

Article

## Synthesis and Biological Evaluation of a $\gamma$ -Cyclodextrin-based Formulation of the Anticancer Agent 5,6,11,12,17,18,23,24-Octahydrocyclododeca[1,2-*b*:4,5-*b'*:7,8-*b''*:10,11-*b'''*]tetraindole (CTet)

Simone Lucarini <sup>1</sup>, Mauro De Santi <sup>2</sup>, Francesca Antonietti <sup>1</sup>, Giorgio Brandi <sup>2</sup>, Giuseppe Diamantini <sup>1</sup>, Alessandra Fraternale <sup>2</sup>, Maria Filomena Paoletti <sup>2</sup>, Andrea Tontini <sup>1</sup>, Mauro Magnani <sup>2</sup> and Andrea Duranti <sup>1,\*</sup>

<sup>1</sup> Dipartimento di Scienze del Farmaco e della Salute, Università degli Studi di Urbino “Carlo Bo” I-61029 Urbino, Piazza del Rinascimento 6, Italy; E-Mails: simone.lucarini@uniurb.it (S.L.); francesca.antonietti@uniurb.it (F.A.); giuseppe.diamantini@uniurb.it (G.D.); andrea.tontini@uniurb.it (A.T.)

<sup>2</sup> Dipartimento di Scienze Biomolecolari, Università degli Studi di Urbino “Carlo Bo” I-61029 Urbino, Via Aurelio Saffi 2, Italy; E-Mails: mauro.desanti@uniurb.it (M.D.S.); giorgio.brandi@uniurb.it (G.B.); alessandra.fraternale@uniurb.it (A.F.); maria.paoletti@uniurb.it (M.F.P.); mauro.magnani@uniurb.it (M.M.)

\* Author to whom the correspondence should be addressed; E-Mail: andrea.duranti@uniurb.it; Tel.: +39 0722 303323; Fax: +39 0722 303313.

Received: 21 April 2010; in revised form: 27 May 2010 / Accepted: 1 June 2010 /

Published: 4 June 2010

---

**Abstract:** 5,6,11,12,17,18,23,24-octahydrocyclododeca[1,2-*b*:4,5-*b'*:7,8-*b''*:10,11-*b'''*]tetraindole (CTet), an indole-3-carbinol (I3C) metabolite endowed with anticancer properties, is poorly soluble in the solvents most frequently used in biological tests. This study indicates that the use of  $\gamma$ -cyclodextrin ( $\gamma$ -CD) avoids this problem. Formulated with  $\gamma$ -CD CTet is a potent inhibitor of DNA synthesis in both estrogen receptor positive (MCF-7) and estrogen receptor negative (MDA-MB-231) human breast cell lines ( $IC_{50} = 1.20 \pm 0.04 \mu\text{M}$  and  $1.0 \pm 0.1 \mu\text{M}$ , respectively).

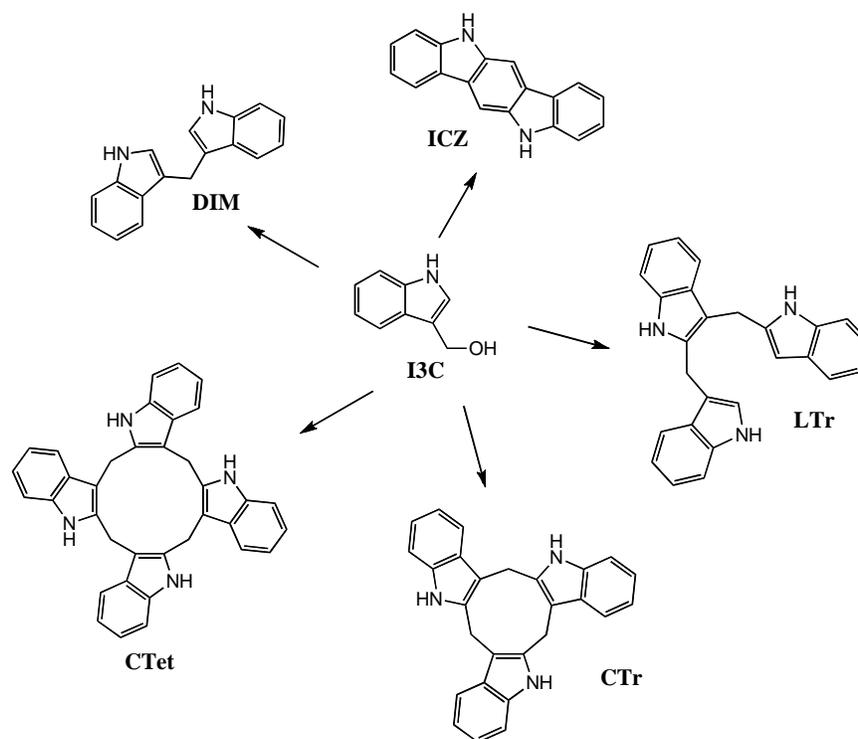
**Keywords:** indole-3-carbinol; indole cyclic tetramer;  $\gamma$ -cyclodextrin; breast cancer

---

## 1. Introduction

Edible cruciferous vegetables of the genus *Brassica* are endowed with chemopreventive and chemotherapeutic properties [1–3]. These actions depend on an autolysis product of 3-indolylmethyl glucosinolate (glucobrassicin), namely indole-3-carbinol (**I3C**, Figure 1) [4,5], and the resulting indole oligomers produced in the acidic environment of the stomach: 3,3'-diindolylmethane (**DIM**) [4,5], indolo[3,2-*b*]carbazole (**ICZ**) [6,7], the linear trimer **LTr** [6,8,9], the cyclic trimer **CTr** [6,8,10], and the cyclic tetramer **CTet** [11,12] (Figure 1) [2]. The current interest of pharmacologists and medicinal chemists in this topic has resulted in several reports which disclosed synthetic analogues of **I3C** [13–15], **DIM** [16–31], and **CTr** [32] possessing anticancer properties.

**Figure 1.** Chemical structures of compounds **I3C**, **DIM**, **ICZ**, **LTr**, **CTr**, and **CTet**.



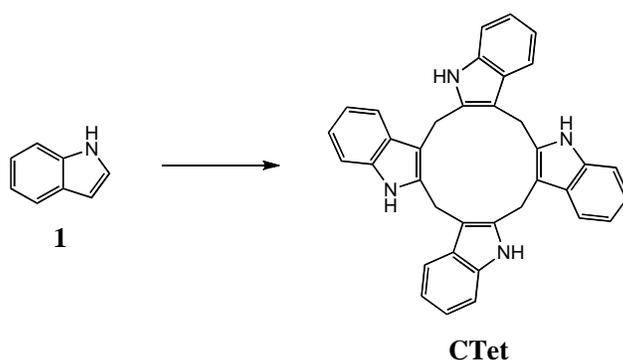
In order to study the antitumor effects of **CTet**, we needed a reliable and gram-scale synthesis of this compound. The methods reported in the literature for the preparation of **CTet** are three. The first involves the one-pot reaction of indole and formaldehyde in acidic methanol, and is in principle advantageous [33]. The second is in fact aimed at obtaining **CTr** and affords **CTet** as a by-product in low yield after recrystallization with DMSO; this method, employing gramine as a source in a basic environment of the presumed 3-methylene indolenine intermediate, is practical and efficient but not all its reagents could be utilized on a large scale synthesis because of their toxicity [34]. The third protocol utilizes **I3C** and acetic acid but the acidic conditions [8] and the purification by means of silica gel column chromatography lead to the formation of polymers and degradation products, so it is not possible to isolate pure **CTet** by recrystallization with methanol [12].

Unfortunately, **CTet** is poorly, if at all, soluble in the most common solvents, in particular those usually employed in biological experiments (acetone: 0.04, pyridine: 0.22, 2-butanol: 0.11, DMSO: 0.1% w/v). Furthermore, in chloroform, ethanol, methanol, and toluene **CTet** solubility is less than 0.1% w/v and the compound is insoluble in water and physiological saline solutions. Several procedures were therefore evaluated to increase **CTet** solubility in a pharmaceutically acceptable formulation. We found the approach with  $\gamma$ -cyclodextrin ( $\gamma$ -CD) promising, therefore it was selected for further investigation.

## 2. Results and Discussion

The synthesis of pure **CTet** was carried out by modifying Bergman *et al.*'s procedure [33]. When we applied this protocol, we repeatedly obtained results not congruent with the reported ones. In particular, the precipitate that separated from the mixture contained only a trace of the desired **CTet**, being instead constituted of numerous side-products, probably formed through polymerization processes. However, the filtrate of the reaction mixture did contain **CTet**, which was isolated by chromatography and recrystallization. In addition, HPLC analysis of the chromatographic fractions showing a single spot on TLC plates demonstrated that **CTet** was present together with **CTr**. Bergman's protocol was modified by prolonging reaction time, due to the presence of the starting material in the mixture after one hour, and by purifying the crude by two rapid passages through short aluminum oxide columns. **CTet** was finally obtained with a purity higher than 99% by recrystallization from acetone, rather than pyridine [33] and DMSO [34], to facilitate solvent removal. The protocol proved to be scalable, in that it was possible to run it using up to 150 mmol of indole (17.5 g); these experiments gave yields and **CTr/CTet** ratios comparable with those reported on a lower scale (amounts of reagents higher than those reported were not used) (Scheme 1).

**Scheme 1.** Synthesis of 5,6,11,12,17,18,23,24-octahydrocyclododeca[1,2-*b*:4,5-*b'*:7,8-*b''*:10,11-*b'''*]tetraindole (**CTet**).



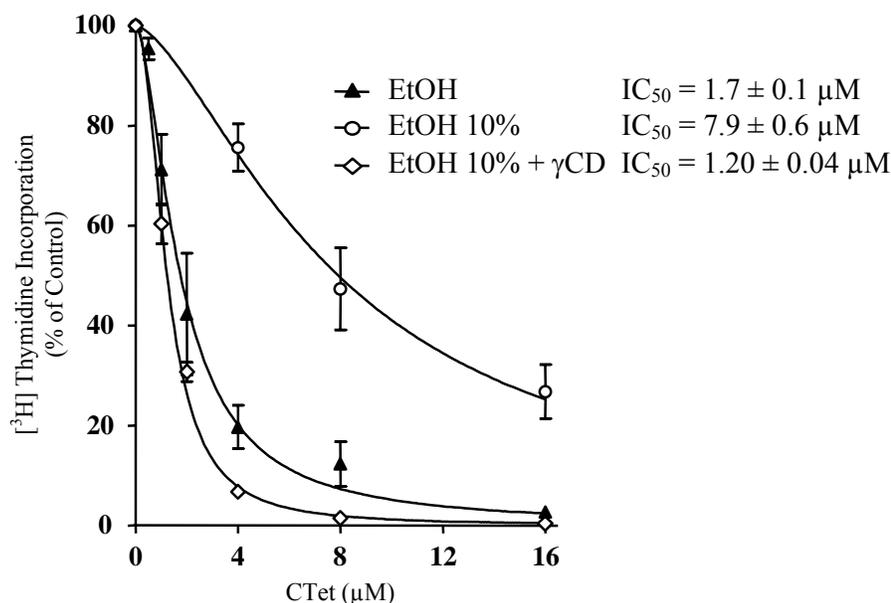
*Reagents and conditions:* 37% HC(O)H, MeOH, 96% H<sub>2</sub>SO<sub>4</sub>, reflux, 2 h.

With the aim of examining the antiproliferative activity of **CTet**, the drug was solubilized in pyridine or suspended in ethanol or DMSO and tested on estrogen receptor positive (ER+) breast cancer cell line MCF-7. It resulted that **CTet** in pyridine could not affect cell proliferation, whereas **CTet** in DMSO did in a dose-dependent manner ( $IC_{50} = 11.3 \pm 1.4 \mu\text{M}$ ). Also, **CTet** suspended in

ethanol showed good antiproliferative activity in the same cell line ( $IC_{50} = 1.7 \pm 0.1 \mu\text{M}$ ) (Figure 2). A pure ethanolic preparation, however, could not be used in clinical studies, thus we considered important to investigate formulations of **CTet** in an aqueous system.

Several protocols such as Solvent Induced Activation (SIA) system with PVP-Cl (polyvinylpyrrolidone-Cl) in different mediums, HP-55 (hydroxypropyl methyl cellulose phthalate), and  $\beta$ - or  $\gamma$ -CD complexation, were investigated. Only  $\gamma$ -CD formulation gave encouraging results. So, while the suspension obtained by diluting the **CTet** mixture in ethanol/water 1:10 showed a significant loss of biological activity ( $IC_{50} = 7.9 \pm 0.6 \mu\text{M}$ ;  $P < 0.001$ ) (Figure 2), when dilution of **CTet** was carried out in a  $\gamma$ -CD EtOH/H<sub>2</sub>O (1:10) solution, the activity of **CTet** resulted superimposable to that of **CTet** suspended in pure ethanol ( $IC_{50} = 1.20 \pm 0.04 \mu\text{M}$ ) (Figure 2).

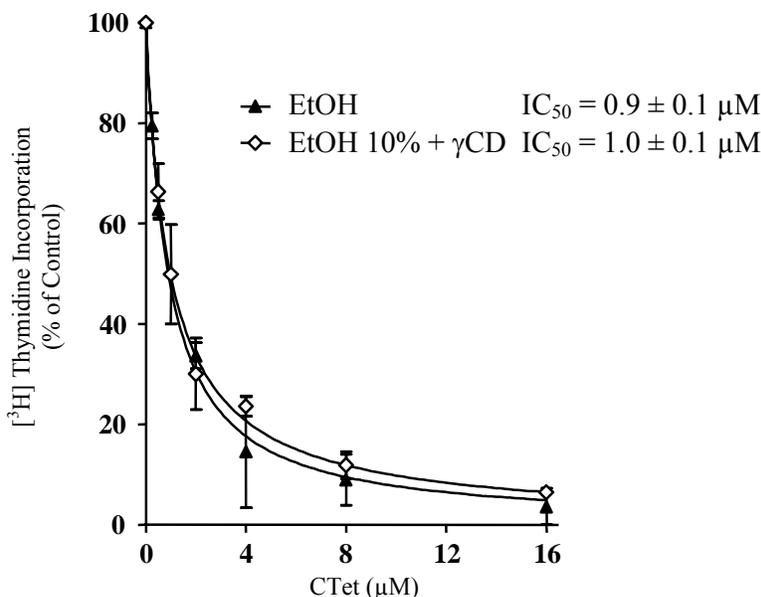
**Figure 2.** Effect of **CTet** formulated in aqueous solutions on DNA synthesis of MCF-7 breast cancer cell line. Cells were treated with various concentrations of **CTet** suspended in 10% EtOH (○), 10% EtOH with 160 mM  $\gamma$ -CD (◇) or pure EtOH (▲); during the last 5 h of treatment, cells were pulsed with [<sup>3</sup>H]thymidine, and the incorporation into DNA was determined (1.5  $\mu\text{Ci}$ ). Data are expressed as percentage of cells treated with vehicle only and are means  $\pm$  SEM of at least three experiments.



The antiproliferative activities of **CTet** both suspended in pure ethanol and formulated in  $\gamma$ -CD 10% ethanol were also tested on an estrogen receptor negative (ER-) breast cancer cell line (MDA-MB-231); the results were comparable with those obtained with MCF-7 cells ( $IC_{50} = 0.9 \pm 0.1$  and  $1.0 \pm 0.1 \mu\text{M}$ , respectively) (Figure 3). Notably, a 10% ethanolic solution of  $\gamma$ -CD did not have any appreciable cytotoxicity in our tests.

Finally, we had ascertained by HPLC that these formulations were stable for many months at room temperature in the dark; this observation is corroborated by the fact that antiproliferative tests in MCF-7 cells were comparable with those reported above (data not shown).

**Figure 3.** Effect of **CTet** formulated in aqueous solutions on DNA synthesis of MDA-MB-231 breast cancer cell line. Cells were treated with various concentrations of **CTet** suspended in 10% EtOH with 160 mM  $\gamma$ -CD ( $\diamond$ ) or pure EtOH ( $\blacktriangle$ ); during the last 5 h of treatment cells were pulsed with [ $^3$ H]thymidine, and the incorporation into DNA was determined (1.5  $\mu$ Ci). Data are expressed as percentage of cells treated with vehicle only and are means  $\pm$  SEM of at least three experiments. A 10% ethanolic solution of  $\gamma$ -CD did not have any appreciable cytotoxicity in our tests.



### 3. Experimental

#### 3.1. General

All reagents were purchased from Sigma-Aldrich or Carlo Erba with the exception of PVP-Cl and HP-55 which were furnished by Eurand,  $\beta$ -cyclodextrin (CAPTISOL<sup>®</sup>, CyDex), and  $\gamma$ -cyclodextrin (CAVAMAX<sup>®</sup> W8, Wacker); they were in the highest quality commercially available. Solvents were RP grade. Melting points were determined on a Büchi B-540 capillary melting point apparatus. The structure of **CTet** was unambiguously assessed by MS,  $^1$ H-NMR, and  $^{13}$ C-NMR. MS (ESI) spectra were recorded with a Waters Micromass ZQ spectrometer in a positive mode using a nebulizing nitrogen gas at 400 L/min and a temperature of 250 °C, cone flow 40 mL/min, capillary 3.5 Kvolts and cone voltage 60 V; only molecular ion in positive ion mode  $[M+H]^+$  is given. Retention time ( $t_R$ ) value was determined by direct HPLC analysis by Waters 2795 Separations Module, Alliance HT and Waters 2996, Photodiode Array Detector spectrometers with a Supelcosil<sup>TM</sup> LC-18 (15 cm  $\times$  4 mm, 3  $\mu$ M; Supelco) column using a combination of acetonitrile and aqueous solution 0.1% formic acid as eluent.  $^1$ H-NMR and  $^{13}$ C-NMR spectra were recorded on a Bruker AC 200 or 50, instrument, respectively, and analyzed using the WIN-NMR software package. Chemical shifts were measured by using the central peak of the solvent. Purification of the crude material was carried out by

column chromatography on aluminum oxide (0.05–0.15 mm, Fluka). TLC analyses were performed on precoated aluminum oxide on aluminum sheets (60 F<sub>254</sub>, neutral; Merck).

### 3.2. Synthesis of 5,6,11,12,17,18,23,24-octahydrocyclododeca[1,2-b:4,5-b':7,8-b'':10,11-b''']tetraindole (CTet)

To a solution of indole (3.12 g, 26.7 mmol) and aqueous 37% HC(O)H (3.2 mL, 40 mmol) in CH<sub>3</sub>OH (240 mL), 96% H<sub>2</sub>SO<sub>4</sub> (1.74 mL) was added. The mixture was stirred at reflux in the dark for 1 h, then further HC(O)H (3.2 mL, 40 mmol) was added, the mixture was stirred in the same conditions for 1 h, cooled to room temperature and concentrated in the dark. Purification of the solid by two short, protected from light, and fast aluminum oxide column chromatographies (cyclohexane/EtOAc 6:4,  $R_f$  = 0.82) and washing with hot CH<sub>3</sub>OH gave a white solid consisting (HPLC/MS) in a 9:1 mixture of **CTr** and **CTet** [HPLC: Supelcosil<sup>TM</sup> LC-18; flow: 0.5 mL/min;  $\lambda_{\max}$ : 284 nm; eluent: CH<sub>3</sub>CN/aqueous solution 0.1% HCOOH with a gradient 7:3 to 9:1 in 9 min;  $t_R$  **CTr**: 4.95 min,  $t_R$  **CTet**: 6.93 min]. Yield: 31% (1.08 g). Recrystallization [(CH<sub>3</sub>)<sub>2</sub>CO, 78 mL] afforded pure **CTet** as a white solid. Mp: chars over 300 °C. MS (ESI)  $m/z$ : 517.2 [M+H]<sup>+</sup>. <sup>1</sup>H-NMR [(CD<sub>3</sub>)<sub>2</sub>CO]:  $\delta$  3.88 (s, 8H, CH<sub>2</sub>), 6.85 (dd, 4H, ArH,  $J_1$  = 7.0 and  $J_2$  = 8.0 Hz), 6.99 (dd, 4H, ArH,  $J_1$  = 7.0 and  $J_2$  = 8.0 Hz), 7.24 (d, 4H, ArH,  $J$  = 8.0 Hz), 7.33 (d, 4H, ArH,  $J$  = 8.0 Hz), 9.95 (s, 4H, NH); <sup>13</sup>C-NMR (pyridine-*d*<sub>5</sub>):  $\delta$  23.6, 109.1, 112.6, 119.4, 120.4, 122.0, 131.4, 137.7, 138.0.

### 3.3. CTet formulations

A suspension of **CTet** (0.0083 g, 0.016 mmol) in pure EtOH (1 mL) was magnetically stirred at room temperature for different times (1 to 3 days, 1,000 rpm). The highest percentage of inhibition was obtained when the suspension was stirred for at least 2 days. This time was routinely used in all further experiments. The emulsion obtained was then diluted (volume ratio 1:10) by an aqueous solution of  $\gamma$ -CD (177 mM); the resulting white emulsion had a final concentration of 1.6 mM. The antiproliferative assays were performed with 10  $\mu$ L of formulated product appropriately diluted in 1 mL of the cellular culture medium.

### 3.4. Cell cultures and antiproliferative assay

The human breast carcinoma ER+ (MCF-7) and ER- (MDA-MB-231) cell lines were cultured in DMEM (Dulbecco's Modified Eagle's Medium) supplemented with 10% FCS (Fetal Calf Serum), 2 mM L-glutamine, 10 g/L NEAA (Non-Essential Amino Acid), 50 mg/L streptomycin, 1,000 U/L penicillin, with (in the case of MCF-7) or without (in the case of MDA-MB-231) 10 mg/L insulin. Cells (30,000/well in 24-well tissue culture plates) were treated with the several **CTet** formulations or respective vehicles for 72 h, and during the last 4 h of treatment were pulsed with 1.5  $\mu$ Ci of [<sup>3</sup>H]thymidine and processed [12].

### 3.5. Statistical analyses

Data are means  $\pm$  SEM of at least three separate experiments. Differences between means were evaluated by Student *t*-test; differences were considered significant at  $P < 0.05$  (Prism5, GraphPad Software Inc., La Jolla, CA, USA).

## 4. Conclusions

A straightforward, reproducible, and scalable synthesis of **CTet** is reported, together with a formulation of **CTet** that allows the molecule to exert its pharmacological potential as an inhibitor of DNA synthesis in both ER+ and ER- human breast cancer cells. It is hypothesized that  $\gamma$ -CD is capable to enhance the otherwise very low solubility of the drug in aqueous systems.

## Acknowledgements

The authors thank Giorgio Tarzia for his helpful hints.

## References

1. National Research Council. *Diet, Nutrition and Cancer*; Peter, F.M., Ed.; National Academy Press: Washington, DC, USA, 1982; pp. 358–370.
2. Weng, J.-R.; Tsai, C.-H.; Kulp, S.K.; Chen, C.-S. Indole-3-carbinol as a chemopreventive and anti-cancer agent. *Cancer Lett.* **2008**, *269*, 153–163.
3. Safe, S.; Papineni, S.; Chintharlapalli, S. Cancer chemotherapy with indole-3-carbinol, bis(3'-indolyl)methane and synthetic analogs. *Cancer Lett.* **2008**, *269*, 326–338.
4. Virtanen, A.I. Studies on organic sulphur compounds and other labile substances in plants. *Pythochemistry* **1965**, *4*, 207–228.
5. Wattenberg, L.W.; Loub, W.D. Inhibition of polycyclic aromatic hydrocarbon-induced neoplasia by naturally occurring indoles. *Cancer Res.* **1978**, *38*, 1410–1413.
6. Bjeldanes, L.F.; Kim, J.-Y.; Grose, K.R.; Bartholomew, J.C.; Bradfield, C.A. Aromatic hydrocarbon responsiveness-receptor agonists generated from indole-3-carbinol *in vitro* and *in vivo*: Comparisons with 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. *Proc. Natl. Acad. Sci. USA* **1991**, *88*, 9543–9547.
7. D'Argy, R.; Bergman, J.; Dencker, L. Effects of immunosuppressive chemicals on lymphoid development in fetal thymus organ cultures. *Pharmacol. Toxicol.* **1989**, *64*, 33–38.
8. De Kruif, C.A.; Marsman, J.W.; Venekamp, J.C.; Falke, H.E.; Noordhoek, J.; Blaauboer, B.J.; Wortelboer, H.M. Structure elucidation of acid reaction products of indole-3-carbinol: detection *in vivo* and enzyme induction *in vitro*. *Chem.-Biol. Interact.* **1991**, *80*, 303–315.
9. Chang, Y.-C.; Riby, J.; Chang, G.H.-F.; Peng, B.C.; Firestone, G.; Bjeldanes, L.F. Cytostatic and antiestrogenic effects of 2-(indol-3-ylmethyl)-3,3'-diindolylmethane, a major *in vivo* product of dietary indole-3-carbinol. *Biochem. Pharmacol.* **1999**, *58*, 825–834.
10. Riby, J.E.; Feng, C.; Chang, Y.-C.; Schaldach, C.M.; Firestone, G.L.; Bjeldanes, L.F. The major cyclic trimeric product of indole-3-carbinol is a strong agonist of the estrogen receptor signaling pathway. *Biochemistry* **2000**, *39*, 910–918.

11. Grose, K.R.; Bjeldanes, L.F. Oligomerization of indole-3-carbinol in aqueous acid. *Chem. Res. Toxicol.* **1992**, *5*, 188–193.
12. Brandi, G.; Paiardini, M.; Cervasi, B.; Fiorucci, C.; Filippone, P.; De Marco, C.; Zaffaroni, N.; Magnani, M. A new indole-3-carbinol tetrameric derivative inhibits cyclin-dependent kinase 6 expression, and induces G<sub>1</sub> cell cycle arrest in both estrogen-dependent and estrogen-independent breast cancer cell lines. *Cancer Res.* **2003**, *63*, 4028–4036.
13. Weng, J.-R.; Tsai, C.-H.; Kulp, S.K.; Wang, D.; Lin, C.-H.; Yang, H.-C.; Ma, Y.; Sargeant, A.; Chiu, C.-F.; Tsai, M.-H.; Chen, C.-S. A potent indole-3-carbinol-derived antitumor agent with pleiotropic effects on multiple signaling pathways in prostate cancer cells. *Cancer Res.* **2007**, *67*, 7815–7824.
14. Jump, S.M.; Kung, J.; Staub, R.; Kinset, M.A.; Cram, E.J.; Yudina, L.N.; Preobrazhenskaya, M.N.; Bjeldanes, L.F.; Firestone, G.L. *N*-Alkoxy derivatization of indole-3-carbinol increases the efficacy of the G<sub>1</sub> cell cycle arrest and of I3C-specific regulation of cell cycle gene transcription and activity in human breast cancer cells. *Biochem. Pharmacol.* **2008**, *75*, 713–724.
15. Guo, W.; Wu, S.; Liu, J.; Fang, B. Identification of a small molecule with synthetic lethality for K-Ras and protein kinase C  $\iota$ . *Cancer Res.* **2008**, *68*, 7403–7408.
16. Ramamoorthy, K.; Navo, M.; Gupta, M.S.; Safe, S.H. AhR-mediated antiestrogenicity of diindolylmethane and analogs *in vivo* and *in vitro*. *Organohalogen Compd.* **1998**, *37*, 321–324.
17. Ramamoorthy, K.; McDougal, A.; Safe, S.H. Structure-Ah receptor agonist/binding activity relationships of various chlorine-substituted diindolylmethane compounds. *Organohalogen Compd.* **1999**, *42*, 363–367.
18. McDougal, A.; Gupta, M.S.; Ramamoorthy, K.; Sun, G.; Safe, S.H. Inhibition of carcinogen-induced rat mammary tumor growth and other estrogen-dependent responses by symmetrical dihalo-substituted analogs of diindolylmethane. *Cancer Lett.* **2000**, *151*, 169–179.
19. McDougal, A.; Gupta, M.S.; Morrow, D.; Ramamoorthy, K.; Lee, J.-E.; Safe, S.H. Methyl-substituted diindolylmethanes as inhibitors of estrogen-induced growth of T47D cells and mammary tumors in rats. *Breast Cancer Res. Treat.* **2001**, *66*, 147–157.
20. Benabadji, S.H.; Wen, R.; Zheng, J.-B.; Dong, X.-C.; Yuan, S.-G. Anticarcinogenic and antioxidant activity of diindolylmethane derivatives. *Acta Pharmacol. Sin.* **2004**, *25*, 666–671.
21. Pisano, C.; Kollar, P.; Gianni, M.; Kalac, Y.; Giordano, V.; Ferrara, F.F.; Tancredi, R.; Devoto, A.; Rinaldi, A.; Rambaldi, A.; Penco, S.; Marzi, M.; Moretti, G.; Vesci, L.; Tinti, O.; Carminati, P.; Terao, M.; Garattini, E. Bis-indols: a novel class of molecules enhancing the cytodifferentiating properties of retinoids in myeloid leukemia cells. *Blood* **2002**, *100*, 3719–3730.
22. Giannini, G.; Marzi, M.; Moretti, G.P.; Penco, S.; Tinti, M.O.; Pesci, S.; Lazzaro, F.; De Angelis, F. Synthesis of cycloalkanoindoles by an unusual DAST-triggered rearrangement reaction. *Eur. J. Org. Chem.* **2004**, 2411–2420.
23. Maciejewska, D.; Rasztawicka, M.; Wolska, I.; Anuszewka, E.; Gruber, B. Novel 3,3'-diindolylmethane derivatives: Synthesis and cytotoxicity, structural characterization in solid state. *Eur. J. Med. Chem.* **2009**, *44*, 4136–4147.
24. Sanderson, J.T.; Slobbe, L.; Lansbergen, G.W.A.; Safe, S.; van der Berg, M. 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin and diindolylmethanes differentially induce cytochrome P450 1A1, 1B1, and 19 in H295R human adrenocortical carcinoma cells. *Toxicol. Sci.* **2001**, *61*, 40–48.

25. Qin, C.; Morrow, D.; Stewart, J.; Spencer, K.; Porter, W.; Smith, R., III; Phillips, T.; Abdelrahim, M.; Samudio, I.; Safe, S. A new class of peroxisome proliferator-activated receptor  $\gamma$  (PPAR $\gamma$ ) agonists that inhibit growth of breast cancer cells: 1,1-Bis(3'-indolyl)-1-(*p*-substituted phenyl)methanes. *Mol. Cancer Ther.* **2004**, *3*, 247–259.
26. Contractor, R.; Samudio, I.J.; Estrov, Z.; Harris, D.; McCubrey, J.A.; Safe, S.H.; Andreeff, M.; Konopleva, M. A novel ring-substituted diindolylmethane, 1,1-bis[3'-(5-methoxyindolyl)]-1-(*p*-*t*-butylphenyl) methane, inhibits extracellular signal-regulated kinase activation and induces apoptosis in acute myelogenous leukemia. *Cancer Res.* **2005**, *65*, 2890–2898.
27. Chintharlapalli, S.; Burghardt, R.; Papineni, S.; Ramaiah, S.; Yoon, K.; Safe, S. Activation of Nur77 by selected 1,1-bis(3'-indolyl)-1-(*p*-substituted phenyl)methanes induces apoptosis through nuclear pathways. *J. Biol. Chem.* **2005**, *280*, 24903–24914.
28. Inamoto, T.; Papineni, S.; Chintharlapalli, S.; Cho S.-D.; Safe, S.; Kamat, A.M. 1,1-Bis(3'-indolyl)-1-(*p*-chlorophenyl)methane activates the orphan nuclear receptor Nurr1 and inhibits bladder cancer growth. *Mol. Cancer Ther.* **2008**, *7*, 3825–3833.
29. Noguchi-Yachide, T.; Tetsuhashi, M.; Aoyama, H.; Hashimoto, Y. Enhancement of chemically-induced HL-60 cell differentiation by 3,3'-diindolylmethane derivatives. *Chem. Pharm. Bull.* **2009**, *57*, 536–540.
30. Chao, W.-R.; Yean, D.; Amin, K.; Green, C.; Jong, L. Computer-aided rational drug design: A novel agent (SR13668) designed to mimic the unique anticancer mechanisms of dietary indole-3-carbinol to block akt signaling. *J. Med. Chem.* **2007**, *50*, 3412–3415.
31. Vincent, E.; Shirani, H.; Bergman, J.; Rannug, U.; Janosik, T. Synthesis and biological evaluation of fused thio- and selenopyrans as new indolocarbazole analogues with aryl hydrocarbon receptor affinity. *Bioorg. Med. Chem.* **2009**, *17*, 1648–1653.
32. Xue, L.; Schaldach, C.M.; Janosik, T.; Bergman, J.; Bjeldanes, L.F. Effects of analogs of indole-3-carbinol cyclic trimerization product in human breast cancer cells. *Chem.-Biol. Interact.* **2005**, *152*, 119–129.
33. Bergman, J.; Högberg, S.; Lindström, J.-O. Macrocyclic condensation products of indole and simple aldehydes. *Tetrahedron* **1970**, *26*, 3347–3352.
34. Staub, R.E.; Bjeldanes, L.F. Convenient synthesis of 5,6,11,12,17,18-hexahydrocyclononal[1,2-*b*:4,5-*b'*:7,8-*b''*]triindole, a novel phytoestrogen. *J. Org. Chem.* **2003**, *68*, 167–169.

*Sample Availability:* A sample of the compound is available from the authors.

© 2010 by the authors; licensee MDPI, Basel, Switzerland. This article is an Open Access article distributed under the terms and conditions of the Creative Commons Attribution license (<http://creativecommons.org/licenses/by/3.0/>).