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Synthesis, molecular docking, and antitumoral activity of alnustone-like compounds against estrogen receptor alpha-positive human breast cancer

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Abstract: Alnustone-like compounds are promising inhibitors for estrogen receptor α (ER-α), which is a novel cancer therapeutic target. Therefore, 10 alnustone-like compounds with substituents at the phenyl rings were synthesized by condensation of 4-phenyl-2-butanones and cinnamaldehydes via in situ enamination. The compounds displayed either protective activity or inhibited cell growth and proliferation of human breast cancer cells. Molecular docking studies indicated that the synthesized compounds interact with ER-α efficiently. In this work, the protective and inhibitive roles of the synthesized compounds were related to their functional groups and to their binding mode of action on ER-α protein. The compounds are potential drug candidates as ER-α antagonists.

Key words: Alnustone-like compounds, MCF-7, breast cancer, estrogen receptor α, diarylheptanoids

1. Introduction

Diarylheptanoids with a typical aryl-C_7-aryl structure occur naturally.[1] Alnustone (**1**), a nonphenolic diarylheptanoid, was first isolated from *Alnus pendula* (Betulaceae) and characterized 4 decades ago (Figure 1).[2,3]

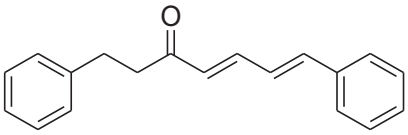

Figure 1. Structure of alnustone (**1**).

The first synthesis of alnustone starting from 3-phenylpropionyl chloride was performed in 5 steps by Sakakibara et al.[4] Then alnustone (**1**) was synthesized starting from benzaldehyde by Vig et al.[5] In subsequent studies, an alternative synthesis for the preparation of alnustone (**1**) was developed.[6,7] In a previous study, we also reported a short and efficient method for synthesis of alnustone (**1**) based on in situ enamination of 4-phenyl-2-butanone (**2**) and cinnamaldehyde (Scheme 1).[8]

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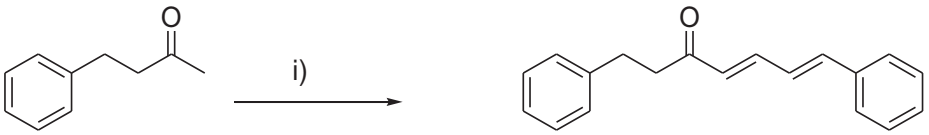


2 **1**

Scheme 1. (i) Pyrrolidine, AcOH, cinnamaldehyde, 60 h, 73%

In a later study, we developed a methodology for preparation of 2 natural alnustone-like compounds.[9] In this context, Baranovsky et al. described 3 alternative strategies for preparation of alnustone-like compounds.[10]

The biological activities of alnustone (**1**) as well as its synthesis have been the subject of numerous studies. Remarkable antihepatotoxic activity of alnustone (**1**) amongst many natural diarylheptanoids was reported.[11]

The anti-inflammatory activity of alnustone (**1**) isolated from *Curcuma xanthorrhiza* was reported by Claeson et al.[12] The antibacterial activity of alnustone (**1**) against well-known bacteria species was reported by Huang et al.[13] They also reported alnustone to have an antiemetic activity.[14] Additionally, weak estrogenic activity of isolated alnustone (**1**) from rhizomes of *Curcuma comosa* was found by Suksamrarn et al.[15] Studies by Grienke et al. revealed that alnustone (**1**) shows neuraminidase inhibitory activity, and it was concluded that the compound may be employed as an antiviral agent.[16] Recently, Li et al. isolated some chemical compounds from *Alpinia katsumadai* Hayata seeds and evaluated their antitumor activities in vitro. Among the isolated compounds, they reported that alnustone (**1**) exhibited significant antitumor activity against Bel-7402 (human hepatocellular carcinoma cells) and LO-2 (human normal liver cells) cell lines.[17]

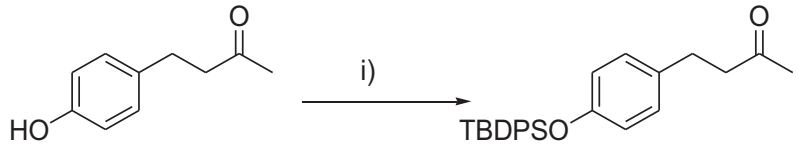
Breast cancer is the most common cancer type worldwide among women. In the United States, breast cancer accounts for 14.1% of all expected cancer cases; in 2013, breast cancer accounted for approximately 232,340 new cancer cases and 39,620 deaths.[18] The prognosis of breast cancer depends on genetic and lifestyle-related factors, aging, and the hormone estrogen. Estrogen stimulates both normal and malignant mammary tissues. ER-α is overexpressed in 70% of breast cancer cases (known as ER-α-positive breast cancer), and the binding of estrogen to the ER-α protein triggers the formation of breast tumors. Therefore, many ER-α antagonists have been developed for blocking ER-α protein in breast cancer treatment.[19–21] In the present study, we aimed to design and synthesize some new alnustone-like compounds and to determine their cytotoxicity against the MCF-7 human ER-α-positive breast cancer cell line. To achieve this goal, 10 new alnustone-like compounds containing different substituents on the phenyl rings at the 1 and 7 positions were synthesized. We proposed that substitution at different positions of the phenyl ring may elevate antitumoral activity; therefore, alnustone-like compounds were designed and compared to alnustone along with the FDA-approved tamoxifen and paclitaxel.

2. Results and discussion

2.1. Synthesis

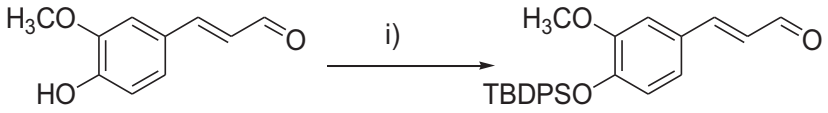
Our starting materials were 4-phenyl-2-butanones and cinnamaldehydes. The starting materials with OH groups at the phenyl rings first underwent group protection by treating with *tert*-butyldiphenylsilyl chloride (TBDPSCl) to give OTBDPS derivatives (Schemes 2 and 3). Condensations of the 4-phenyl-2-butanones and cinnamaldehydes were performed via in situ enamination using pyrrolidine and AcOH in Et_2O (Scheme 4). Thus, 10 different alnustone-like compounds (**1**, **7**, **9**, **11**, **13**, **15**–**19**), differing with respect to the aryl

substituents, were prepared in a variety of yields. The prepared alnustones with OTBDPS derivatives (**7**, **9**, **11**, **13**) were transformed to the corresponding OH derivatives (**8**, **10**, **12**, **14**) by treating with *tetra-n*-butylammonium fluoride (TBAF) (Scheme 5).



3 **4**

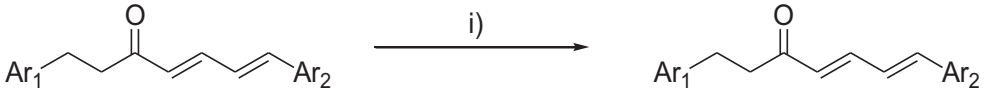
Scheme 2. Protection of **3**. (i) TBDPSCl, imidazole, CH_2Cl_2, 20 °C, 24 h, 96%.



5 **6**

Scheme 3. Protection of **5**. (i) TBDPSCl, imidazole, CH_2Cl_2, 20 °C, 24 h, 88%.

Scheme 4. Synthesis of alnustones. (i) Pyrrolidine, AcOH, Et_2O, 0 °C, rt, 60–96 h. (For Ar_1 and Ar_2 see Table 1).



7, 9, 11, 13 **8, 10, 12, 14**

(Ar_1 or Ar_2 with OTBDPS) (Ar_1 or Ar_2 with OH)

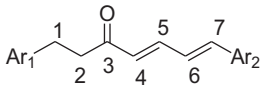
Scheme 5. Deprotection of OTBDPS groups. (i) TBAF, THF, 0 °C → rt, 30 min–1 h. (For Ar_1 and Ar_2 see Table 1).

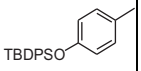
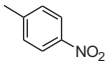
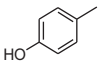
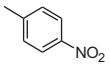
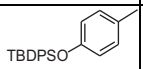
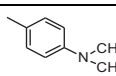
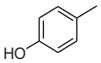
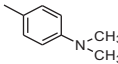
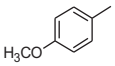
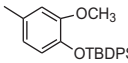
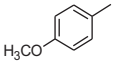
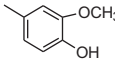
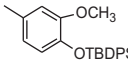
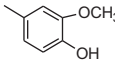
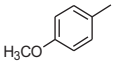
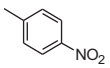
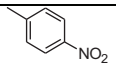
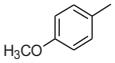
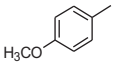
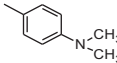
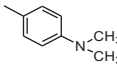
2.2. NMR structural elucidations

The C_1–C_7 skeletons of the alnustones and their OTBDPS derivatives showed similar ^1H and ^{13}C spectra (Table 1). In this context, the 4 hydrogens attached to C-1 and C-2 resonate as an A_2B_2 system at δ 3.01–2.79 ppm. The hydrogens at C-4 resonate as doublets at δ 6.15–6.38 ppm with $J_{4,5}$ = 15.4–16.3 Hz. The H-5 protons were observed as dd at the downfield region (δ 7.26–7.38 ppm), as expected due to representing the β-hydrogen of an α,β-unsaturated system. The $J_{5,6}$ varied from 7.0 Hz to 11.0 Hz. H-6 and H-7 usually resonate as an AB system at δ 6.66–7.03 ppm.

In the same manner, the ^{13}C NMR spectra of the C_1–C_7 skeletons of the alnustones showed similar chemical shifts (δ). The C-1 carbons resonate at δ 29.4–30.7 ppm, and the C-2 carbons resonate at δ 42.2–43.4 ppm. The C-3 carbons, which are carbonyl carbons, resonate at δ 199.3–199.9 ppm. The C-5 carbons (δ 141.4–144.5 ppm) and C-7 carbons (δ 138.2–143.4 ppm) resonate downfield, as expected from the structure of the conjugated dienone system. The C-6 carbons (δ 127.0–132.0 ppm) and C-4 carbons (δ 122.1–131.1 ppm) appear in the olefinic region.

Table 1. ^{1}H NMR and ^{13}C NMR data of the alnustone skeletons.



			δ (ppm) Values of protons and carbons				
	Ar_1	**Ar_2**	**1 2**	**3**	**4**	**5**	**6 7**
1			3.01–2.91(A_2B_2) 30.4 42.6	– 199.6	6.29 (d, 15.4) 126.3	overlapped 143.0	6.87 6.93 (dd, 15.5, 10.0) (d, 15.5) 129.7 141.6
7			2.84 (A_2B_2) 29.5 43.1	– 199.6	6.33 (d, 15.4) 131.1	7.27 (dd, 15.6, 8.8) 141.2	6.80–7.01 (AB, m) 132.0 138.3
8			2.91 (A_2B_2) 29.2 43.0	– 199.4	6.37 (d, 16.3) 130.8	7.28 (ddd, 16.3, 7.0, 1.5) 141.1	6.90–6.99 (AB, m) 131.9 138.2
9			2.83–2.80 (A_2B_2) 29.6 42.2	– 199.7	6.15 (d, 15.4) 122.1	7.31 (dd, 15.0, 11.0) 144.1	overlapped 6.85 – (d, 15.8) 127.0 142.3
10			2.98–2.80 (A_2B_2) 30.0 43.4	– 199.8	6.20 (d, 15.4) 123.6	7.38 (dd, 15.4, 10.6) 145.0	6.82 6.97 (dd, 15.4, 10.6) (d, 15.4) 128.6 143.4
11			2.98–2.79 (A_2B_2) 29.4 42.5	– 199.5	6.20 (d, 15.4) 124.7	7.26 (dd, 15.2, 10.8) 143.1	6.66 6.77 (d, 15.4,10.8) (d, 15.4) 129.3 141.6
12			2.93–2.85 (A_2B_2) 29.7 42.8	– 199.9	6.23 (d, 15.4) 124.7	7.30 (dd, 15.4, 11.0) 143.4	6.72 6.85 (dd, 15.4, 10.6) (d, 15.4) 128.7 141.8
13			2.98–2.87 (A_2B_2) 30.3 42.3	– 199.4	6.21 (d, 15.4) 124.7	overlapped 143.2	6.66 6.82 (dd, 15.6, 10.8) (d, 15.6) 128.5 141.0
14			3.00–2.90 (A_2B_2) 30.5 42.5	– 199.7	6.25 (d, 15.5) 124.7	7.31 (dd, 15.5, 10.6) 143.4	6.73 6.86 (dd, 15.7, 10.6) (d, 15.7) 128.63 141.8
15			2.96–2.87 (A_2B_2) 29.4 43.2	– 199.5	6.36 (d, 15.9) 131.1	7.31 (dd, 15.9, 9.6) 141.2	6.92–7.03 (AB, m) 131.9 138.3
16			3.01–2.88 (A_2B_2) 30.3 42.9	– 199.3	6.38 (d, 15.4) 131.0	overlapped 141.3	6.88–7.03 (AB, m) 131.9 138.4
17			3.00–2.90 (A_2B_2) 29.5 42.8	– 199.8	6.28 (d, 15.7) 126.9	overlapped 142.9	6.82–6.95 (AB, m) 129.8 141.6
18			2.95–2.83 (A_2B_2) 29.8 42.6	– 199.8	6.20 (d, 15.4) 122.3	7.34 (dd, 15.4, 11.0) 144.5	6.66–6.78 (AB, m) 127.2 142.6
19			3.01–2.90 (A_2B_2) 30.7 42.3	– 199.7	6.20 (d, 15.4) 122.3	overlapped 144.5	overlapped 6.88 (d, 15.2) 127.1 142.6

2.3. Anticancer activity of new alnustone-like compounds

2.3.1. In vitro studies

A variety of drugs may be employed for different therapeutic strategies against breast cancer tumor genesis. Paclitaxel is widely used in breast cancer chemotherapy to destroy malignant cells, and tamoxifen is used to

prevent unregulated cell growth and to block estrogen receptor alpha (ER-α) to stop the transformation of normal cells into malignant cells. Therefore, tamoxifen is prescribed to patients after surgical treatment and chemotherapy. Thus, this ER-α antagonist is used in breast cancer patients as a protective agent.

In our experimental set up, we employed tamoxifen and paclitaxel as positive controls to compare with the alnustone derivatives. Tamoxifen can bind to the ER-α ligand binding site, but paclitaxel may not fit into this ligand binding cavity. However, paclitaxel treated MCF-7 cells decrease the concentration of the ER-α protein, as previously reported in the literature. Therefore, we did not include paclitaxel in our docking calculations but employed this compound in the cell proliferation assay.[22] The cell proliferation assay showed that **1**, **10**, **12**, **14**, and **17** (Group A) displayed paclitaxel-like activity, and **15**, **16**, and **19** (Group B) displayed tamoxifen-like activity. Compounds **15** and **18** (Group C) displayed a transitional activity between these drugs (Figure 2).

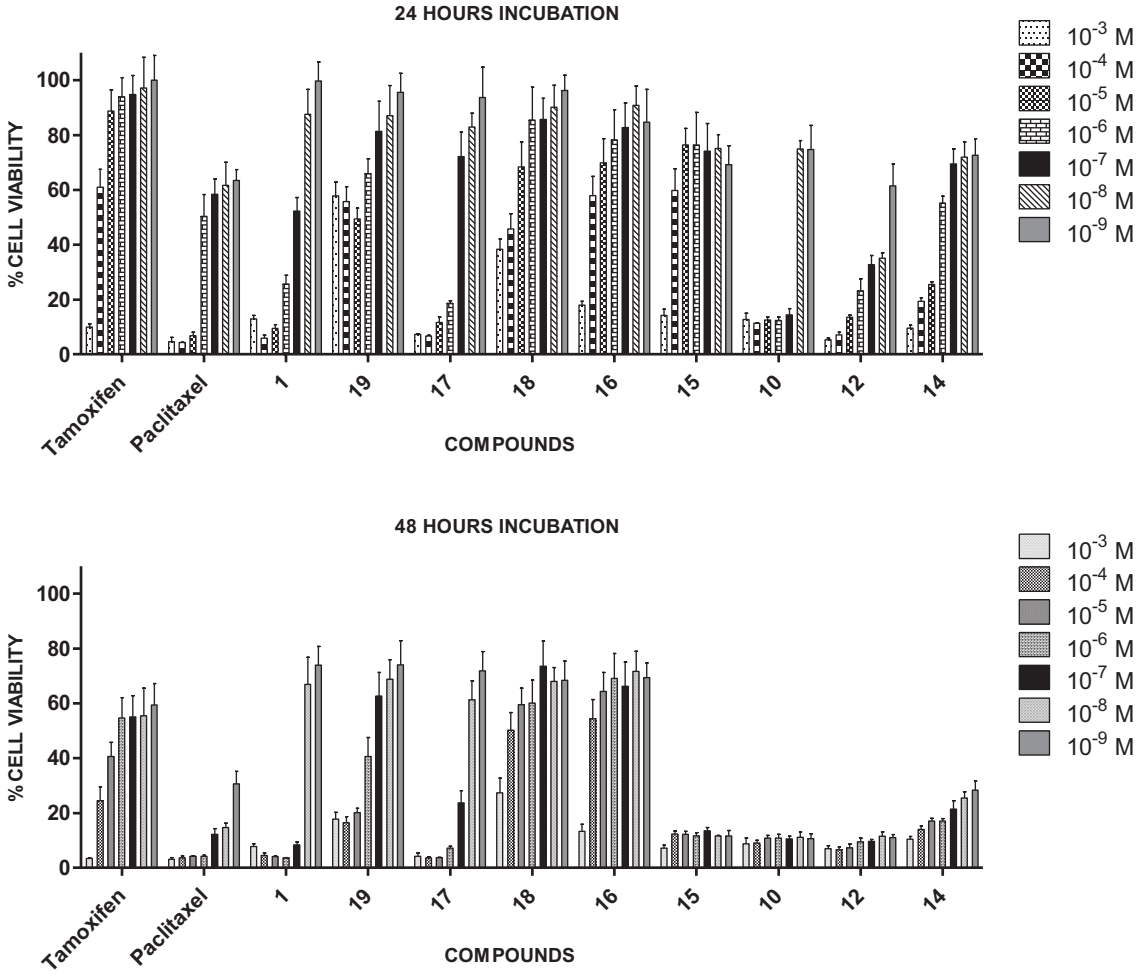


Figure 2. Anticancer activities of tamoxifen, paclitaxel, and 10 alnustone-like compounds on the MCF-7 cell line after 24 and 48 h of incubation. Compound **8** did not provide consistent statistical results; therefore it is omitted from the evaluation.

Paclitaxel and Group A compounds at 0.1 to 1 mM concentration showed cytotoxic effects on MCF-7 cells and killed almost 90% of the total number of cells after 24 h. At lower concentration of the compounds (1 nM to 1 μM) the cytotoxic effect on MCF-7 cells was around 20%, and after 48 h the survival percentage

was decreased to that of the millimolar levels. To better compare the results IC_{50} values are given in Table 2 as well.

Table 2. IC_{50} values of tamoxifen, paclitaxel, and 10 alnustone-like compounds for the MCF-7 cell line.

Compounds	IC_{50} Value
Tamoxifen	0.5 mM
Paclitaxel	2.09 μM
1	0.08 μM
19	0.26 μM
17	0.25 μM
18	15.39 μM
16	$>$ 0.5 mM
15	0.12 mM
10	0.69 μM
12	$<$ 1 nM
14	2.66 μM

In contrast to paclitaxel, tamoxifen and Group B compounds' impact on MCF-7 cells was tolerable, and 90% of the cells survived after 24 h; after 48 h, the survival was decreased to 65%. This cell survival percentage comes from the aforementioned preventive action of the ER-α antagonist compound. This competitive antagonist action blocks breast cancer cell growth rather than killing the malignant cells.

Compounds **15** and **18** displayed cytotoxic effects between Groups A and B. This behavior may be related to the conformational state of the ER-α protein, as will be further discussed in the docking section.

Group A compounds carry hydroxyl and methoxy groups, and paclitaxel has several hydroxyl groups. These radical groups generate oxidative stress through reactive oxygen species (ROS) in the cell line. ROS may inhibit cancer cell proliferation and may induce apoptosis or autophagy. Tamoxifen and Group B compounds do not have hydroxyl groups, and this may reduce their ability to exert a higher cytotoxic effect on MCF-7 cells. Tamoxifen carries a dimethylamino group, and this group may decrease the cytotoxic effect of the compound, as evidenced by comparing synthesized compounds **10** and **17**–**19**. The addition of a dimethylamino group to compound **17** forms compound **18**, and the elimination of a hydroxyl group from compound **10** forms compound **19**. In both cases, we observed a decrease in cell survival percentage and the cytotoxicity effect. This strategy may be advantageous for drugs designed to be used as protective agents against breast cancer rather than to be used for malignant cell killing.

2.3.2. Docking calculations

Alnustone (**1**) and its derivatives were ligated to human ER-α protein by docking studies. The studies monitored the binding of the ligands and 2 types of binding were determined, as observed in Figure 3. Group A compounds bind ER-α protein rather differently than Group B compounds bind ER-α protein (Figure 3).

Group A compounds preferentially bind to the pocket formed by Thr347, Ala357, Leu387, and Leu525, and Group B compounds interact with Glu323, Ile326, Glu353, Arg394, and Phe445. Group B binds to the binding pocket approximately 15 Å higher than Group A compounds. Thus, Group A compounds tighten helixes 1, 4, 5, and 6, and this action inhibits the allosteric signaling of the protein and probably decreases its specific interaction with DNA in the nucleus by not allowing the proper zinc finger conformational change (Figure 3). However, Group B ligands interact with the protein at the upper site of the binding pocket, possibly

allowing more flexible allosteric movement to the protein, and ER-α may partly maintain its DNA binding function. Compounds **15** and **18** bind to a region between Group A and Group B and display a transitional behavior.

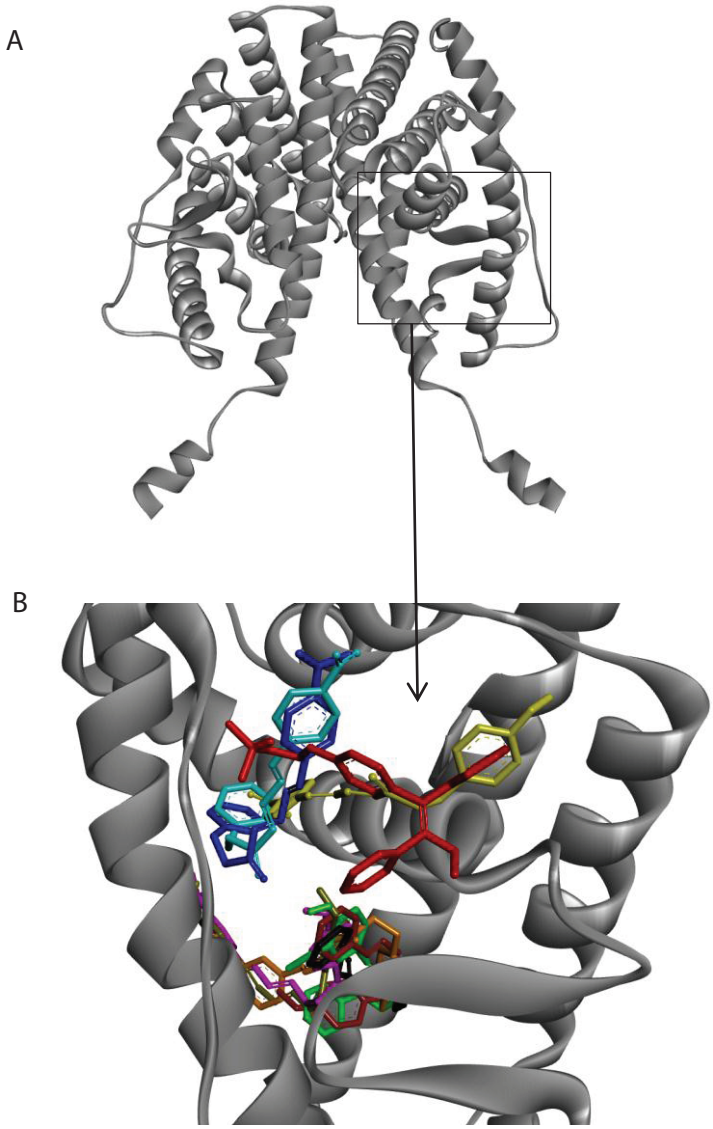


Figure 3. A) Structure of human estrogen receptor alpha (ER-α) **B)** Binding regions of tamoxifen and alnustone-like compounds (Tamoxifen: red, 1: black, 19: blue, 16: cyan, 15: yellow, 17: brown, 18: cement, 10: pink, 12: orange, 14: green).

Our theoretical calculations (Table 3) showed that all compounds bind to the protein with similar binding energies, and the differences in the function originate from the mode of the binding region. The binding region determines the protein allosteric movement accessibility and its function. Therefore, the loss of ER-α function decreases MCF-7 cell survival, and this may explain why Group A compounds kill more cancer cells than Group B compounds do.

Table 3. Docking calculation results of tamoxifen and 10 alnustone-like compounds on human ER-α protein.

Compounds	Est. free energy of binding	Est. inhibition constant, Ki
Tamoxifen	–7.14 kcal/mol	5.88 μM
1	–8.44 kcal/mol	648.68 nM
19	–7.25 kcal/mol	903.35 nM
17	–7.96 kcal/mol	1.47 μM
18	–7.73 kcal/mol	2.17 μM
16	–7.68 kcal/mol	512.80 nM
15	–7.97 kcal/mol	2.37 μM
10	–8.58 kcal/mol	2.36 μM
12	–8.99 kcal/mol	1.39 μM
14	–8.20 kcal/mol	982.27 nM

3. Experimental

3.1. General

The chemicals used in the synthesis of the new alnustone-like compounds designed in this study were as follows: 4-hydroxy-3-methoxycinnamaldehyde, imidazole, *tert*-butyldiphenylsilyl chloride (TBDPSCl), 4-phenyl-2-butanone, 4-(4-hydroxyphenyl)-2-butanone, pyrrolidine, 4-nitrocinnamaldehyde, *tetra-n*-butylammonium fluoride 1.0 M in THF (TBAF) (Aldrich), dichloromethane, hexane, sodium sulfate, acetic acid, diethyl ether, hydrochloric acid 37%, methanol, tetrahydrofuran (THF), silica gel for preparative TLC (254–366 mesh ASTM), silica gel 60 for column chromatography (70–230 mesh ASTM) (Merck), ethyl acetate (Riedel-de Haen), 4-(dimethylamino)cinnamaldehyde (Fluka), and cinnamaldehyde, 4-(4-methoxyphenyl)-2-butanone (SAFC). The melting points were measured on an Electrothermal 9100 melting point apparatus (IA9100, UK). ^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz) spectra were recorded with a Varian 400 MHz FT spectrometer (Danbury, CT, USA). The chemical shifts are reported as (δ) ppm. The assignments of the numbered hydrogens and carbons were performed by comparing similar structures. TMS was used as the internal standard. Coupling constants (J) are reported in Hertz. The elemental analyses were performed on a Leco CHNS-932.

The MCF-7 cell line was obtained from ATCC (American Type Culture Collection, USA). Dulbecco's modified Eagle's medium (DMEM) was from Sigma-Aldrich. Fetal bovine serum and trypsin–EDTA were purchased from Biological Industries Ltd. (Haemek, Israel). L-glutamine–penicillin–streptomycin solution was from Sigma-Aldrich (Steinheim am Albuch, Germany). The XTT (2,3-bis-(2-methoxy-4-nitro-5-sulfophenyl)-2H-tetrazolium-5-carboxanilide) cell proliferation kit was obtained from Roche. Tamoxifen (brand name: Nolvadex) and paclitaxel (brand name: Taxol) were supplied from AstraZeneca and Kocak Farma, respectively.

3.2. Synthesis

3.2.1. 4-(4-tert-Butyldiphenylsilyloxyphenyl)-2-butanone (4)

A 3.000-g (18.3 mmol) sample of 4-(4-hydroxyphenyl)-2-butanone (**3**), 2.488 g (36.5 mmol) of imidazole, and 4.755 g (17.3 mmol) of TBDPSCl were dissolved in dichloromethane (50 mL). The reaction mixture was stirred at room temperature for 24 h. The progress of the reaction was followed by TLC using an EtOAc–hexane system. The reaction mixture was extracted with CH_2Cl_2 (2 $\times$ 20 mL) and dried (Na_2SO_4). Evaporation of the solvent and chromatography of the residue on a silica gel (70-230 mesh) column eluted with 7.5:2.5

hexane–EtOAc gave 4-(4-tert-butyldiphenylsilyloxyphenyl)-2-butanone (**4**) (6.686 g, 96%) (Colorless solid with mp 75–76 °C). ^{1}H NMR (400 MHz, $CDCl_3$)δ (ppm): 7.71 (dd, 4H, 2 × H-2″/H-6″, J = 8.0, 1.5 Hz), 7.44–7.34 (m, 6H, 2 × H-3″/H-5″, 2 × H-4″), 6.90 (dm, 2H, H-2′/6′, J = 7.8 Hz), 6.67 (dm, 2H, H-3′/5′, J = 7.8 Hz), 2.77–2.63 (A_2B_2 system, m, 4H, 2 × H-3 and 2 × H-4), 2.09 (s, 3H, CH_3), 1.09 (s, 9H, $C(CH_3)_3$). ^{13}C NMR (100 MHz, $CDCl_3$)δ (ppm): 208.5 (C-2), 154.1 (C-4′), 135.8 (C-2″/6″), 133.5 (C-1′), 133.3 (C-1″), 130.1 (C-2′/6′), 129.2 (C-4″), 127.9 (C-3″/5″), 119.8 (C-3′/5′), 45.6 (C-3), 30.4 (C-1), 29.2 (C-4), 26.7 ($C(\underline{CH}_3)_3$), 19.7 ($C(CH_3)_3$).

3.2.2. 3-[4-(tert-Butyldiphenylsilyloxy)-3-methoxyphenyl]propenal (6)

The procedure above described for synthesis of **4** was applied to **5** to afford **6**. Crude compound was purified on a silica gel (70–230 mesh) column eluted with 8:2 hexane-EtOAc gave 3-[4-(tert-butyldiphenylsilyloxy)-3-methoxyphenyl]propenal (**6**) (88%) (Brownish solid with mp 92–94 °C). ^{1}H NMR (400 MHz, $CDCl_3$)δ (ppm): 9.61 (d, 1H, H-1, $J_{1,2}$ = 7.7 Hz), 7.70 (dd, 4H, 2 × H-2″ and 2 × H-6″, J = 7.7, 1.3 Hz), 7.44–7.34 (m, 6H, 2 × H-3″, 2 × H-4″, 2 × H-5″), 7.33 (d, 1H, H-3, $J_{2,3}$ = 15.7 Hz), 6.96 (d, 1H, H-2′, $J_{2',6'}$ = 1.8 Hz), 6.90 (dd, 1H, H-6′, $J_{5',6'}$ = 8.0 Hz, $J_{2',6'}$ = 1.8 Hz), 6.71 (d, 1H, H-5′, $J_{5'6'}$ = 8.0 Hz), 6.54 (dd, 1H, H-2, $J_{2,3}$ = 15.7 Hz, $J_{1,2}$ = 7.7 Hz), 3.62 (s, 3H, OCH_3), 1.12 (s, 9H, $C(\underline{CH}_3)_3$). ^{13}C NMR (100 MHz, $CDCl_3$)δ (ppm): 193.9 (C-1), 153.4 (C-3), 151.2 (C-4′), 148.6 (C-3′), 135.5 (C-2″/6″), 133.2 (overlapped C-1′ and C-1″), 130.1 (C-4″), 127.9 (C-3″/5″), 126.9 (C-2), 123.1 (C-6′), 120.7 (C-5′), 111.4 (C-2′), 55.6 (OCH_3), 26.8 ($C(\underline{CH}_3)_3$), 20.0 ($\underline{C}(CH_3)_3$).

3.2.3. General procedure for the preparation of the alnustones

The corresponding ketone (1 mmol) in 5 mL of Et_2O was added dropwise over 10 min at 0 °C to a solution of 1.1 mmol of pyrrolidine and 1.1 mmol of acetic acid in 5 mL of Et_2O. After additional stirring for 30 min, a solution of 1 mmol cinnamaldehyde in 5 mL of Et_2O was added dropwise over 30 min followed by stirring for 60–96 h at room temperature. Then 1.0 M HCl (2 mL) was added to the reaction mixture. The organic phase was extracted with Et_2O (2 × 50 mL), then washed with H_2O (2 × 30 mL), and dried (Na_2SO_4). Evaporation of the solvent and chromatography of the residue on a silica gel (70–230 mesh) column eluted with hexane–EtOAc gave alnustones.

1,7-Diphenylhepta-4,6-dien-3-one (Alnustone) (**1**)[8]: 60 h, 73%. Yellow solid. mp 60–62 °C. ^{1}H NMR (400 MHz, $CDCl_3$)δ (ppm): 7.47 (d, H-2″/6″, 2H, J = 7.6 Hz), 7.39–7.21 (m, 9H, H-3″/4″/5″, 5 × PhH and H-5), 6.93 (d, 1H, H-7, $J_{6,7}$ = 15.5 Hz), 6.87 (dd, 1H, H-6, $J_{6,7}$ = 15.5, $J_{5,6}$ = 10.0 Hz), 6.29 (d, 1H, H-4, $J_{4,5}$ = 15.4 Hz), 3.01–2.91 (A_2B_2 system, m, 4H, 2 × H-1 and 2 × H-2). ^{13}C NMR (100 MHz, $CDCl_3$)δ (ppm): 199.6 (C-3), 143.0 (C-5), 141.6 (C-7), 141.5 (C-1′), 136.2 (C-1″), 129.7 (C-6), 129.4 (C-3′/5′), 129.1 (C-2′/6′), 128.7 (C-2″/6″), 128.6 (C-4″), 127.5 (C-3″/5″), 126.9 (C-4′), 126.3 (C-4), 42.6 (C-2), 30.4 (C-1). Anal. Calcd. for $C_{19}H_{18}O$ (MW 262.35): C, 86.99; H, 6.92. Found: C, 87.19; H, 7.26.

1-[4-(*tert*-Butyldiphenylsilyloxy)phenyl]-7-(4-nitrophenyl)hepta-4,6-dien-3-one (**7**): 70 h, 32%. Yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$)δ (ppm): 8.21 (d, 2H, H-3″/5″, J = 8.8 Hz), 7.71 (d, 4H, 2 × H-2″/H-6″, J = 8.0 Hz), 7.59 (d, 2H, H-2″/6″, J = 8.8 Hz), 7.43–7.34 (m, 6H, 2 × H-3‴, 2 × H-4‴, 2 × H-5‴), 7.27 (dd, 1H, H-5, $J_{4,5}$ = 15.6 Hz, $J_{5,6}$ = 8.8 Hz), 7.01–6.80 (AB system, m, H-6 and H-7), 6.93 (d, 2H, H-2′/6′, J = 8.4 Hz), 6.69 (d, 2H, H-3′/5′, J = 8.4 Hz), 6.33 (d, 1H, H-4, J = 15.4 Hz), 2.84 (A_2B_2 system, quasi

s, 4H, 2 × H-1 and 2 × H-2), 1.08 (s, 9H, C(CH_3)$_3$). ^{13}C NMR (100 MHz, $CDCl_3$)δ (ppm): 199.6 (C-3), 154.1 (C-4′), 147.8 (C-4″), 142.4 (C-1″), 141.2 (C-5), 138.3 (C-7), 135.7 (C-2‴/6‴), 133.5 (C-1‴), 133.2 (C-1′), 132.0 (C-6), 131.1 (C-4), 130.1 (C-2′/6′), 129.2 (C-4‴), 128.0 (C-3‴/5‴), 127.8 (C-3″/5″), 124.4 (C-2″/6″), 119.8 (C-3′/5′), 43.1 (C-2), 29.5 (C-1), 26.7 (C($\underline{C}H_3$)$_3$), 19.7 ($\underline{C}$(CH_3)$_3$).

1-[4-(*tert*-Butyldiphenylsilyloxy)phenyl]-7-[4-(dimethylamino)phenyl]hepta-4,6-dien-3-one (**9**): 90 h, 30%. Orange oil. ^{1}H NMR (400 MHz, $CDCl_3$)δ (ppm): 7.72 (dd, 4H, 2 × H-2‴/6‴, J = 8.1, 1.5 Hz), 7.42–7.31 (m, 6H, 2 × H-3‴/H-5‴, 2 × H-4‴), 7.31 (dd, 1H, H-5, $J_{5,6}$ = 15.0 Hz, $J_{4,5}$ = 11.0 Hz), 6.93 (dm, 4H, H-2′/6′ and H-2″/6″, J = 8.8 Hz), 6.85 (d, 1H, H-7, $J_{6,7}$ = 15.8 Hz), 6.76–6.64 (m, 5H, H-3′/5′, H-3″/5″ and H-6), 6.15 (d, 1H, H-4, $J_{4,5}$ = 15.4 Hz), 3.01 (s, 6H, N(CH_3)$_2$), 2.83–2.80 (A_2B_2 system, m, 4H, 2 × H-1 and 2 × H-2), 1.09 (s, 9H, C(CH_3)$_3$). ^{13}C NMR (100 MHz, $CDCl_3$)δ (ppm): 199.7 (C-3), 153.7 (C-4′), 151.0 (C-4″), 144.1 (C-5), 142.3 (C-7), 135.5 (C-2‴/6‴), 133.8 (C-1‴), 133.0 (C-1′), 129.8 (C-2′/6′), 129.0 (C-4‴), 128.8 (C-2″/6″), 127.7 (C-3‴/5‴), 127.0 (C-6), 124.1 (C-1″), 122.1 (C-4), 119.5 (C-3′/5′) 112.0 (C-3″/5″), 42.2 (C-2), 40.2 (N(CH_3)$_2$), 29.6 (C-1), 26.5 (C($\underline{C}H_3$)$_3$), 19.4 ($\underline{C}$(CH_3)$_3$).

7-[4-(*tert*-Butyldiphenylsilyloxy)-3-methoxyphenyl]-1-(4-methoxyphenyl)hepta-4,6-dien-3-one **(11):** 92 h**,** 43%. Yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 7.70 (dd, 4H, 2 × H-2‴/H-6‴, J = 6.8, 1.2 Hz), 7.43–7.33 (m, 6H, 2 × H-3‴/H-5‴, 2 × H-4‴), 7.26 (dd, 1H, H-5, $J_{4,5}$ = 15.2 Hz, $J_{5,6}$ = 10.8 Hz), 7.13 (dm, 2H, H-2′/6′, J = 8.8 Hz), 6.87 (d, 1H, H-2″, J_2'', 6″ = 2.0 Hz), 6.83 (dm, 2H, H-3′/5′, J = 8.8 Hz), 6.77 (d, 1H, H-7, $J_{6,7}$ = 15.4 Hz), 6.76 (dd, 1H, H-6″, J_5'' ,6″ = 8.4 Hz, J_2'', 6″ = 2.0 Hz), 6.67 (d, 1H, H-5″, J_5'', 6″ = 8.4 Hz), 6.66 (dd, 1H, H-6, $J_{6,7}$ = 15.4, $J_{5,6}$ = 10.8 Hz), 6.20 (d, 1H, H-4, $J_{4,5}$ = 15.4 Hz), 3.78 (s, 3H, OCH_3), 3.61 (s, 3H, OCH_3), 2.98–2.79 (A_2B_2 system, m, 2 × H-1 and 2 × H-2), 1.11 (s, 9H, C(CH_3)$_3$). ^{13}C NMR (100 MHz, $CDCl_3$)δ (ppm): 199.5 (C-3), 157.9 (C-4′), 150.8 (C-4″), 146.6 (C-3′), 143.1 (C-5), 141.6 (C-7), 135.3 (C-2‴/6‴), 133.4 (C-1‴), 133.2 (C-1′), 129.7 (C-2′/6′), 129.3 (C-6), 128.4 (C-4‴), 127.6 (C-3‴/5‴), 124.7 (C-4), 120.9 (C-6″), 120.4 (C-5″), 113.9 (C-3′/5′), 110.4 (C-2″), 55.4 (OCH_3), 55.2 (OCH_3), 42.5 (C-2), 29.4 (C-1), 26.6 (C($\underline{C}H_3$)$_3$), 19.8 ($\underline{C}$(CH_3)$_3$).

7-[4-(*tert*-Butyldiphenylsilyloxy)-3-methoxyphenyl]-1-phenylhepta-4,6-dien-3-one (**13**): 124 h, 31%. Yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$)δ (ppm): 7.72–7.69 (dm, 4H, 2 × H-2‴/H-6‴, J = 8.0 Hz), 7.43–7.18 (m, 12H, 2 × H-3‴/H-5‴ 2 × H-4‴, 5 × PhH and H-5), 6.84 (bs, 1H, H-2″), 6.82 (d, 1H, H-7, $J_{6,7}$ = 15.6 Hz), 6.79 (bd, 1H, H-6″, $J_{5'',6''}$ = 8.2 Hz), 6.67 (d, 1H, H-5″, $J_{5'',6''}$ = 8.2 Hz), 6.66 (dd, 1H, H-6, $J_{6,7}$ = 15.6 Hz, $J_{5,6}$ = 10.8 Hz), 6.21 (d, 1H, H-4, $J_{4,5}$ = 15.4 Hz), 3.61 (s, 3H, OCH_3), 2.98–2.87 (A_2B_2 system, m, 4H, 2 × H-1 and 2 × H-2), 1.11 (s, 9H, C(CH_3)$_3$). ^{13}C NMR (100 MHz, $CDCl_3$)δ (ppm): 199.4 (C-3), 150.8 (C-4″), 146.6 (C-3″), 143.2 (C-5), 141.0 (C-7), 135.3 (C-2‴/6‴), 133.2 (C-1‴), 129.7 (C-2′/6′ and C-3′/5′), 129.6 (C-1″), 128.5 (C-6), 128.4 (C-4‴), 127.6 (C-3‴/5‴), 126.0 (C-4′), 124.7 (C-4), 120.9 (C-6″), 120.3 (C-5″), 110.4 (C-2″), 55.4 (OCH_3), 42.3 (C-2), 30.3 (C-1), 26.6 (C($\underline{C}H_3$)$_3$), 19.8 ($\underline{C}$(CH_3)$_3$).

1-(4-Methoxyphenyl)-7-(4-nitrophenyl)hepta-4,6-dien-3-one (**15**): 90 h, 16%. Orange solid. mp 122–124 °C. ^{1}H NMR (400 MHz, $CDCl_3$)δ (ppm): 8.21 (d, 2H, H-3″/5″, J = 8.8 Hz), 7.59 (d, 2H, H-2″/6″, J = 8.8 Hz), 7.31 (dd, 1H, H-5, $J_{4,5}$ = 15.9 Hz, $J_{5,6}$ = 9.6 Hz), 7.13 (d, 2H, H-2′/6′, J = 8.4 Hz), 7.03–6.92 (AB system, m, 2H, H-7 and H-6), 6.83 (d, 2H, H-3′/5′, J = 8.4 Hz), 6.36 (d, 1H, H-4, J = 15.9 Hz), 3.78 (s, 3H, OCH_3), 2.96–2.87 (A_2B_2 system, m, 4H, 2 × H-1 and 2 × H-2). ^{13}C NMR (100 MHz, $CDCl_3$)δ (ppm): 199.5 (C-3), 158.2 (C-4′), 147.8 (C-4″), 142.4 (C-1″), 141.2 (C-5), 138.3 (C-7), 133.3 (C-1′), 131.9 (C-6), 131.1

(C-4), 129.5 (C-2′/6′), 127.8 (C-3″/5″), 124.4 (C-2″/6″), 114.1 (C-3′/5′), 55.5 (OCH_3), 43.2 (C-2), 29.4 (C-1). Anal. Calcd. for $C_{20}H_{19}NO_4$ (MW 337.37): C, 71.20; H, 5.68; N, 4.15;. Found: C, 70.86; H, 5.54; N, 4.15.

7-(4-Nitrophenyl)-1-phenylhepta-4,6-dien-3-one (**16**): 93 h**,** 12%. Orange solid. mp 88–90 °C. ^{1}H NMR (400 MHz, $CDCl_3$)δ (ppm): 8.23 (d, 2H, H-3″/5″, J = 8.5 Hz), 7.60 (d, 2H, H-2″/6″, J = 8.5 Hz), 7.34–7.19 (m, 6H, 5 × PhH and H-5), 7.03–6.88 (AB system, m, 2H, H-6 and H-7), 6.38 (d, 1H, H-4, J = 15.4 Hz), 3.01–2.88 (A_2B_2 system, m, 4H, 2 × H-1 and 2 × H-2). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 199.3 (C-3), 147.8 (C-4″), 142.4 (C-1′), 141.3 (C-5), 138.4 (C-7), 131.9 (C-6), 131.0 (C-4), 128.8 (C-2′/6′), 128.6 (C-3′/5′), 127.9 (C-2″/6″), 126.4 (C-4′), 124.4 (C-3″/5″), 42.9 (C-2), 30.3 (C-1). Anal. Calcd. for $C_{19}H_{17}NO_3$ (MW 307.34): C, 74.25; H, 5.58; N, 4.56; Found: C, 74.29; H, 6.01; N, 4.38.

1-(4-Methoxyphenyl)-7-phenylhepta-4,6-dien-3-one (**17**): 65 h, 90%. Yellow solid. mp 71–75 °C. ^{1}H NMR (400 MHz, $CDCl_3$)δ (ppm): 7.47 (d, 2H, H-2″/6″, J = 7.0 Hz), 7.38–7.25 (m, 4H, H-3″/4″/5″ and H-5), 7.14 (d, 2H, H-2′/6′, J = 8.4 Hz), 6.95–6.82 (m, 4H, H-3′/5′, H-6 and H-7), 6.28 (d, 1H, H-4, $J_{4,5}$ = 15.7 Hz), 3.78 (s, 3H, OCH_3), 3.00–2.90 (A_2B_2 system, m, 4H, 2 × H-1 and 2 × H-2). ^{13}C NMR (100 MHz, $CDCl_3$)δ (ppm): 199.8 (C-3), 158.1 (C-4′), 142.9 (C-5), 141.6 (C-7), 136.2 (C-1′), 133.5 (C-1″), 129.8 (C-6), 129.6 (C-2′/6′), 129.5 (C-3″/5″), 129.1 (C-2″/6″), 127.5 (C-4″), 126.9 (C-4), 114.1 (C-3′/5′), 55.4 (OCH_3), 42.8 (C-2), 29.5 (C-1). Anal. Calcd. for $C_{20}H_{20}O_2$ (MW 292.37): C, 82.16; H, 6.89; Found: C, 82.06; H, 6.56.

7-[4-(Dimethylamino)phenyl]-1-(4-methoxyphenyl)hepta-4,6-dien-3-one (**18**): 65 h, 17%. Orange solid. mp 115–117 °C. ^{1}H NMR (400 MHz, $CDCl_3$)δ (ppm): 7.38 (dm, 2H, H-2″/6″, J = 8.9 Hz), 7.34 (dd, 1H, H-5, $J_{4,5}$ = 15.4 Hz, $J_{5,6}$ = 11.0 Hz), 7.16 (dm, 2H, H-2′/6′, J = 8.8 Hz), 6.85 (dm, 2H, H-3′/5′, J = 8.8 Hz), 6.78–6.66 (4H, m, H-3″/5″, H-6 and H-7), 6.20 (d, 1H, H-4, $J_{4,5}$ = 15.4 Hz), 3.79 (s, 3H, OCH_3), 3.02 (s, 6H, $N(CH_3)_2$), 2.95–2.85 (A_2B_2 system, 4H, m, 2 × H-1 and 2 × H-2). ^{13}C NMR (100 MHz, $CDCl_3$)δ (ppm): 199.8 (C-3), 158.1 (C-4′), 151.3 (C-4″), 144.5 (C-5), 142.6 (C-7), 133.8 (C-1′), 131.0 (C-1″), 129.6 (C-2′/6′), 129.5 (C-2″/6″), 127.2 (C-6), 122.3 (C-4), 114.1 (C-3′/5′), 112.2 (C-3″/5″), 55.5 (OCH_3), 42.6 (C-2), 40.4 ($N(CH_3)_2$), 29.8 (C-1). Anal. Calcd. for $C_{22}H_{25}NO_2$ (MW 335.44): C, 78.77; H, 7.51; N, 4.18; Found: C, 79.09; H, 7.65; N, 4.02.

7-[4-(Dimethylamino)phenyl]-1-phenylhepta-4,6-dien-3-one (**19**): 96 h, 24%. Reddish solid. mp 96–98 °C. ^{1}H NMR (400 MHz, $CDCl_3$)δ (ppm): 7.39–7.19 (m, 8H, 5 × PhH, H-5 and H-2′/6′), 6.88 (d, 1H, H-7, $J_{6,7}$ = 15.2 Hz), 6.76–6.66 (m, 3H, H-6 and H-3′/5′), 6.20 (d, 1H, H-4, $J_{4,5}$ = 15.4 Hz), 3.02 (s, 6H, $N(CH_3)_2$), 3.01–2.90 (A_2B_2 system, m, 4H, 2 × H-1 and 2 × H-2). ^{13}C NMR (100 MHz, $CDCl_3$)δ (ppm): 199.7 (C-3), 151.3 (C-4″), 144.5 (C-5), 142.6 (C-7), 141.7 (C-1′), 131.0 (C-1″), 129.0 (C-2′/6′ and C-3′/5′), 128.7 (C-2″/6″), 127.1 (C-6), 126.2 (C-4′), 122.3 (C-4), 112.2 (C-3″/5″), 42.3 (C-2), 40.4 ($N(CH_3)_2$), 30.7 (C-1). Anal. Calcd. for $C_{21}H_{23}NO$ (MW 305.41): C, 82.58; H, 7.59; N, 4.59. Found: C, 82.55; H, 7.50; N, 4.41.

3.2.4. General procedure for removing the protecting group TBDPS from the resulting compounds

To a solution of the protected compounds with TBDPS (1 mmol) in THF (20 mL), TBAF (1.2 mmol) was added at 0 °C under N_2 atm. The reaction mixture was stirred at room temperature for 30–60 min. The progress of the reaction was followed by TLC using an EtOAc–hexane system. NH_4Cl solution was added dropwise to the

reaction mixture, which was then stirred. After evaporating the reaction mixture, the residue was extracted with EtOAc (3 × 20 mL), then washed with H_2O (3 × 20 mL), and dried (Na_2SO_4). Evaporation of the EtOAc and chromatography of the residue on a silica gel (70–230 mesh) column eluted with hexane–EtOAc gave the deprotected compound.

As an exception, after purifying compound **10** by column chromatography, it was recrystallized from CH_2Cl_2 at 4 °C.

1-(4-Hydroxyphenyl)-7-(4-nitrophenyl)hepta-4,6-dien-3-one (**8**): 1 h, 90%. Orange solid. mp 151–154 °C. ^{1}H NMR (400 MHz, $CDCl_3$)δ (ppm): 8.22 (d, 2H, H-3″/5″, J = 8.8 Hz), 7.59 (d, 2H, H-2″/6″, J = 8.8 Hz), 7.28 (ddd, 1H, H-5, $J_{4,5}$ = 16.3, $J_{5,6}$ = 7.0, $J_{5,7}$ = 1.5 Hz), 7.08 (dm, 2H, H-2′/6′, J = 8.4 Hz), 6.99–6.90 (AB system, m, 2H, H-6 and H-7), 6.76 (dm, 2H, H-3′/5′, J = 8.4 Hz), 6.37 (d, 1H, H-4, $J_{4,5}$ = 16.3 Hz), 4.74 (bs, 1H, OH), 2.91 (A_2B_2 system, quasi s, 4H, 2 × H-1 and 2 × H-2). ^{13}C NMR (100 MHz, $CDCl_3$)δ (ppm): 199.4 (C-3), 153.9 (C-4′), 142.2 (C-4″), 141.1 (C-5), 138.2 (C-7), 133.1 (C-1′), 131.7 (C-6), 130.8 (C-4), 129.5 (C-2′/6′), 127.6 (C-3″/5″), 124.2 (C-2″/6″), 115.3 (C-3′/5′), 43.0 (C-2), 29.2 (C-1). Anal. Calcd. for $C_{19}H_{17}NO_4$ (MW 323.34): C, 70.58; H, 5.30; N, 4.33; Found: C, 70.42; H, 5.55; N, 4.15.

7-[4-(Dimethylamino)phenyl]-1-(4-hydroxyphenyl)hepta-4,6-dien-3-one (**10**): 1 h, 58%. Brownish solid. mp 189–193 °C. ^{1}H NMR (400 MHz, acetone-d_6)δ (ppm): 8.10 (bs, 1H, OH), 7.41 (dm, 2H, H-2′/6′, J = 8.8 Hz), 7.38 (dd, 1H, H-5, $J_{4,5}$ = 15.4 Hz, $J_{5,6}$ = 10.6 Hz), 7.07 (dm, 2H, H-2″/6″, J = 6.6 Hz), 6.97 (d, 1H, H-7, $J_{6,7}$ = 15.4 Hz), 6.82 (dd, 1H, H-6, $J_{6,7}$ = 15.4 Hz, $J_{5,6}$ = 10.6 Hz), 6.81–6.70 (m, 4H, H-3′/5′ and H-3″/5″), 6.20 (d, 1H, H-4, $J_{4,5}$ = 15.4 Hz), 3.00 (s, 6H, $N(CH_3)_2$), 2.98–2.80 (A_2B_2 system, m, 4H, 2 × H-1 and 2 × H-2). ^{13}C NMR (100 MHz, acetone-d_6)δ (ppm): 199.8 (C-3), 157.0 (C-4′), 152.8 (C-4″), 145.0 (C-5), 143.4 (C-7), 133.9 (C-1′), 130.7 (C-2′/6′), 130.1 (C-2″/6″), 128.6 (C-6), 125.7 (C-1″), 123.6 (C-4), 116.6 (C-3′/5′), 113.5 (C-3″/5″), 43.4 (C-2), 40.8 ($N(CH_3)_2$), 30.0 (C-1). Anal. Calcd. for $C_{21}H_{23}NO_2$ (MW 321.41): C, 78.47; H, 7.21; N, 4.36; Found: C, 77.91; H, 7.29; N, 4.12.

7-[(4-Hydroxy-3-methoxy)phenyl]-1-(4-methoxyphenyl)hepta-4,6-dien-3-one (**12**): 1 h, 90%. Orange solid. mp 122–124 °C. ^{1}H NMR (400 MHz, $CDCl_3$)δ (ppm): 7.30 (dd, 1H, H-5, J = 15.4, J = 11.0 Hz), 7.14 (d, 2H, H-2′/6′, J = 8.4 Hz), 7.00 (dd, 1H, H-6″, $J_{5,''',6''}$ = 8.0 Hz, $J_{2'',6''}$ = 1.5 Hz), 6.97 (bs, 1H, H-2″), 6.90 (d, 1H, H-5″, $J_{5'',6''}$ = 8.0 Hz), 6.85 (d, 1H, H-7, $J_{6,7}$ = 15.4 Hz), 6.84 (d, 2H, H-3′/5′, J = 8.4 Hz), 6.72 (dd, 1H, H-6, $J_{6,7}$ = 15.4 Hz, $J_{5,6}$ = 10.6 Hz), 6.23 (d, H-4, $J_{4,5}$ = 15.4 Hz), 3.93 (s, 3H, OCH_3), 3.78 (s, 3H, OCH_3), 2.93–2.85 (A_2B_2 system, m, 2 × H-1 and 2 × H-2). ^{13}C NMR (100 MHz, $CDCl_3$)δ (ppm): 199.9 (C-3), 158.2 (C-4′), 147.3 (C-4″), 147.0 (C-3″), 143.4 (C-5), 141.8 (C-7), 133.6 (C-1′), 129.5 (C2′/6′), 128.9 (C-1″), 128.7 (C-6), 124.7 (C-4), 122.1 (C-6″), 115.0 (C-5″), 114.1 (C-3′/5′), 108.9 (C-2″), 56.2 (OCH_3), 55.5 (OCH_3), 42.8 (C-2), 29.7 (C-1). Anal. Calcd. for $C_{21}H_{22}O_4$ (MW 338.40): C, 74.54; H, 6.55; Found: C, 74.44; H, 6.69.

7-[(4-Hydroxy-3-methoxy)phenyl]-1-phenylhepta-4,6-dien-3-one (**14**): 30 min, 87%. Viscous brownish oil. ^{1}H NMR (400 MHz, $CDCl_3$)δ (ppm): 7.31 (dd, 1H, H-5, $J_{4,5}$ = 15.5, $J_{5,6}$ = 10.6 Hz), 7.32–7.18 (m, 5H, 5 × PhH), 7.00 (dd, 1H, H-6″, $J_{5'',6''}$ = 8.4 Hz, $J_{2'',6''}$ = 1.7 Hz), 6.97 (bs, 1H, H-2″), 6.91 (d, 1H, H-5″, $J_{5'',6''}$ = 8.4 Hz), 6.86 (d, 1H, H-7, $J_{6,7}$ = 15.7 Hz), 6.73 (dd, 1H, H-6, $J_{6,7}$ = 15.7 Hz, $J_{5,6}$ = 10.6 Hz), 6.25 (d, 1H, H-4, $J_{4,5}$ = 15.5 Hz), 5.82 (bs, 1H, OH), 3.94 (s, 3H, OCH_3), 3.00–2.90 (A_2B_2 system, m, 4H, 2 × H-1 and 2 × H-2). ^{13}C NMR (100 MHz, $CDCl_3$)δ (ppm): 199.7 (C-3), 147.3 (C-4″), 147.0 (C-3″), 143.4 (C-5), 141.8 (C-7), 141.6 (C-1′), 128.9 (C-1″), 128.7 (C-3′/5′), 128.63 (C-6), 128.61 (C-2′/6′), 126.3 (C-4′), 124.7 (C-4),

122.1 (C-6″), 115.0 (C-5″), 108.9 (C-2″), 56.2 (OCH_3), 42.5 (C-2), 30.5 (C-1). Anal. Calcd. for $C_{20}H_{20}O_3$ (MW 308.37): C, 77.90; H, 6.54; Found: C, 77.92; H, 6.79.

3.2.5. Exceptions to the general procedures

When 4-(dimethylamino)cinnamaldehyde or 4-nitrocinnamaldehyde was used as the cinnamaldehyde derivative, the starting compounds were added in aliquots over 30 min due to insolubility in Et_2O. In the syntheses of **7**, **13**, **15**, **16**, **17**, and **19**, the organic phase was extracted with EtOAc. In the syntheses of **17** and **18**, the final compounds were purified by chromatotron after column chromatography. In the purification of **9**, the compound was purified by column chromatography followed by preparative chromatography with the hexane:EtOAc (4:1) system.

3.3. In vitro studies

3.3.1. Culture of the MCF-7 breast cancer cells

The MCF-7 cell line was cultured in DMEM medium with 10% fetal bovine serum, 1% l-glutamine, 100 IU/mL penicillin, and 10 mg/mL streptomycin. Cells were cultivated in a humidified incubator at 37 °C and 5% CO_2. The cells were washed with sterile PBS, and they were removed from the flask surface with a 0.25% trypsin–EDTA solution. The cells were centrifuged and counted in a Thoma counting chamber. At this stage, harvested cells were ready for the cell proliferation assay.

3.3.2. Cell proliferation assay

The XTT cell proliferation kit was used to measure the cytotoxic activities of 10 alnustone-like compounds, tamoxifen, and paclitaxel on the MCF-7 cell line. Metabolic active cancer cells reduce yellow colored tetrazolium salt (XTT) into water-soluble orange colored formazan salt. Sterile 96-well culture plates were seeded with 10 $\times$ 10^4 MCF-7 cells, and the wells were treated with decreasing concentrations (from 10^{-3} M to 10^{-9} M) of compounds in 200 μL of medium. After 24 and 48 h of incubation, the medium was removed, and the wells were washed with sterile phosphate buffered saline. Then, 100 μL of colorless medium carrying 50 μL of XTT reagent was added to each well, and the plate was incubated for 4 h. The absorbance was measured using a micro plate reader (Thermo) at 450 nm, and then the percentage of cell viability was calculated.[23,24] IC_{50} values of tamoxifen, paclitaxel, and the 10 alnustone-like compounds were calculated by GrapPad Prism software.

3.3.3. Molecular docking studies

The docking server (http://www.dockingserver.com/) was employed for molecular docking of tamoxifen and the 10 alnustone-like compounds with the human ER-α protein. The binding energy, inhibition constant, and intermolecular interactions were estimated using the docking server. The X-ray crystallographic structure of the human ER-α protein was obtained from the Protein Data Bank (pdb code: 1A52). Ligand docking studies were performed on the binding pocket of the protein. The alnustone-like compounds and tamoxifen structures were drawn using Marvin Sketch software (Chemaxon). The structures at this stage were energy minimized by Discovery Studio 3.5 Client software (Accelrys) and recorded in pdb format. Then the structures were uploaded to the docking server (experimental parameters are tstep 0.2, qstep 5.0, d.step 5.0, rmstol 2.0, ga_pop_size 150, ga_num_evals 250000, ga_num_generations 540000, ga_run 10). After the runs, the docking models were visualized and edited by Discovery Studio 3.5 Client software.

3.3.4. Statistical analysis

Differences in the mean values of the measured activities were evaluated statistically using SPSS 17.0 (univariate variance analyses and Pearson correlation). Probability values of $P < 0.05$ were considered significant.

4. Conclusions

Ten alnustone-like compounds were synthesized systematically and their structures were elucidated via ^{1}H and ^{13}C NMR spectra for the first time. They showed potent antitumor activity against MCF-7 cell lines. Synthesized alnustone-like compounds may be categorized into 2 groups: paclitaxel-like (Group A) and tamoxifen-like (Group B). This classification was made according to 2 criteria: the presence/absence of radical groups (hydroxyl and/or methoxy groups) or dimethylamine groups and the binding region of the derivatives in the ER-α pocket. The structure and function of the designed compounds correlate with the biochemical behavior of breast cancer cell survival. The designed compounds may be developed for drug resistance and may potentially decrease the side effects of commercially available drugs in the future.

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