67 (td, 1H, *J* = 7.5, 1.3 Hz). MS (ESI) calcd for $C_{23}H_{13}N_2O_2$ $[M+H]^+$ 439, found 439. Anal. calcd for $C_{23}H_{12}N_2O_2$ C, 79.30; H, 3.47; N, 8.04. Found C, 79.48; H, 3.41; N, 7.99.

4.1.6. Benzo[5,6]indolo[2,1-*b*]quinazoline-6,14-dione (1e, benzo[*b*]tryptanthrin)

4.1.6.1. Method A. Yellow powder (51%): mp 286 °C. ^{1}H NMR ($CDCl_3$, 250 MHz) δ 9.00 (s, 1H), 8.65 (d, 1H, *J* = 7.8 Hz), 8.52 (s, 1H), 8.12–8.03 (m, 2H), 7.79 (td, 1H, *J* = 7.8, 1.3 Hz), 7.69–7.64 (m, 2H), 7.41 (t, 1H, *J* = 7.5 Hz). MS (ESI) calcd for $C_{19}H_{11}N_2O_2$ $[M+H]^+$ 299, found 299. Anal. calcd for $C_{19}H_{10}N_2O_2$ C, 76.50; H, 3.38; N, 9.39. Found C, 76.53; H, 3.35; N, 9.34.

4.1.7. Benzo[*g*]benzo[5,6]indolo[2,1-*b*]quinazoline-7,15-dione (1f, dibenzo[*b,k*]tryptanthrin)

4.1.7.1. Method A. Yellow powder (54%): mp >400 °C. ^{1}H NMR (DMSO-d_6, 600 MHz) δ 9.09 (s, 1H), 8.94 (s, 1H), 8.66 (s, 1H), 8.61 (s, 1H), 8.36 (d, 1H, *J* = 7.8 Hz), 8.24 (d, 1H, *J* = 9.0 Hz), 8.23 (d, 1H, *J* = 9.0 Hz), 8.19 (d, 1H, *J* = 8.4 Hz), 7.80–7.73 (m, 3H), 7.63 (td, 1H, *J* = 7.8, 1.2 Hz). MS (ESI) calcd for $C_{23}H_{13}N_2O_2$ $[M+H]^+$ 439, found 439. Anal. calcd for $C_{23}H_{12}N_2O_2$ C, 79.30; H, 3.47; N, 8.04. Found C, 79.34; H, 3.46; N, 8.09.

4.1.8. Benzo[6,7]indolo[2,1-*b*]quinazoline-7,13-dione (1g, Benzo[*a*]tryptanthrin)

4.1.8.1. Method A. Yellow powder (54%): mp 297 °C. ^{1}H NMR ($CDCl_3$, 250 MHz) δ 9.11 (d, 1H, *J* = 7.5 Hz), 8.43 (d, 1H, *J* = 7.5 Hz), 7.99 (d, 1H, J = 7.5 Hz), 7.90–7.79 (m, 4H), 7.71–7.60 (m, 3H). MS (ESI) calcd for $C_{19}H_{11}N_2O_2$ $[M+H]^+$ 299, found 299. Anal. calcd for $C_{19}H_{10}N_2O_2$ C, 76.50; H, 3.38; N, 9.39. Found C, 76.47; H, 3.40; N, 9.41.

4.1.9. Benzo[*g*]benzo[6,7]indolo[2,1-*b*]quinazoline-7,15-dione (1h, dibenzo[*a,k*]tryptanthrin)

4.1.9.1. Method A. Yellow powder (56%): mp >400 °C. ^{1}H NMR ($CDCl_3$, 250 MHz) δ 9.07 (s, 1H), 8.98 (d, 1H, *J* = 7.5 Hz), 8.57 (s, 1H), 8.32 (d, 1H, *J* = 7.5 Hz), 8.24 (d, 1H, *J* = 7.5 Hz), 8.13 (d,1H, *J* = 7.5 Hz), 8.06 (d, 1H, *J* = 7.5 Hz), 7.86 (d, 1H, *J* = 7.5 Hz), 7.81 (d, 1H, *J* = 7.5 Hz), 7.74–7.70 (m, 3H). MS (ESI) calcd for $C_{23}H_{13}N_2O_2$ $[M+H]^+$ 439, found 439. Anal. calcd for $C_{23}H_{12}N_2O_2$ C, 79.30; H, 3.47; N, 8.04. Found C, 79.36; H, 3.49; N, 8.02.

4.2. Cyclic voltammetry

A glassy carbon working, a silver–silver chloride reference electrode and a platinum auxiliary electrode were used in a 5 mL glass cell. All samples were dissolved in dry CH_3CN (dried over P_2O_5) with 0.1 M tetrabutylammonium hexafluorophosphate (Aldrich) as the supporting electrolyte. The stock solution with each tryptanthrin analogue was prepared at a concentration of 1 mM and was degassed with nitrogen for 5 min prior to analysis. Samples were run at several scans ranging from 20 to 1000 mV/s.[33] Cyclic voltammograms were recorded using A Hewlett–Packard X–Y recorder. Halfwave potentials ($E_{1/2}$) were measured as the average of the anodic and cathodic peak potentials.

4.3. Biology

4.3.1. DNA relaxation assay of topoisomerase I

The test compounds were dissolved in 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 0.2 units of calf thymus DNA topoisomerase I (Fermentas, USA) was incubated without and with the prepared compounds at 37 °C for 30 min in the relaxation buffer (35 mM Tris–HCl (pH 8.0), 72 mM KCl, 5 mM $MgCl_2$, 5 mM dithiothreitol, 2 mM spermidine, 0.01% bovine serum albumin). The reaction in the final volume of 10 μL was terminated by adding 2.5 μL of the stop solution containing 10% SDS, 0.2% bromophenol blue, 0.2% xylene cyanol and 30% 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 Corporation).

4.3.2. 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, USA) 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 EDTA, 1 mM ATP, and 15 μg/mL bovine serum albumin) for 30 min at 37 °C. The reaction in a final volume of 10 μL was terminated by the addition of 3 μL of 7 mM EDTA. Reaction products are 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 Corporation).

Acknowledgements

Financial support from Korean Science Foundation (KRF-2008-521-E00189) and Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology (2010-0002646) are gratefully acknowledged.

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