Synthesis and Evaluation of 2-Naphthaleno *trans*-Stilbenes and Cyanostilbenes as Anticancer Agents

Nikhil R. Madadi[a], **Narsimha R. Penthala**[a], **Amit Ketkar**[b], **Robert L. Eoff**[b], **Vicenta Trujullo-Alonso**[c], **Monica L. Guzman**[c], **Peter A. Crooks**[a,*]

[a]Department of Pharmaceutical Sciences, College of Pharmacy, University of Arkansas for Medical Sciences, Little Rock, AR 72205, USA;

[b]Department of Biochemistry and Molecular Biology, University of Arkansas for Medical Sciences, Little Rock, AR 72205-7199, USA;

[c]Weill Cornell Medical College, New York, NY 10021, USA

Abstract

Background: Naphthalene is a good structural replacement for the isovanillin moiety (*i.e.* the 3-hydroxy-4-methoxyphenyl unit) in the combretastatin A-4 molecule, a natural product structurally related to resveratrol, which consistently led to the generation of highly cytotoxic naphthalene analogues when combined with a 3,4,5-trimethoxyphenyl or related aromatic system. Also, the naphthalene ring system is present in many current drug molecules that are utilized for anti-tumor, anti-arrhythmia and antioxidant therapy.

Objective: In our continuing quest to improve the potencies of naturally occurring anti-cancer molecules through chemical modification, we have now synthesized a small library of 2-naphthaleno *trans*- stilbenes and cyanostilbenes that are structurally related to both resveratrol and DMU-212, and have evaluated these novel analogs against a panel of 54 human tumor cell lines.

Method: A series of 2-naphthaleno-containing *trans*-stilbenes **3a-3h** (Scheme 1) were synthesized by Wittig reaction of a variety of aromatic substituted benzyl-triphenylphosphonium bromide reactants with 2-naphthaldehyde using n-BuLi as a base in THF. A second series of 2-naphthaleno *trans*-cyanostilbenes analogs **5a-5h** was synthesized by reaction of 2-naphthaldehyde (**2**; 1 mmol) with an appropriately substituted 2-phenylacrylonitrile **4a-4h**; 1 mmol) in 5% sodium methoxide/methanol. The reaction mixture was stirred at room temperature for 2–3 hours and the reaction allowed to go to completion (TLC monitoring), during which time the desired product

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ETHICS APPROVAL AND CONSENT TO PARTICIPATE
The studies with human cancer cells were carried out by the National Cancer Institute of the USA, and were not done in my laboratory.

HUMAN AND ANIMAL RIGHTS
No Animals/Humans were used for studies that are the basis of this research.

CONSENT FOR PUBLICATION
Not applicable.

CONFLICT OF INTEREST
The authors declare no conflict of interest, financial or otherwise.

precipitated out of the solution as a solid. The resulting precipitate was filtered off, washed with water and dried to yield the desired compound in yields ranging from 70–95% (Scheme 2).

Results: The percentage growth inhibition of 54 human cancer cell lines in a primary NCI screen after exposure to compounds **3a**, **3d**, **5b** and **5c** was carried out. The results showed that only compounds **5b** and **5c** met the criteria for subsequent testing to determine growth inhibition values (GI50) in dose-response studies. At 10–5 M concentration, compounds **5b** and **5c** exhibited cytotoxic activity against leukemia cell lines HL-60(TB) and SR, lung cancer cell line NCI-H522, colon cancer cell lines COLO 205 and HCT-116, CNS-cancer cell line SF-539, melanoma cell line MDA-MB-435, and breast cancer cell line BT-549. The naphthalene *trans*-stilbene analogue **3a**, exhibited significant growth inhibition against only one cell line, melanoma cell line MDA-MB-435 (96 % growth inhibition). Compound **3d** was inactive in the 10^{-5} M single dose screen.

Conclusion: We have synthesized a small set of novel 2-naphthaleno stilbenes and cyanostilbenes and evaluated several of these compounds for their anticancer properties against a panel of 54 human tumor cell lines. The most active analogs, **5b** and **5c**, showed significantly improved growth inhibition against the human cancer cells in the NCI panel when compared to DMU-212. Of these compounds, analog **5c** was found to be the most potent anticancer agent and exhibited significant growth inhibitory effects against COLO 205, CNS SF 539 and melanoma SK-MEL 5 and MDA-MB-435 cell lines with GI_{50} values 25 nM. Analog **5b** also exhibited GI_{50} values in the range 25–41 nM against CNS SF 295 and melanoma MDA-MB-435 and UACC-62 cell lines. Compounds **5b** and **5c** were also cytotoxic towards the MV4–11 leukemia cell line with LD_{50} value of 450 nM and 200 nM, respectively, and demonstrated >50% inhibition of tubulin polymerization at concentrations below their LD_{50} values in these cells. *In silico* docking studies suggest that compounds **5b** and **5c** bind favorably at the colchicine-binding pocket of the tubulin dimer, indicating that both **5b** and **5c** may inhibit tubulin polymerization through a mechanism similar to that exhibited by colchicine. Derivative **5c** demonstrated more favorable binding based on the docking score and buried surface area, as compared to compound **5b**, in agreement with the higher observed potency of **5c** against a broader range of tumor cell lines. Based on these results, analog **5c** is considered to be a lead compound for further optimization as a clinical candidate for treating a variety of cancers.

Keywords

1. INTRODUCTION

Resveratrol (Fig. 1A) is a phytoalexin which was first isolated and characterized from the medicinal plant *Veratrum album* by Michio Takaoka in 1939 [1]. Since then, this natural product has been isolated from more than 80 other plant species [2]. Resveratrol has received extensive news coverage and is widely used as a supplement drug after being credited with the cardio-protective effects of red wine [3]. In 1991, researchers reported that resveratrol was highly effective in curing skin tumors in mice [4]. Since then, numerous biologically beneficial properties of resveratrol for the treatment of a wide variety of diseases have been reported, including anti-cancer activity, as modulators of platelet aggregation, as an antioxidant [5–7], cardiovascular diseases [8], and in neurodegenerative

diseases [9] in humans. Unfortunately, resveratrol cannot be utilized as a drug product because of its extremely low bioavailability and rapid clearance from the circulation [10].

Extensive anticancer structure-activity relationship (SAR) and metabolic stability studies on resveratrol analogs has revealed that maintaining the stilbene skeleton and replacing the extensively metabolized hydroxyl groups with methoxyl groups improves metabolic stability and retains the medicinal properties of reseveratrol [11, 12]. From such SAR studies *trans*-3,4,5,4 tetramethoxystilbene (DMU-212) (Fig. 1B) has been shown to possess more favorable pharmacokinetic properties than resveratrol and exhibits more potent anti-proliferative/proapoptotic activities in a variety of cancer cells, including K562 (leukemia), HT29 (colo-rectal), and HePG2 (hepatoma) HeLa (cervical), LnCaP (prostate), HepG2 (hepatoma) and MCF-7 (breast) cancer cells [13, 14]. Recently, we have reported a comprehensive evaluation of DMU-212 as an anticancer agent against a panel of 54 human tumor cell lines. DMU-212 was found to be an effective antitumor agent against SNB-75 (CNS), MDA-MB-435 (melanoma), A498 (renal) and MCF7 (breast cancer) cell lines, with GI_{50} values ranging from 0.74 to 1.88 μM [15].

More recently, we have reported a series of (*Z*)-benzothiophene acrylonitrile derivatives of resveratrol (Fig. 1C); these compounds are potent anti-cancer agents which do not act as substrates for Pglycoproteins [16] involved in cellular efflux mechanisms. Interestingly, Maya *et al.* reported naphthalene to be a good structural replacement for the isovanillin moiety (*i.e.* the 3-hydroxy-4-methoxyphenyl unit) in the combretastatin A-4 molecule, a natural product structurally related to resveratrol, which consistently led to the generation of highly cytotoxic naphthalene analogues (Fig. 1D) when combined with 3,4,5-trimethoxyphenyl or related aromatic systems [17, 18]. Also, the naphthalene ring system is present in many current drug molecules that are utilized for anti-tumor, anti-arrhythmia and antioxidant therapy [19, 20].

In our continuing quest to improve the potencies of naturally occurring anti-cancer molecules through chemical modification, we have now synthesized a small library of 2-naphthaleno *trans*-stilbenes and cyanostilbenes that are structurally related to both resveratrol and DMU-212, and have evaluated these novel analogs against a panel of 54 human tumor cell lines.

2. MATERIALS AND METHODS

2.1. Chemical Synthesis

All starting materials were purchased from different commercial sources and were used as received without further purification. TLC 60 F254 plates were used for thin layer chromatography (TLC) and silica gel (230–400 mesh) was used for column chromatography. NMR spectra were recorded in $CDCl_3$, $CDCl_3+CD_3OD$, or DMSO-d_6 at 400 MHz for ^{1}H NMR and 100 MHz for ^{13}C-NMR on an Agilent-400 spectrometer. Chemical shifts (δ) were expressed in parts per million (*ppm*) and coupling constants (*J*) were reported in Hertz (Hz).

2.2. Synthesis of Compounds 3a-3h

The series of 2-naphthaleno-containing *trans*-stilbenes **3a-3h** (Scheme 1) were synthesized by Wittig reaction of a series of aromatic substituted benzyl-triphenylphosphonium bromide reactants with 2-naphthaldehyde using n-BuLi as a base in THF.

2.3. General Procedure for the Synthesis of Compounds 3a-3h

In a round-bottomed flask, an appropriately substituted benzyl-triphenylphosphonium bromide salt (**1a-1h**; 1 mmol) was added to 5 volumes of anhydrous THF under nitrogen gas. The solution was cooled to 0°C in an ice bath and n-BuLi (1.2 mmol) was added slowly over a period of 10 min. The reaction was allowed to stir at 0°C for 30 min and 2-naphthaldehyde (**2**; 1 mmol) was added. The reaction was monitored by TLC, and once the reaction was completed, ice water was added to quench the reaction. Ethyl acetate was then added and the organic phase was separated and evaporated under vacuum to afford a crude residue. Silica gel flash chromatography was performed on the crude product to isolate the resulting 2-naphthaleno *trans*-stilbene. Reaction yields obtained for compounds **3a-3h** were within the range 50–70% (Scheme 1).

2.4. Analytical Data of Compounds 3a-3h

(E)-2-(3,5-Dimethoxystyryl)naphthalene (3a)—^{1}H NMR (400 MHz, CDCl$_3$): δ 3.85 (s, 6H, OCH$_3$), 6.4 (s, 1H, ArH), 6.72 (s, 2H, ArH), 7.18 (m, 2H, Ar-H), 7.46 (m, 2H, ArH), 7.74 (m, 5H, ArH) *ppm*. ^{13}C NMR (100 MHz, CDCl$_3$): δ 55.73 (-OCH$_3$), 100.41 (C4), 104.94 (C1 and C6), 123.83, 126.29, 126.69, 127.12, 128.04, 128.35, 128.66, 129.33, 129.62, 133.43, 134.02, 134.97, 139.71, 161.36 (C3 and C5), *ppm*. HRMS calcd. for C$_{20}$H$_{19}$O$_2$, (M+H)$^+$: 291.1387 Found: 291.1395.

(E)-2-(4-Ethoxystyryl)naphthalene (3b)—^{1}H NMR (400 MHz, CDCl$_3$+CD$_3$OD): δ 1.40 (t, *J* = 7.2 Hz, 3H, -CH$_3$), 4.03 (dd, *J* = 13.6 Hz, *J* = 6.8 Hz, 2H, -CH$_2$), 6.89 (d, *J* = 8.4 Hz, 2H, ArH), 7.10 (d, *J* = 9.2 Hz, 2H, ArH), 7.40–7.49 (m, 4H, ArH), 7.70 (d, 1H, *J* = 8.4 Hz, ArH), 7.79 (m, 4H, ArH) *ppm*. ^{13}C-NMR (100 MHz, CDCl$_3$+CD$_3$OD): 14.63 (CH$_3$), 63.45 (-OCH$_2$), 114.62 (C3 and C5), 123.33, 125.55, 125.95, 126.15, 126.43, 127.53 (-CH=CH-), 127.64, 127.76, 128.10, 128.48, 129.94, 132.73, 133.65, 135.06, 158.57 (C4) *ppm*. HRMS calcd. for C$_{20}$H$_{19}$O, (M+H)$^+$: 275.1438 Found: 275.1451.

(E)-Methyl4-(2-(naphthalen-2-yl)vinyl)benzoate (3c)—^{1}H NMR (400 MHz, CDCl$_3$+CD$_3$OD): δ 3.93 (s, 3H, -OCH$_3$), 7.28 (s, 1H, ArH), 7.36 (d, *J* = 7.6 Hz, 1H, ArH), 7.47 (m, 2H, ArH), 7.61 (d, *J* = 6.8 Hz, 2H, ArH), 7.74–7.85 (m, 4H, ArH), 7.89 (s, 1H, ArH), 8.03 (d, *J* = 6.8 Hz, 2H, ArH) *ppm*. ^{13}C NMR (100 MHz, CDCl$_3$+CD$_3$OD): 52.27 (-OCH$_3$), 123.51, 126.41 (-CH=CH-), 126.52, 126.62, 127.50, 127.85, 127.91, 128.24, 128.60, 128.84, 130.20, 131.57, 133.47, 133.77, 134.36, 142.21, 167.49 (-CO) *ppm*. HRMS calcd. for C$_{20}$H$_{17}$O$_2$, (M+H)$^+$: 289.1230 Found: 289.1252.

(E)-2-(4-Methoxystyryl)naphthalene (3d)—^{1}H NMR (400 MHz, CDCl$_3$): δ 3.82 (s, 3H, -OCH$_3$), 6.90 (d, *J* = 8.8 Hz, 2H, ArH), 7.14 (d, *J* = 9.2 Hz, 2H, ArH), 7.41–7.50 (m, 4H, ArH), 7.69 (dd, *J* = 1.2 Hz and 8.4 Hz, 1H, ArH), 7.78–7.80 (m, 4H, ArH) *ppm*. ^{13}C NMR (100 MHz, CDCl$_3$): 55.98 (-OCH$_3$), 114.83 (C3 and C5), 124.12, 126.33, 126.77,

126.92, 127.32, 128.33 (-CH=CH-), 128.41, 128.56, 128.89, 129.21, 130.81, 133.50, 134.42, 135.81, 160.01(C4) *ppm*. HRMS calcd. for $C_{19}H_{17}O$, (M+H)$^+$: 261.1281 Found: 261.1291.

(E)-2-(3-Methylstyryl)naphthalene (3e)—^{1}H NMR (400 MHz, DMSO-d_6): δ 2.34 (s, 3H, -CH$_3$), 7.09–7.26 (d, *J* = 7.2 Hz, 1H, ArH), 7.26 (t, *J* = 7.2 Hz, 1H, ArH), 7.37 (d, *J* = 6.4 Hz, 2H, ArH), 7.43–7.51 (m, 4H, ArH), 7.85–7.92 (m, 4H, ArH), 8.02 (s, 1H, ArH) *ppm*. ^{13}C NMR (100 MHz, DMSO-d_6): 21.48 (-CH$_3$), 124.02, 124.26, 126.41, 126.76, 126.89, 127.49, 128.04, 128.29 (-CH=CH-), 128.64, 128.90, 129.07, 129.47, 132.99, 133.73, 135.15, 137.41, 138.23 (C3) *ppm*. HRMS calcd. for $C_{19}H_{17}$, (M+H)+: 245.1332 Found: 245.1309.

(E)-2-(2-Methylstyryl)naphthalene (3f)—^{1}H NMR (400 MHz, DMSO-d_6): δ 2.45 (s, 3H, -CH$_3$), 7.19–7.31 (m, 4H, ArH), 7.48–7.59 (m, 3H, ArH), 7.73 (d, *J* = 7.6 Hz, 1H, ArH), 7.89–7.92 (m, 4H, ArH), 8.03 (s, 1H, ArH) *ppm*. ^{13}C NMR (100 MHz, DMSO-d_6): 20.03 (CH$_3$), 124.25, 125.55, 126.43, 126.65, 126.89, 126.92, 128.03 (-CH=CH-), 128.32 128.63, 130.11, 130.87, 133.03, 133.73, 135.33, 136.07, 136.28 (C1) *ppm*. HRMS calcd. for $C_{19}H_{17}$, (M+H)$^+$: 245.1332 Found: 245.1322.

(E)-2-(4-Fluorostyryl)naphthalene (3g)—^{1}H NMR (400 MHz, CDCl$_3$): δ 7.04–7.08 (t, *J* = 8.8 Hz, 2H, ArH), 7.17 (s, 2H, ArH), 7.43–7.52 (m, 4H, ArH), 7.69 (dd, *J* = 1.6 Hz, 8.4 Hz, 1H, ArH), 7.80–7.82 (m, 4H, ArH) *ppm*. ^{13}C NMR (100 MHz, CDCl$_3$): 116.22 (C3), 116.43 (C5), 124.05, 126.60, 127.03, 127.24, 128.36 (-CH=CH-), 128.44, 128.63, 128.70, 129.01, 129.20, 129.22, 133.69, 134.18, 134.21, 134.34, 135.30, 161.79, 164.26 (d, C4) *ppm*. HRMS calcd. for $C_{18}H_{14}F$, (M+H)$^+$: 249.1081 Found: 249.1173.

(E)-2-(4-Chlorostyryl)naphthalene (3h)—^{1}H NMR (400 MHz, CDCl$_3$): δ 7.17 (d, *J* = 14.0 Hz 2H, ArH), 7.32–7.34 (m, 2H, ArH), 7.44–7.47 (m, 4H, Ar-H), 7.69 (dd, *J* = 1.6 Hz and 8.4 Hz, 1H, ArH), 7.79–7.83 (m, 4H, ArH) *ppm*. ^{13}C NMR (100 MHz, CDCl$_3$): 123.40, 126.08, 126.44, 126.85, 127.69 (-CH=CH-), 127.70, 127.74, 128.05, 128.41, 128.91, 129.40, 133.14, 133.24, 133.69, 134.49 (C4-Cl), 135.89 (C1) *ppm*. HRMS calcd. for $C_{18}H_{14}Cl$, (M+H)$^+$: 265.0786 Found: 265.0792.

2.5. General Procedure for the Synthesis of Compounds 5a-5h

A second series of 2-naphthaleno *trans*-cyanostilbenes analogs **5a–5h** were synthesized by the reaction of 2-naphthaldehyde (**2**; 1 mmol) with an appropriately substituted 2-phenylacrylonitrile **4a–4h**; 1 mmol) in 5% sodium methoxide/methanol [16].

The reaction mixture was stirred at room temperature for 2–3 hours and the reaction allowed to go to completion (TLC monitoring), during which time the desired product precipitated out of the solution as a solid. The resulting precipitate was filtered off, washed with water and dried to yield the desired compound in yields ranging from 70–95% (Scheme 2).

2.6. Analytical Data for Compounds 5a–5h

(Z)-3-(Naphthalen-2-yl)-2-(4-nitrophenyl)acrylonitrile (5a)—^{1}H NMR (400 MHz, CDCl$_3$+CD$_3$OD): δ 7.56–7.62 (m, 2H, ArH), 7.86–790 (m, 4H, ArH), 7.93 (d, *J* = 9.2 Hz,

2H, ArH), 8.12 (d, J = 8.8 Hz, 1H, ArH), 8.30 (d, J = 8.8 Hz, 2H, ArH), 8.35 (s, 1H, ArH) *ppm*. ^{13}C NMR (100 MHz, CDCl$_3$+CD$_3$OD): 117.67 (=CCN), 124.42 (-CN), 124.64, 125.24, 126.98, 127.38, 128.09, 128.63, 129.24, 129.30, 130.68, 131.95, 133.23, 134.87, 140.99, 145.99 (C4-NO$_2$), 148.07 (stilbene =CH) *ppm*. HRMS calcd. for C$_{19}$H$_{13}$N$_2$O$_2$, (M +H)$^+$: 301.0979 Found: 301.0756.

(Z)-2-(3,4-Dimethoxyphenyl)-3-(naphthalen-2-yl)acrylonitrile (5b)—^{1}H NMR (400 MHz, CDCl$_3$): δ 3.93 (s, 3H, -OCH$_3$), 3.97 (s, 3H, -OCH$_3$), 6.91 (d, J = 8.0 Hz, 1H, ArH), 7.19 (d, J = 2.4 Hz, 1H, ArH), 7.29–7.31 (dd, J = 2.0 Hz and 8.4 Hz, 1H, ArH), 7.51–7.54 (m, 2H, ArH), 7.57 (s, 1H, ArH), 7.84–7.91 (m, 3H, ArH), 8.058.07 (dd, J = 1.6 Hz and 8.4 Hz, 1H, ArH), 8.26 (s, 1H, Ar-H) *ppm*. ^{13}C NMR (100 MHz, CDCl$_3$): 56.37 (-OCH$_3$), 56.39 (-OCH$_3$), 109.09 (=C-CN), 111.62 (C6), 111.70 (C1), 118.66 (-CN), 119.41, 125.56, 127.09, 127.78, 127.82, 128.07, 128.99, 129.01, 130.40, 131.74, 133.45, 134.27, 140.70 (stilbene =CH), 149.63 (C3), 150.42 (C4) *ppm*. HRMS calcd. for C$_{21}$H$_{18}$NO$_2$, (M+H)$^+$: 316.1339 Found: 316.1347.

(Z)-3-(Naphthalen-2-yl)-2-(3,4,5-trimethoxyphenyl)acrylonitrile (5c)—^{1}H NMR (400 MHz, CDCl$_3$): δ 3.90 (s, 3H, -OCH$_3$), 3.95 (s, 6H, -OCH$_3$), 6.91 (s, 2H, ArH), 7.54 (m, 2H, ArH), 7.61 (s, 1H, ArH), 7.84–7.92 (m, 3H, ArH), 8.06 (d, J = 1.2 Hz, 1H, ArH), 8.27 (s, 1H, ArH) *ppm*. ^{13}C NMR (100 MHz, CDCl$_3$): 56.65 (OCH$_3$), 61.33 (OCH$_3$), 104.4 (=C-CN), 111.84, 118.54 (-CN), 125.49, 127.16, 127.97, 128.09, 129.05, 130.59, 130.71, 131.48, 133.41, 134.40, 139.46, 142.03 (stilbene =CH), 153.91 (C3 and C5) *ppm*. HRMS calcd. for C$_{22}$H$_{20}$NO$_3$, (M+H)$^+$: 346.1445 Found: 346.1436.

(Z)-2-(4-Methoxyphenyl)-3-(naphthalen-2-yl)acrylonitrile (5d)—^{1}H NMR (400 MHz, CDCl$_3$): δ 3.84 (s, 3H, -OCH$_3$), 6.95 (d, J = 8.0 Hz, 2H, ArH), 7.50–7.53 (m, 2H, ArH), 7.55 (s, 1H, ArH), 7.63 (d, J = 8.8 Hz, 2H, ArH), 7.83–7.89 (m, 3H, ArH), 8.03–8.06 (dd, J = 1.2 Hz and 8.8 Hz, 1H, ArH), 8.24 (s, 1H, ArH) *ppm*. ^{13}C NMR (100 MHz, CDCl$_3$): 55.77 (OCH$_3$), 111.51 (=C-CN), 114.78 (C3 and C5), 118.66 (-CN), 125.58, 127.06, 127.43, 127.66, 127.76, 128.06, 128.95, 129.01, 130.30, 131.82, 133.45, 134.24, 140.41 (stilbene =CH), 160.77 (C4) *ppm*. HRMS calcd. for C$_{20}$H$_{16}$NO, (M+H)$^+$: 286.1234 Found: 286.1258.

(Z)-3-(Naphthalen-2-yl)-2-phenylacrylonitrile (5e)—^{1}H NMR (400 MHz, DMSO-d_6): δ 7.47 (t, J = 7.2 Hz, 1H, ArH), 7.54 (t, J = 6.8 Hz, 2H, ArH), 7.60–7.63 (m, 2H, ArH), 7.83 (d, J = 7.2 Hz, 2H, ArH), 7.99 (t, J = 13.6 Hz, 1H, ArH), 8.07 (d, J = 8.4 Hz, 2H, ArH), 8.13 (d, J = 8.4 Hz, 1H, ArH), 8.21 (s, 1H, ArH), 8.43 (s, 1H, ArH) *ppm*. ^{13}C NMR (100 MHz, DMSO-d_6): 110.91 (=C-CN), 118.51 (-CN), 125.42, 126.23, 126.29, 127.46, 128.16, 128.22, 128.97, 129.69, 129.67, 130.80, 131.81, 133.02, 134.01, 134.30, 143.32, 143.36 (stilbene =CH) *ppm*. HRMS calcd. for C$_{19}$H$_{14}$N, (M+H)$^+$: 256.1128 Found: 256.1114.

(Z)-3-(Naphthalen-2-yl)-2-(3(trifluoromethyl)phenyl)acrylonitrile (5f)—^{1}H NMR (400 MHz, CDCl$_3$): δ 7.53–7.60 (m, 3H, ArH), 7.64 (d, J = 7.2 Hz 1H, ArH), 7.71 (s, 1H, ArH), 7.84–7.93 (m, 5H, ArH), 8.08–8.11 (dd, J = 2.0 Hz and 9.2 Hz, 1H, ArH), 8.29 (s, 1H, ArH) *ppm*. ^{13}C NMR (100 MHz, CDCl$_3$): 110.69 (=C-CN), 118.34 (-CN), 123.24, 123.28, 125.71 (-CF$_3$), 126.38, 126.42, 127.61, 128.45, 128.65, 129.54, 129.99, 130.01, 130.35,

131.34, 131.69, 133.67, 134.99, 136.16, 144.43 (stilbene =CH) *ppm*. HRMS calcd. for $C_{20}H_{13}F_3N$, $(M+H)^+$: 324.1002 Found: 324.1026.

(Z)-2-(Benzo[d][1,3]dioxol-5-yl)-3-(naphthalen-2-yl)acrylonitrile (5g)—^{1}H NMR (400 MHz, CDCl$_3$): δ 5.33 (s, 1H, ArH), 5.86 (d, *J* = 12.8 Hz, 2H, OCH$_2$O), 6.57 (d, *J* = 8.0 Hz, 1H, ArH), 6.76 (d, *J* = 1.6 Hz, 1H, ArH), 6.84–6.87 (dd, *J* = 1.6 Hz and 8.4 Hz, 1H, ArH), 7.34–7.37 (dd, *J* = 1.6 Hz and 8.8 Hz, 1H, ArH), 7.50–7.52 (m, 2H, ArH), 7.62–7.83 (m, 3H, ArH), 7.88 (s, 1H, =CH) *ppm*. ^{13}C NMR (100 MHz, CDCl$_3$): 101.52 (-OCH$_2$O-), 108.41 (=C-CN), 108.44, 121.62 (CN), 121.87, 126.09, 126.61, 126.87, 127.75, 128.19, 128.45, 128.54, 129.33, 130.64, 133.08, 147.87, 148.22 (stilbene =CH) *ppm*. HRMS calcd. for $C_{20}H_{14}NO_2$, $(M+H)^+$: 300.1026 Found: 300.1045.

(Z)-2-(4-Hydroxyphenyl)-3-(naphthalen-2-yl)acrylonitrile (5h)—^{1}H NMR (400 MHz, CDCl$_3$+CD$_3$OD): δ 6.92–6.95 (dd, *J* = 2.0 Hz and 6.8 Hz, 2H, ArH), 7.53–7.57 (m, 3H, ArH), 7.61–7.63 (dd, *J* = 2.0 Hz and 6.4 Hz, 2H, ArH), 7.89–8.01 (m, 4H, ArH), 8.12 (s, 1H, ArH), 9.94 (s, 1H, -OH) *ppm*. ^{13}C NMR (100 MHz, CDCl$_3$+CD$_3$OD): 115.22 (=C-CN), 116.23, 118.25 (-CN), 123.70, 125.71, 125.82, 126.58, 127.04, 127.06, 127.86, 129.10, 130.55, 131.74, 131.78, 133.72, 138.03 (stilbene =CH), 158.54 (C4-OH) *ppm*. HRMS calcd. for $C_{19}H_{14}NO$, $(M+H)^+$: 272.1077 Found: 272.1084.

3. BIOLOGICAL EVALUATION

3.1. Anti-cancer Activity Screening

The National Cancer Institute (NCI) employs an effective triage system for the evaluation of potential anticancer agents based on duplicates already screened and ADME-TOX algorithm results prior to selection of compounds for initial single dose screening assays against a large panel of human cancer cell lines [21]. Of the sixteen 2-naphthaleno *trans*-stilbene and cyanostilbene compounds synthesized, compounds **3a, 3d, 5b** and **5c** were selected for anticancer screening against the NCI human cancer cell panel. *In vitro* screening of the above compounds utilized the sulforhodamine B (SRB) assay procedure described by Rubinstein *et al* [21]. Growth inhibitory or cytotoxicity effects of the test molecules were measured by determining percentage cell growth (PG) inhibition after 48 hours incubation. Optical density (OD) measurements of SRBderived color just before exposing the cells to the test compound (OD_{tzero}) and after 48 hours exposure to the test compound (OD_{test}) or the control vehicle (OD_{ctrl}) were recorded. Compounds were initially screened at 10^{-5} M to determine growth inhibition, and only compounds that showed more than 60% growth inhibition in at least eight cell lines from the panel were selected for a complete dose-response study at five different concentrations, *i.e.* 10^{-4} M, 10^{-5} M, 10^{-6} M, 10^{-7} M and 10^{-8} M, to determine growth inhibition (GI_{50}) values.

The percentage growth inhibition of 54 human cancer cell lines in the primary screen after exposure to compounds **3a**, **3d**, **5b** and **5c** are presented in Tables 1S–4S (Supplementary Data). The results showed that only compounds **5b** and **5c** met the criteria for subsequent testing to determine growth inhibition values (GI_{50}) in dose-response studies. At 10^{-5} M concentration, compounds **5b** and **5c** exhibited cytotoxic activity against leukemia cell lines HL-60(TB) and SR, lung cancer cell line NCI-H522, colon cancer cell lines COLO 205 and

HCT-116, CNS-cancer cell line SF-539, melanoma cell line MDA-MB-435, and breast cancer cell line BT-549. The naphthalene *trans*-stilbene analogue **3a**, exhibited significant growth inhibition against only one cell line, melanoma cell line MDA-MB-435 (96 % growth inhibition). Compound **3d** was inactive in the 10^{-5} M single dose screen.

Table 1 provides data from the five-dose study for compounds **5b, 5c** and the positive control, **DMU-212**, against the NCI panel of 54 human tumor cell lines. From this study (*Z*)-3-(naphthalen-2-yl)2-(3,4,5-trimethoxyphenyl)acrylonitrile (**5c**) was identified as the most active compound from the series of 2-naphthaleno *trans*-cyanostilbene analogs, and was found to have significant growth inhibitory effects against a wide variety of human cancer cell lines. Growth inhibition data for **5c** against 4 human cancer cell lines is shown in Fig. (2); GI_{50} values were in the range 21–25 nM.

Compound **5c** afforded GI_{50} values less than 100 nM against 98% of all the human cancer cell lines in the panel, and exhibited GI_{50} values below 50 nM in 73% of the cell lines in the panel. Analog **5c** was significantly effective against the following cancer cell lines: non-small cell lung cancer cell line NCI-H522 (GI_{50} = 27 nM), colon cancer cell line COLO 205 (GI_{50} = 22 nM), CNS cancer cell lines SF 295 and SF 539 (GI_{50} = 28 and 25 nM, respectively), melanoma cell lines M14, MDA-MB-435, and SK-MEL-5 (GI_{50} = 26, 21 and 23 nM, respectively), and breast cancer cell lines MDAMB-231/ATCC and MDA-MB-468 (GI_{50} = 26 and 27 nM). Compound **5b** showed significant growth inhibitory effects against several human cancer cell lines in the panel, but was generally less potent when compared to lead analog **5c**. Compound **5b** exhibited GI_{50} values less than 100 nM against 40% of the human cancer cells in the panel, and afforded GI_{50} values less than 50 nM against 14% of the cancer cells in the panel. Analog **5b** specifically afforded significant growth inhibitory effects against colon cancer cell line HCT-116 (GI_{50} = 45 nM), CNS cancer cell lines SF 295 and SF 539 (GI_{50} = 41 and 49 nM, respectively), melanoma cell lines M14, MDA-MB-435, and UACC-62 (GI_{50} = 48, 25, and 35 nM, respectively), and renal cancer cell line A498 (GI_{50} = 49 nM). Both **5c** and **5b** showed significantly improved growth inhibitory and cytotoxicity effects against almost all the 54 human tumor cell lines when compared to DMU-212.

3.2. Cytotoxicity of 5b and 5c Against MV4–11 Cells and Inhibition of Tubulin Polymerization

Compounds **5b** and **5c** were also screened for their *in vitro* cytotoxicity against cultured MV4–11 cells, and for their ability to inhibit tubulin polymerization utilizing both an immunofluorescence assay and antibody against tubulin (a marker for dynamic microtubules) in MV4–11 cells (Fig. 3) [22]. Cultured MV4–11 cells were treated with the above two compounds at seven different doses. Compound **5c** exhibited greater cytotoxicity (LD_{50}) towards the MV4–11 leukemia cell line compared to compound **5b** (Fig. 3A). In separate experiments cultured MV4–11 cells were exposed to compounds **5b** and **5c** for 2 hours and cell-based tubulin depolymerization assays performed. Polymerized tubulin in the cell pellet (P) and unpolymerized tubulin in the supernatant (S) were detected by immunoblotting using antibody against tubulin. Compounds **5b** and **5c** both demonstrated

>50% inhibition of tubulin polymerization at concentrations below their LD_{50} values, with **5c** being a more potent inhibitor of tubulin polymerization than **5b** (Fig. 3B).

3.3. Methodology for Determining the Cytotoxicity of Compounds 5b and 5c Against Cultured MV4–11 Cells

MV4–11 cells were cultured in Iscove's Modified Dulbecco's Media (IMDM) supplemented with 100 U/ml penicillin, 100 U/ml streptomycin, and 10% fetal bovine serum (Life technologies). Cells were seeded at 5×10^5/ml, and treated with the test compounds. At 48 hours post treatment, cells were stained with annexin V-FITC (BD Biosciences) and 1 μg/ml 7-AAD (Life Technologies). Percent dead cells were determined by flow cytometry as the percent of annexin V+ cells. Data were analyzed using Flowjo 9.3.2 for Mac OS X (TreeStar). Cell death was represented relative to vehicle control (DMSO).

3.4. Tubulin Polymerization Inhibition Assay

MV4–11 cells were treated with the indicated doses of compounds **5b** and **5c** for 2 hours. Cells were then lysed in microtubulestabilizing buffer (100 mM PIPES, 1 mM EGTA, 1 mM $MgSO_4$, 30% glycerol, 5% DMSO, 1 mM DTT, 0.02% NaN_3, 0.125% NP-40, pH 6.9) at 37 °C. Free tubulin (supernatants, SN) and polymerized tubulin (pellets, P) were separated and examined by immunoblotting using tubulin antibody. Both microtubule stabilizing and microtubule-destabilizing drugs inhibit hypoxia inducible factor-1 alpha accumulation and activity by disrupting microtubule function [23].

4. MOLECULAR DOCKING

In order to determine the molecular mechanism of tubulin inhibition by compounds **5b** and **5c**, we performed *in silico* docking of these molecules to their target, the αβ-tubulin dimer. Docking was performed using the web server-based SwissDock [24, 25] as described previously [26]. The tubulin dimer crystal structure PDB 1SA0 was used as the target molecule. The resulting docked poses were ranked based on their Fullfitness (FF) scores, and only the highest-scoring poses for each molecule were considered for further analyses. Manual inspection of the binding modes, determination of atomic interactions, surface area and distance measurements were accomplished using PyMOL (*The PyMOL Molecular Graphics system, v1.8, Schrödinger LLC*).

Similar to our observations earlier with some related resveratrol analogues [26], both compounds **5b** and **5c** docked to the colchicine-binding pocket at the tubulin dimer interface (Fig. 4). As shown in Fig. 4, **5b** and **5c** share most of the atomic interactions with the same set of amino acid residues of tubulin. These interactions consist entirely of van der Waals' contacts and no polar contacts are observed, once again consistent with our observations with related resveratrol analogues. The docking scores of both compounds **5b** and **5c** (Table 2), are in agreement with their observed potencies in the cellular cytotoxicity assay. As indicated in Table 2, compound **5c** shows a higher FF score, as well as a larger buried surface area (859 $Å^2$), as compared to **5b** (814 $Å^2$).

CONCLUSION

In conclusion, we have synthesized a small set of novel 2-naphthaleno stilbenes and cyanostilbenes and evaluated several of these compounds for their anticancer properties against a panel of 54 human tumor cell lines. The most active analogs, **5b** and **5c**, showed significantly improved growth inhibition against the human cancer cells in the NCI panel when compared to DMU-212. Of these compounds, analog **5c** was found to be the most potent anticancer agent and exhibited significant growth inhibitory effects against COLO 205, CNS SF 539 and melanoma SK-MEL 5 and MDA-MB-435 cell lines with GI_{50} values 25 nM. Analog **5b** also exhibited GI_{50} values in the range 25–41 nM against CNS SF 295 and melanoma MDA-MB-435 and UACC-62 cell lines. Compounds **5b** and **5c** were also cytotoxic towards the MV4–11 leukemia cell line with LD_{50} value of 450 nM and 200 nM, respectively, and demonstrated >50% inhibition of tubulin polymerization at concentration below their LD_{50} values in these cells. *In silico* docking studies suggest that compounds **5b** and **5c** bind favorably at the colchicine-binding pocket of the tubulin dimer, indicating that both **5b** and **5c** may inhibit tubulin polymerization through a mechanism similar to that exhibited by colchicine. Derivative **5c** demonstrated more favorable binding based on the docking score and buried surface area, as compared to compound **5b**, in agreement with the higher observed potency of **5c** against a broader range of tumor cell lines. Based on these results, analog **5c** is considered to be a lead compound for further optimization as a clinical candidate for treating a variety of cancers.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

ACKNOWLEDGEMENTS

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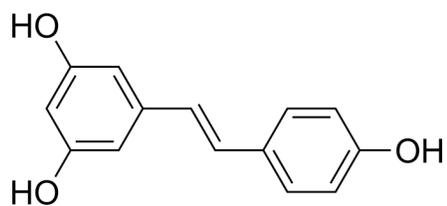
(A) Resveratrol
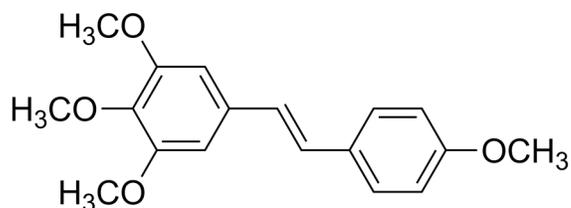
(B) 3,4,5,4'-trans-tetramethoxystilbene (DMU-212)
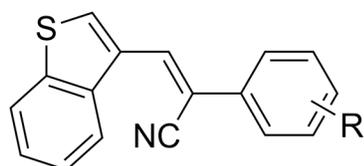
(C) (*Z*)-Benzothiophene acrylonitriles
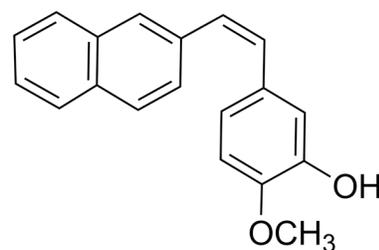
(D) Naphthalene analogues of combretastatin A-4

Fig (1).
Structures of resveratrol (**A**), DMU-212 (**B**), (*Z*)-benzothiopheneacrylonitriles (**C**) and the 2-naphthaleno analog of combretastatin A-4 (**D**).
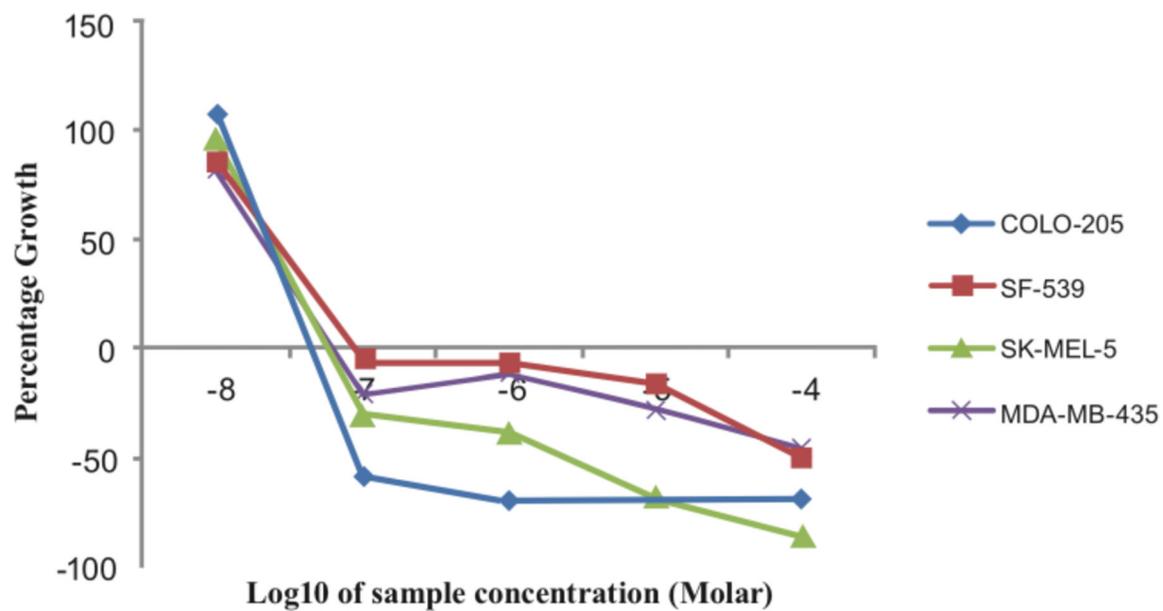
Fig. (2).
Dose-response curves for compound **5c** against human colon, CNS, and melanoma cancer cell lines.
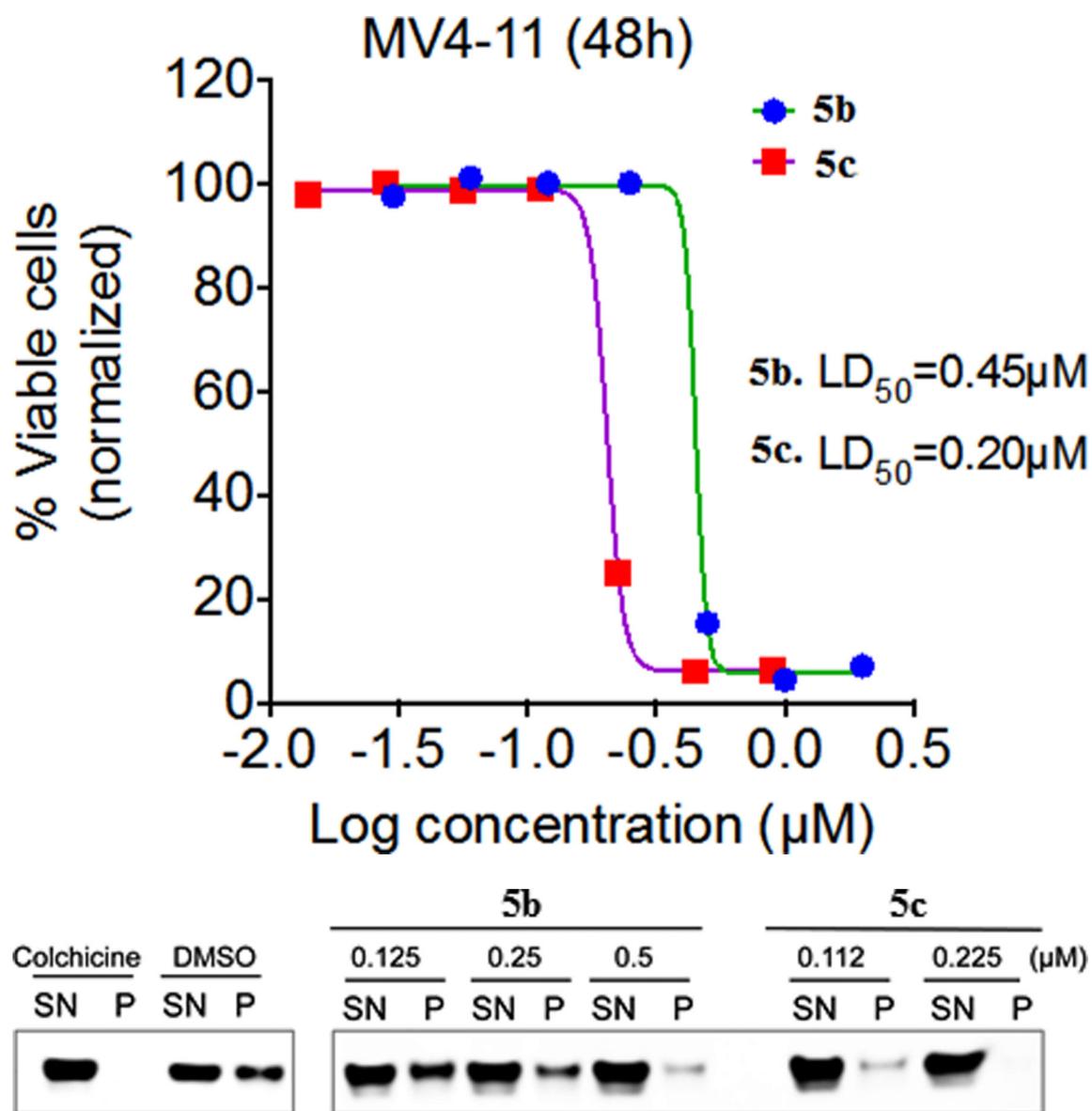
Fig. (3).
Effect of **5b** and **5c** on cell viability **(A)** and tubulin polymerization inhibition **(B)** in MV4–11 leukemia cells.
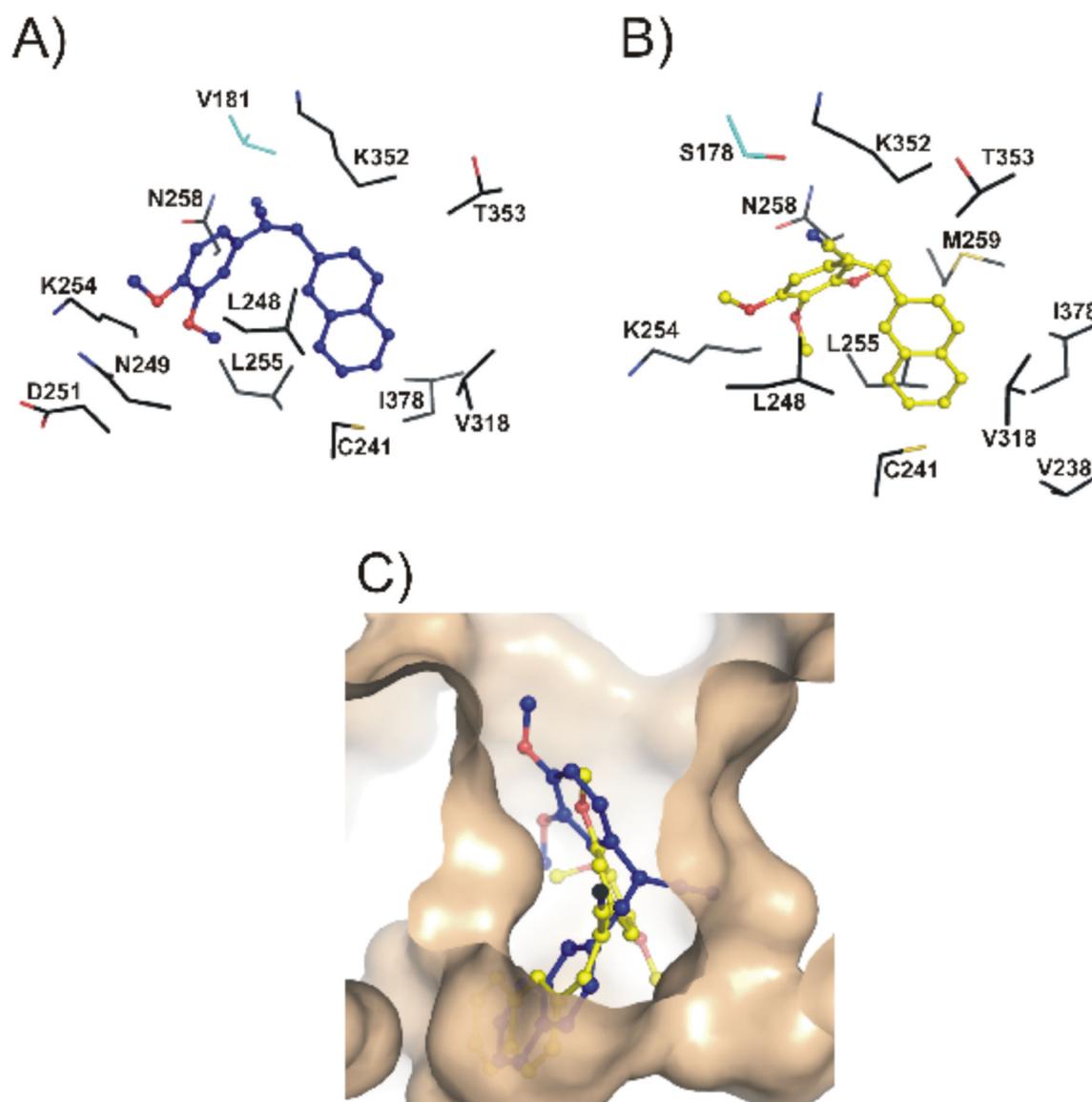
Fig. (4).

Compounds **5b**, and **5c** both docked to the colchicine-binding pocket of tubulin. The highest-scoring docked poses of compounds **5b** (*blue*) and **5c** (*yellow*) are shown as ball-and-stick models in panels **A** and **B**, respectively. The amino-acid residues of tubulin that are involved in van der Waals' interactions with each molecule are labeled, and are shown as sticks, where α-tubulin residues are *cyan*, and the β-tubulin residues are *black*. Panel C shows the top poses of these molecules bound at the colchicine-binding pocket of tubulin, which is shown as a solid surface in *wheat*. Compound **5b** can be observed to be slightly less deeply buried in the pocket, as compared to **5c**.

Scheme 1.
Synthesis of 2-naphthaleno *trans*-stilbenes **3a-3h.**

1a : R₁ = R₃ = OCH₃, R₂ = R₄ = H
1b : R₂ = OEt, R₁ = R₃ = R₄ = H
1c : R₂ = COOCH₃, R₁ = R₃ = R₄ = H
1d : R₂ = OMe, R₁ = R₃ = R₄ = H
1e : R₃ = Me, R₁ = R₂ = R₄ = H
1f : R₄ = Me, R₁ = R₂ = R₃ = H
1g : R₂ = F, R₁ = R₃ = R₄ = H
1h : R₂ = Cl, R₁ = R₃ = R₄ = H

3a : R₁ = R₃ = OCH₃, R₂ = R₄ = H
3b : R₂ = OEt, R₁ = R₃ = R₄ = H
3c : R₂ = COOCH₃, R₁ = R₃ = R₄ = H
3d : R₂ = OMe, R₁ = R₃ = R₄ = H
3e : R₃ = Me, R₁ = R₂ = R₄ = H
3f : R₄ = Me, R₁ = R₂ = R₃ = H
3g : R₂ = F, R₁ = R₃ = R₄ = H
3h : R₂ = Cl, R₁ = R₃ = R₄ = H

Scheme 1.
Synthesis of 2-naphthaleno *trans*-stilbenes **3a-3h.**

1a : $R_1 = R_3 = OCH_3$, $R_2 = R_4 = H$
1b : $R_2 = OEt$, $R_1 = R_3 = R_4 = H$
1c : $R_2 = COOCH_3$, $R_1 = R_3 = R_4 = H$
1d : $R_2 = OMe$, $R_1 = R_3 = R_4 = H$
1e : $R_3 = Me$, $R_1 = R_2 = R_4 = H$
1f : $R_4 = Me$, $R_1 = R_2 = R_3 = H$
1g : $R_2 = F$, $R_1 = R_3 = R_4 = H$
1h : $R_2 = Cl$, $R_1 = R_3 = R_4 = H$

3a : $R_1 = R_3 = OCH_3$, $R_2 = R_4 = H$
3b : $R_2 = OEt$, $R_1 = R_3 = R_4 = H$
3c : $R_2 = COOCH_3$, $R_1 = R_3 = R_4 = H$
3d : $R_2 = OMe$, $R_1 = R_3 = R_4 = H$
3e : $R_3 = Me$, $R_1 = R_2 = R_4 = H$
3f : $R_4 = Me$, $R_1 = R_2 = R_3 = H$
3g : $R_2 = F$, $R_1 = R_3 = R_4 = H$
3h : $R_2 = Cl$, $R_1 = R_3 = R_4 = H$

4a : R₂ = NO₂, R₁ = R₃ = H
4b : R₂ = R₃ = OMe, R₁ = H
4c : R₁ = R₂ = R₃ = OMe
4d : R₂ = OMe, R₁ = R₃ = H
4e : R₁ = R₂ = R₃ = H
4f : R₁ = CF₃, R₂ = R₃ = H
4g : R₂R₃ = OMeO, R₁ = H
4h : R₂ = OH, R₁ = R₃ = H

5a : R₂ = NO₂, R₁ = R₃ = H
5b : R₂ = R₃ = OMe, R₁ = H
5c : R₁ = R₂ = R₃ = OMe
5d : R₂ = OMe, R₁ = R₃ = H
5e : R₁ = R₂ = R₃ = H
5f : R₁ = CF₃, R₂ = R₃ = H
5g : R₂R₃ = OMeO, R₁ = H
5h : R₂ = OH, R₁ = R₃ = H

Scheme 2.
Synthesis of 2-naphthaleno *trans*-cyanostilbenes **5a-5h.**

Table 1.

Growth inhibition GI_{50}[a] data for compounds **5b**, **5c** and **DMU-212** against a panel of 54 human cancer cell types.

Panel/cell line	5b GI_{50}[a,b]	5c GI_{50}[a,b]	DMU-212 GI_{50}[a]
Leukemia CCRF-CEM	0.297	**0.052**	2.89
HL-60(TB)	**0.060**	**0.031**	3.14
MOLT-4	1.010	**0.061**	3.22
RPMI-8226	0.342	**0.053**	6.42
SR	**0.068**	**0.032**	3.93
Non-Small Cell Lung Cancer A549/ATCC	0.146	**0.056**	3.70
HOP-62	**0.095**	**0.044**	3.14
HOP-92	1.410	**0.038**	7.23
NCI-H226	16.100	NA	4.44
NCI-H23	0.598	**0.048**	3.37
NCI-H522	**0.067**	**0.027**	3.70
Colon Cancer COLO 205	0.131	**0.022**	2.07
HCC-2998	0.331	**0.039**	3.59
HCT-116	**0.045**	**0.030**	3.23
HCT-15	**0.057**	**0.031**	2.82
KM12	**0.080**	**0.039**	2.32
SW-620	**0.054**	**0.038**	3.63
CNS Cancer SF-268	0.651	**0.051**	7.59
SF-295	**0.041**	**0.028**	2.18
SF-539	**0.048**	**0.025**	2.18
SNB-19	0.414	**0.061**	4.85
SNB-75	**0.091**	**0.044**	1.88
U251	0.218	**0.036**	3.07
Melanoma LOX IMVI	0.145	**0.031**	4.89
M14	**0.048**	**0.026**	2.81
MDA-MB-435	**0.025**	**0.021**	1.04
SK-MEL-2	0.558	NA	3.95
SK-MEL-28	0.155	**0.049**	3.86
SK-MEL-5	**0.066**	**0.023**	2.50
UACC-62	**0.035**	**0.039**	2.37
Ovarian Cancer IGROV1	0.660	**0.076**	5.29
OVCAR-3	**0.051**	**0.036**	3.45
OVCAR-4	0.927	**0.077**	4.00

Panel/cell line	5b GI$_{50}$[a,b]	5c GI$_{50}$[a,b]	DMU-212 GI$_{50}$[a]
OVCAR-5	0.244	**0.033**	3.24
OVCAR-8	0.435	**0.043**	4.54
NCI/ADR-RES	0.244	**0.031**	3.01
SK-OV-3	0.113	**0.030**	3.34
Renal Cancer 786–0	**0.062**	**0.035**	5.42
A498	**0.049**	**0.033**	0.74
ACHN	0.306	**0.046**	4.51
CAKI-1	**0.091**	**0.051**	3.00
RXF 393	**0.077**	**0.054**	2.36
SN 12C	0.888	**0.056**	5.52
TK-10	17.800	**0.057**	3.98
UO-31	0.761	2.04	3.69
Prostate Cancer PC-3	0.160	**0.041**	3.22
DU-145	0.381	**0.038**	4.23
Breast Cancer MCF7	0.157	**0.032**	1.66
MDA-MB-231/ATCC	0.376	**0.026**	3.74
HS 578T	0.154	**0.038**	3.09
BT-549	0.164	**0.037**	5.13
T-47D	**0.064**	**0.048**	3.55
MDA-MB-468	**0.094**	**0.027**	2.22

NA: Not analyzed,

[a] GI$_{50}$: 50% Growth inhibition, concentration of drug resulting in a 50% reduction in net protein increase compared with control cells.

[b] GI$_{50}$ values <100 nM are bolded.

Table 2.

FullFitness (FF) scores and buried surface areas of the highest-scoring pose for **5b** and **5c** molecules docked to tubulin. FF scores were obtained from SwissDock, while the buried surface area was calculated using PyMOL.

Compound	FF score (kcal/mol)	Buried surface area ($Å^2$)
5b	4224.9	814
5c	4298.7	859