Original Article

Cytotoxicity and reversal of multidrug resistance by tryptanthrin-derived indoloquinazolines

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Aim: To evaluate the effects and elucidate the mechanisms of a series of indoloquinazolines as novel anticancer agents.

Methods: Condensation of the substituted isatoic anhydride with the substituted isatin was performed to prepare compounds 1–4, followed by adding malononitrile to prepare compounds 5–7. Cytotoxicity was measured by MTT assays. Apoptosis induction was evaluated using DNA fragmentation, cell cycle assay, caspase 3/7 activity and Western blot.

Results: Compounds 3, 4, and 5 display cytotoxicity against MCF-7, HeLa, SKOV3, and A498 cancer cells. DNA ladders appear in cells treated with compounds 3, 4, and 5. Within those, compound 4 exhibits the greatest activity in regards to sub-G1 accumulations in the cell cycle and the activation of caspase-3/7. Furthermore, Fas and Fas ligand levels are elevated by compound 4, implying that the apoptosis is in part mediated through the signals. On the other hand, compounds 1 and 7 display chemosensitizing activity since cytotoxicity of doxorubicin and etoposide is enhanced in combination with compound 1 and 7, respectively, in MCF-7/adr (doxorubicin-resistant) and MCF-7/vp (etoposide-resistant).

Conclusion: The cytotoxicity of indoloquinazolines is structure-dependent rather than cell type-dependent due to the similar degree of cytotoxicity induced by the individual compounds in all four cell lines. Further modification of the tryptanthrin skeleton is important to develop novel anticancer agents bearing either cytotoxicity against MCF-7 cells or drug resistance reversal in MCF-7/adr and MCF-7/vp.

Keywords: apoptosis, cytotoxicity, drug resistance, MCF-7, tryptanthrin

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Introduction

In the last decade, basic cancer research has produced remarkable advances in our understanding of cancer biology and cancer genetics. Among the most important advances is that apoptosis and the genes that regulate it have a profound effect on the malignant phenotype. For example, it is now clear that some oncogenic mutations interrupt apoptosis, leading to tumor initiation, progression or metastasis. Finally, it is now well documented that most cytotoxic anticancer agents induce apoptosis, raising the possibility in apoptotic programs.

Apoptosis is a form of programmed cell death that plays a fundamental role in many normal biological processes as well as several disease states. Apoptosis can be induced by various stimuli that all produce the same end result: systematic and deliberate cell death[3]. Recent developments strongly impli-
P-glycoprotein and the phenotype of MDR in tissue culture models[9]. Development of adjuvant agents to overcome multidrug resistance (MDR) becomes a new trend in cancer chemotherapy.

Indigo plants have been a familiar and important source of dye for Asians since ancient times. Honda et al first isolated an indoloquinazoline, tryptanthrin, from Indigo plant Strobilanthes cusia[10]. Tryptanthrin with chemical name indolo[2,1-b]quinazolin-6,12-dione was reported to carry anti-microbial activity against a variety of microorganisms including bacteria, yeasts, dermatophytes and phytopathogens[10, 11]. Recently, tryptanthrin has been paid much attention as an aryl hydrocarbon receptor agonist[12], anti-inflammatory agent[13], inducer of caspase-3/Fas mediated apoptosis[14], cytostatic agent[15] and drug resistance reversal agent[16]. In this study, a series of tryptanthrin derivatives were synthesized and designated as compounds 1–7. Anticancer and chemosensitizing activities were examined in MCF-7 cells. Results showed that compounds 3, 4, and 5 exhibited cytotoxicity at micromolar or lower concentrations. DNA fragmentation and cell cycle progression were determined by treating with compounds 3, 4, and 5. Changes in Fas/Fas ligand and caspase-3/7 activities reveal the anticancer mechanisms involving Fas-mediated apoptosis pathway. On the other hand, compounds 1 and 7 which are not (or mild) cytotoxic are capable of sensitizing drug-resistant MCF-7 cells.

Materials and methods

Chemicals

Synthesis of 6,12-dihydro-6,12-dioxoindolo-[2,1-b]quinazoline (trypanthrin) and its analogues were as described with modifications[15, 17]. As outlined in Scheme 1, compounds 1–4 were prepared by condensation of the substituted isatoic anhydride with the substituted isatin in N-methylmorpholine, N,N'-diisopropyl carbodiimide and pyridine. The solution of N,N'-diisopropyl carbodiimide in anhydrous pyridine was added with the substituted isatoic anhydride and N-methylmorpholine to increase solubility. The resulting solution was added with the substituted isatoic anhydride and substituted isatin, then subjected to reflux at 120–130 °C for 6 h. Compounds 5–7 containing dicyanomethylene substituent were made as showed in Scheme 2 by treating substituted 6-hydroxyindolo-2,1-b quinazoline derivatives with malononitrile in DMSO or NaH/THF, and then stirring for 5 h. The structures of tryptanthrin and its analogues were determined by 1H and 13C NMR spectra (Bruker AX-400, AX-300 spectrometer, Billerica, MA). The substituents of the tryptanthrin skeleton were systematically modified as in Schemes 1 and 2 (Figure 1), and tabulated in Table 1.

Cell lines and cell culture

MCF-7/wt, MCF-7/adr and MCF-7/vp cell lines were provided by Dr Chih-hsin YANG (National Taiwan University Hospital, Taiwan, China). HeLa, A498, and SKOV3 cell lines were purchased from ATCC (http://www.atcc.org). Cells were maintained in DMEM medium with 10% fetal calf serum and 100 ng/mL penicillin and streptomycin (Invitrogen, Carlsbad, CA) at 37 °C in 5% CO2. MCF-7/adr and MCF-7/vp cells were grown in the presence of 0.1 μmol/L of doxorubicin or etoposide that was removed from the medium 1 week before each assay.

Cell survivability assay

To detect growth inhibition by the series of compounds, cells were seeded onto 96-well plates at a density of 5×10^3/well before compound treatment. Cytotoxicity of the series of compounds in a variety of cell lines was determined using the MTT assay after incubation of cells with these compounds at various concentrations for 3 d. IC₅₀ (the concentration of 50% inhibition of cell growth) was determined by interpolation of the dose-response curves.

DNA fragmentation

MCF-7/wt cells were plated at 4×10^5 cells/well onto each of 6-well plates. After overnight growth, cells were treated with tryptanthrons for various time periods. DNA was extracted by a Wizard® Genomic DNA Purification Kit (Promega, Madison, WI) and then dissolved in TE buffer. The purified DNA was quantified and equally loaded onto a 2% agarose gel for electrophoresis. The resulting bands were stained with ethidium bromide and visualized under UV light.

Cell cycle analysis

Cells were treated with various concentrations of tryptanthrin derivatives for 1 d, and then harvested by 0.25% trypsin and washed with PBS. Cells (2×10^5) were fixed in 70% ice-cold EtOH/PBS for 20 min on ice, and then washed with PBS and incubated in PI solution (69 mmol/L PI, 388 mmol/L sodium citrate, 100 μg/mL RNase A). Then the cells were analyzed immediately using a FACS Caliber (Beckton Dickinson, USA).

Caspase 3/7 assay

Caspase 3/7 activity was measured using the Caspase-glo 3/7 assay kit (Promega, Madison, WI). After treatment with tryptanthrins at different time points, the cells were incubated...
with Caspase-glo reagent for 1 h in the dark, and the luminescence was measured using a luminometer (Berthold Technologies, Bad Wildbad, Germany). Equal amount of cells were analyzed by counting a parallel set of cells and determining the total cell number for each sample.

Western blot analysis

The MCF-7 cells were treated with 10⁻⁶ mol/L tryptanthrins for various time periods. At the end of each treatment, the cells were trypsinized from the subconfluent monolayers, and their proteins were extracted using CelLytic™ M Cell Lysis Reagent (Sigma-Aldrich, St Louis, MO). The protein concentration of each sample was determined using a Bio-rad protein assay kit (Bio-Rad Laboratories, Hercules, CA). Total cellular proteins (80 μg/lane) were electrophoresized on 10% SDS-polyacrylamide gels and transferred to a PVDF membrane (GE Healthcare, Little Chalfont, Buckinghamshire, UK). Proteins were labeled with primary antibody: anti-Fas B-10 and anti-Fas Ligand (Santa Cruz, Santa Cruz, CA). Immunoreactive bands were detected by anti-mouse HRP or anti-rabbit peroxidase-conjugated secondary antibody (Chemicon, Temecula, CA). Proteins were visualized via enhanced chemiluminescence (ECL detection kit, GE Healthcare, Buckinghamshire, UK).

Statistical analysis

Data are presented as mean±SD for the indicated number of separate experiments. Comparisons between groups were analyzed via Student’s t-tests. Probability values of P<0.05 are considered statistically significant.

Results

Effect of tryptanthrin derivatives on inhibition of proliferation in several cancer cell lines

Tryptanthrin-derived indoloquinazolines were synthesized by condensation. Compounds 1–4 were prepared via Scheme 1 and compounds 5–7 containing dicyanomethylene substituent were made via Scheme 2. Growth inhibition of the series of compounds at 1 μmol/L was measured in MCF-7, HeLa, A498, and SKOV3 cells (Table 1). Compounds 3–5 demonstrate higher than 40% inhibition of proliferation in four cell lines. The IC₅₀ of compounds 3–5 is lower than 1×10⁻⁶ mol/L in four cell lines among which A498 and MCF-7 are more sensitive to the compounds. Compound 4 is the most potent tryptanthrin derivative presenting cytotoxic activity. The IC₅₀ is 3.10×10⁻⁷ mol/L in A498 and 3.9×10⁻⁷ mol/L in MCF-7, respectively (Table 2).

Apoptotic biomarkers in MCF-7 cells treated with compounds 3–5

DNA fragmentation is a characteristic feature of apoptosis. Fragmented DNA, subjected to electrophoresis on an agarose gel, is in a manner of ladders, suggesting the formation of nucleosomes in apoptosis progress. When MCF-7 cells were treated with compound 4 at 10⁻⁶ mol/L for 6 h, DNA ladders appeared. The intensity of the ladders significantly increased after the cells were longer exposed to compound 4 up to 9 h (Figure 2A). A delayed appearance (up to 40–44 h) of the DNA ladders was shown in cells treated compounds 3 or 5.

Cell cycle analysis of MCF-7 cells was performed after 24 h of incubation with compounds 3, 4, or 5 at 10⁻⁶ mol/L. As compared to the untreated cells, compounds 3 and 5 did not induce considerable cell cycle arrest, while an important sub-G₁ peak appeared in cells treated with compound 4 at 10⁻⁶ mol/L for 24 h (Figure 2B).

Activity of caspase-3/7 which is essential for apoptosis was determined in MCF-7 cells treated with compounds 3, 4, and 5. After exposure to compound 4 at 10⁻⁶ mol/L for 36 h, caspase-3/7 activity was elevated about five folds, compared to the control (Figure 2C). Only a slight increase in caspase-3/7 activity was observed in cells with compound 3 or 5.

Changes in Fas and Fas ligand levels in MCF-7 cells treated with compound 4

To verify if compound 4-induced apoptosis was mediated

Table 1. Structures and anticancer activities of tryptanthrin derivatives.

| Compound | R₁ | R₂ | X | R₃ | R₄ | Y | Growth Inhibition (%)<sup>a</sup> |
|----------|----|----|---|----|----|---|-------------------|
| 1        | H  | H  | C | H  | H  | O | 26 24 17 8          |
| 2        | OMe| OMe| C | H  | H  | O | 0   12 -8 6         |
| 3        | H  | H  | C | I  | H  | O | 40 48 68 47         |
| 4        | H  | H  | N | H  | H  | O | 77 46 78 50         |
| 5        | H  | H  | C | H  | H  | A<sup>b</sup> | 41 49 45 46       |
| 6        | OMe| OMe| C | H  | H  | A<sup>b</sup> | 17 3 5 8          |
| 7        | Cl  | H  | C | H  | H  | A<sup>b</sup> | 5 10 10 17       |
| Doxo-cl  | H  | H  | C | H  | H  | A<sup>b</sup> | 95 95 84 86       |

<sup>a</sup> Growth inhibition was calculated as the ratio of cell viability of the treated cells over the untreated cells. Data are representative of three independent experiments. The compounds tested were at the concentration of 1 μmol/L.

<sup>b</sup> A=NC-CN

Table 2. The IC₅₀ of compounds 3–5 (μmol/L).

| Cell line | Doxorubicin | Compound 3 | Compound 4 | Compound 5 |
|-----------|-------------|------------|------------|------------|
| MCF-7     | 0.14±0.03   | 1.50±0.28  | 0.39±0.31  | 1.40±0.12  |
| HeLa      | 0.59±0.02   | 1.86±0.21  | 2.34±0.36  | 1.26±0.17  |
| SKOV3     | 0.33±0.03   | 1.20±0.29  | 2.00±0.39  | 8.51±0.23  |
| A498      | 0.58±0.04   | 0.65±0.04  | 0.31±0.02  | 1.48±0.13  |

Cell viability was determined using the MTT assay. Data are presented as mean±SD of triplicate determinations.
through Fas/Fas ligand pathway, Fas and Fas ligand proteins were determined in MCF-7 cells treated with compound 4. Immunoblots against anti-Fas B-10 and anti-Fas Ligand showed that Fas level was raised within 2 h after cells treated with compound 4 and followed by an increase in Fas ligand level in cells after 4 h exposure to compound 4. The promptly elevated Fas did not sustain since a drop of Fas levels was detected in cells with 4–8 h exposure to compound 4 (Figure 3).

Figure 2. Apoptotic biomarkers induced by compounds 3, 4, and 5 in MCF-7 cells. (A) DNA fragmentation in MCF-7 cells treated with compounds 3, 4, and 5. DNA extracts from the cells treated with the compounds for various time periods as indicated were electrophoresed on a 2% agarose gel and stained with ethidium bromide. (B) The cell cycle analysis of MCF-7 cells with compounds 3, 4, and 5. DNA contents of MCF-7 cells were analyzed by flow cytometry. (C) Caspase 3/7 activity assays. MCF-7 cells were exposed to 10^{-6} mol/L compound 3 (●), 4 (○), or 5 (▼) for 0–36 h and subjected to caspase 3/7 assay. Assays were performed in triplicates for each treatment.

Table 3. MTT assays of tryptanthrin derivatives in MCF-7 and MCF-7/adr cells.

| Compound | IC_{50} (μmol/L) | Growth inhibition |
|----------|------------------|------------------|
|          | MCF-7             | MCF-7/adr        | Compounds | MCF-7/adr | Compounds+Dox | MCF-7 | MCF-7/adr |
| 1        | 8.23±0.37         | 5.63±0.53        | 26%       | 18%       | 95%           | 63%   |
| 2        | >100              | >100             | 0%        | 0%        | 99%           | 25%   |
| 6        | >10               | >10              | 17%       | 1%        | 97%           | 12%   |
| 7        | >100              | >100             | 5%        | 0%        | 92%           | 53%   |
| Dox      | 0.14±0.03         | 9.13±0.51        | 95%       | 19%       |               |       |

10^{-6} mol/L tryptanthrin derivatives and 10^{-6} mol/L doxorubicin were used for growth inhibition assays. Data are from three independent experiments.

Effect of tryptanthrin and its derivatives in drug-resistant MCF-7 cells

Instead of compounds 3–5 bearing cytotoxicity through activation of apoptosis process, the rest tryptanthrin derivatives, compounds 1, 2, 6, and 7, were not as potent as to inhibit cell proliferation. The IC_{50} of compound 1 was 8.23 μmol/L and compounds 2, 6, and 7 existed greater than 10 μmol/L of IC_{50} in MCF-7 cells, compared to doxorubicin of which the IC_{50} was 0.14 μmol/L in MCF-7 cells. In terms of non-cytotoxic agents, compounds 1, 2, 6, and 7 did not or slightly show growth inhibition in neither MCF-7 nor MCF-7/adr cells (Table 3). However, in combination with doxorubicin, compounds 1 and 7 at 10^{-6} mol/L enhanced growth inhibition activity of doxorubicin in MCF-7/adr cells from 19% to 63% and 53%, respectively.
Etoposide, which is also a cytotoxic agent, is capable of inducing drug resistance in MCF-7 cells, named MCF-7/vp. MCF-7/vp cells were still viable when the cells were treated with compound 1, 7, or etoposide, while the cells turned out to be sensitive to the combination of either compound 1 plus etoposide or compound 7 plus etoposide at concentrations of 1 μmol/L of each (Figure 4). Compound 7 raised a higher chemosensitizing activity than compound 1 in MCF-7/vp cells. On the contrary, MCF-7/adr cells are more susceptible to compound 1 for enhancing cytotoxicity of doxorubicin.

### Figure 4.
Effect of compounds 1 and 7 on sensitizing drug-resistant cells to doxorubicin or etoposide. Cells were treated with 10⁻⁶ mol/L doxorubicin (D), 10⁻⁶ mol/L etoposide (E), 10⁻⁵ mol/L verapamil (V), and 10⁻⁶ mol/L compound 1 (1) or 7 (7), either alone or in combination as indicated for 5 d. The percentage of cell viability of MCF-7 (□), MCF-7/adr (■), and MCF-7/vp (△) was determined by the MTT assay. Data are presented as mean±SD of triplicate determinations.

Similar to compounds 1 and 7, a known MDR reversing agent verapamil at 10⁻⁵ mol/L did not exist cytotoxicity though, it enhanced cytotoxicity of doxorubicin and etoposide in MCF-7/adr and MCF-7/vp, respectively, with various degrees.

### Discussion
Tryptanthrin and its derivatives have been reported to exhibit anticancer effects[15, 17]. In this study, we synthesized a series of tryptanthrin-derived indoloquinoxazolines, designated as compounds 1–7 and screened for anticancer activity. Compounds 1–4 were prepared by condensation as shown in Scheme 1 and compounds 5–7 containing dicyanomethylene substituents were made via Scheme 2. Among those, either compound 3 bearing an iodine substituent at R₃ or compound 4 with nitrogen containing A ring demonstrated effective growth inhibition activity. The oxygen or dicyanomethylene substitution at Y was indifferent to the cytotoxicity in four cell lines. In fact, the malononitrile derivatives, including compounds 5–7 with dicyanomethylene substitution at Y, exhibited various degree of cytotoxicity. Methoxy group at R₁ and R₂ or chlorine at R₁ did not influence the growth inhibition activity. According to structure-activity relationship analysis, modification of tryptanthrin skeleton with substituents in A ring or D ring enhances the cytotoxicity, except the dimethoxy substitution in A ring. Nitrogen-containing A ring seems to be important to increase cytotoxicity. The cytotoxicity is structure-dependent rather than cell line-dependent because a compound presents similar degree of cytotoxicity in all four cell lines. Tryptanthrin derivatives exhibited double-stranded DNA binding activity[17], which may in part account for structure-dependent cytotoxicity.

Although the tryptanthrin derivatives are not as potent as doxorubicin to kill the cancer cells, compounds 3–5 exhibit growth inhibition (40%–77%) activity in MCF-7, HeLa, A498, and SKOV3 cells. The apoptotic biomarkers, including DNA fragmentation, elevated sub-G₁ accumulation and an increase in caspase 3/7 activity, appear in the cells treated with compounds 3–5 with various intensities. Among them, compound 4 is of most importance. Mechanisms other than apoptosis may be involved in cell growth inhibition by compounds 3 and 5. The MCF-7 cell line which has been described to be Fas-sensitive[18] undergoes apoptosis through Fas/Fas ligand activation. The Fas/Fas ligand system is a key signaling transduction pathway of apoptosis in cells and tissues. Engagement of Fas by a Fas-ligand lead to the formation of a protein complex known as death-inducing signaling complex and permit acute execution of apoptosis by caspase-8 activation[19]. Reimer et al[20] reported that the selection process leading to highly aggressive breast tumor variants might be enhanced by Fas ligand-mediated tumor fratricide, eventually a possible target for novel therapeutic strategies. Our results show the consistent elevation of Fas ligand levels by treating compound 4 in different time periods (4 and 8 h), in spite of the various levels of Fas with time. Elevation of Fas ligand levels may be important to engage Fas and activate the Fas/Fas ligand-mediated apoptosis afterwards. Results may suggest that compound 4 resents cytotoxicity in part through Fas/Fas ligand pathway. It is possible of crosstalk between extrinsic pathway and intrinsic pathway through mitochondria. It would be worth examining if tryptanthrin derivatives induce mitochondria-related signals such as cytochrome c, caspase 9, etc. To differentiate activity of caspase 8 from caspase 9 enables us to confirm which pathway induced by compound 4. It would be interesting to check this issue in the future experiments.

Resistance to chemotherapy has been remaining a major cause of treatment failure in cancer patients. Development of adjuvant agents to circumvent MDR becomes a new trend in cancer chemotherapy. Many attempts, such as inhibition of MDR-related genes, to overcome MDR have been proposed[21]. Inhibition of MDR1 gene function, either by blocking P-gp function or inhibiting MDR1 gene expression, has been one of the most extensive studies[22, 28]. In this report, compounds 1 and 7 in combination with cytotoxic agents such as doxorubicin and etoposide enhance cytotoxicity of the cytotoxic agents against drug-resistant MCF-7 cells. It suggests that compounds 1 and 7 can sensitize drug-resistant cells to the cytotoxic agents, which may contribute to reverse multidrug resistance. It is still unclear the mechanisms involved in MDR reversal. As compound 1 largely sensitizes MCF-7/adr rather
than MCF-7/vp, it is speculated that compound 1 may act on MDRI gene or its function. Nevertheless, compound 7 sensitizes both MCF-7/adr and MCF-7/vp, implying either MDRI-related or MRP-related pathway is affected by compound 7.

In summary, tryptanthrin-derived indoloquinazolines synthesized in this study undergo multiple ways to display their anticancer activity via inducing cytotoxicity against MCF-7 cells by compounds 3–5 or evoking resistance reversal activity by compounds 1 and 7 in MCF-7/adr and MCF-7/vp cells. Further modification of the tryptanthrin skeleton, based on the structure-activity relationships sketched in this study, becomes important to develop novel anticancer agents bearing either cytotoxicity or resistance reversal activity.

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Author contribution
Yen-hui CHEN designed and corresponded to the study. Ji-wang CHERN and Hui-ting CHEN synthesized a series of indoloquinazoline compounds. Sung-tsai YU and Yi-fan CHIU performed the experiments. Sung-tsai YU, Tzer-ming CHEN, and Yen-hui CHEN analyzed data and prepared the manuscript.

References
1 Zakeri Z, Lockshin RA. Cell death: history and future. Adv Exp Med Biol 2008; 615: 1–11.
2 Debatin KM. Apoptosis pathways in cancer and cancer therapy. Cancer Immunol Immunother 2004; 53: 153–9.
3 Thorburn A. Death receptor-induced cell killing. Cell Signal 2004; 16: 139–44.
4 Nagata S, Golstein P. The Fas death factor. Science 1995; 267: 1449–56.
5 Hengartner MO. The biochemistry of apoptosis. Nature 2000; 407: 770–6.
6 Janicke RU, Sprengart ML, Wati MR, Porter AG. Caspase-3 is required for DNA fragmentation and morphological changes associated with apoptosis. J Biol Chem 1998; 273: 9357–60.
7 Ashkenazi A, Dixit VM. Death receptors: signaling and modulation. Science 1998; 281: 1305–8.
8 Liu X, Zou H, Slaughter C, Wang X. DFF, a heterodimeric protein that functions downstream of caspase-3 to trigger dna fragmentation during apoptosis. Cell 1997; 89: 175–84.
9 Luqmani YA. Mechanisms of drug resistance in cancer chemotherapy. Med Princ Pract 2005; 14: 35–48.
10 Honda G, Tabata M. Isolation of antifungal principle tryptanthrin, from Strobilanthes cusia O. Kuntze. Planta Med 1979; 36: 85–90.
11 Honda G, Tabata M, Tsuda M. The antimicrobial specificity of tryptanthrin. Planta Med 1979; 37: 172–4.
12 Schrenk D, Riebniger D, Till M, Vetter S, Fiedler H-P. Tryptanthrins: A novel class of agonists of the aryl hydrocarbon receptor. Biochem Pharmacol 1997; 54: 165–71.
13 Ishihara T, Kohn K, Ushio S, Iwaki K, Ikeda M, Kurimoto M. Tryptanthrin inhibits nitric oxide and prostaglandin E2 synthesis by murine macrophages. Eur J Pharmacol 2000; 407: 197–204.
14 Kimoto T, Hino K, Koya-Miyata S, Yamamoto Y, Takeuchi M, Nishizaki Y, et al. Cell differentiation and apoptosis of monocytic and promyelocytic leukemia cells (U-937 and HL-60) by tryptanthrin, an active ingredient of Polygonum tinctorium Lour. Pathol Int 2001; 51: 315–25.
15 Sharma VM, Prasanna P, Seshu KV, Renuka B, Rao CV, Kumar GS, et al. Novel indolo[2,1-b]quinazoline analogues as cytostatic agents: synthesis, biological evaluation and structure-activity relationship. Bioorg Med Chem Lett 2002; 12: 2303–7.
16 Yu ST, Chen TM, Tseng SY, Chen YH. Tryptanthrin inhibits MDR1 and reverses doxorubicin resistance in breast cancer cells. Biochem Biophys Res Commun 2007; 358: 79–84.
17 Chen GS, Bhagwat BV, Liao PY, Chen HT, Lin SB, Chem JW. Specific stabilization of DNA triple helices by indolo[2,1-b]quinazolin-6,12-dione derivatives. Bioorg Med Chem Lett 2007; 17: 1769–72.
18 Gibson S, Tu S, Oyer R, Anderson SM, Johnson GL. Epidermal growth factor protects epithelial cells against Fas-induced apoptosis. requirement for Akt activation. J Biol Chem 1999; 274: 17612–8.
19 Nagata S. Fas ligand-induced apoptosis. Annu Rev Genet 1999; 33: 29–55.
20 Reimer T, Herrnring C, Koczan D, Richter D, Gerber B, Kabelitz D, et al. Fasl: Fas ratio – a prognostic factor in breast carcinomas. Cancer Res 2000; 60: 822–8.
21 Gottesman MM, Ling V. The molecular basis of multidrug resistance in cancer: The early years of P-glycoprotein research. FEBS Lett 2006; 580: 998–1009.
22 Kimura Yasuhisa MS-yMMUK. Mechanism of multidrug recognition by MDR1/ABCB1. Cancer Sci 2007; 98: 1303–10.
23 Xu D, Hyunmin K, Michael F, Juliano RL. Strategies for inhibition of MDR1 gene expression. Mol Pharmacol 2004; 66: 268–75.