

FULL PAPER

Synthesis and Cytotoxic Evaluation of Novel 1,2,3-Triazole-4-Linked (2*E*,6*E*)-2-Benzylidene-6-(4-nitrobenzylidene)cyclohexanones

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This work describes the synthesis of novel 1,2,3-triazole-4-linked (2*E*,6*E*)-2-benzylidene-6-(4-nitrobenzylidene)cyclohexanones starting from cyclohexanone. 1-(Cyclohex-1-en-1-yl)piperidine, the enamine from cyclohexanone and piperidine, reacted with 4-nitrobenzaldehyde to obtain 2-(4-nitrobenzylidene)cyclohexanone. Condensation of the latter compound with (prop-2-yn-1-yloxy)benzaldehyde derivatives under acidic conditions gave (4-nitrobenzylidene)-[(prop-2-yn-1-yloxy)-benzylidene]cyclohexanones. Finally, ‘click reaction’ of these derivatives and various organic azides led to the title compounds. All compounds were examined by MTT assay for cytotoxic activity in one human breast cancer cell line, MDA-MB-231.

Keywords: (*E,E*)-2-Benzylidene-6-(4-nitrobenzylidene)cyclohexanones, 1,2,3-Triazoles, Click chemistry, Cyclohexanone, Cytotoxic activity.

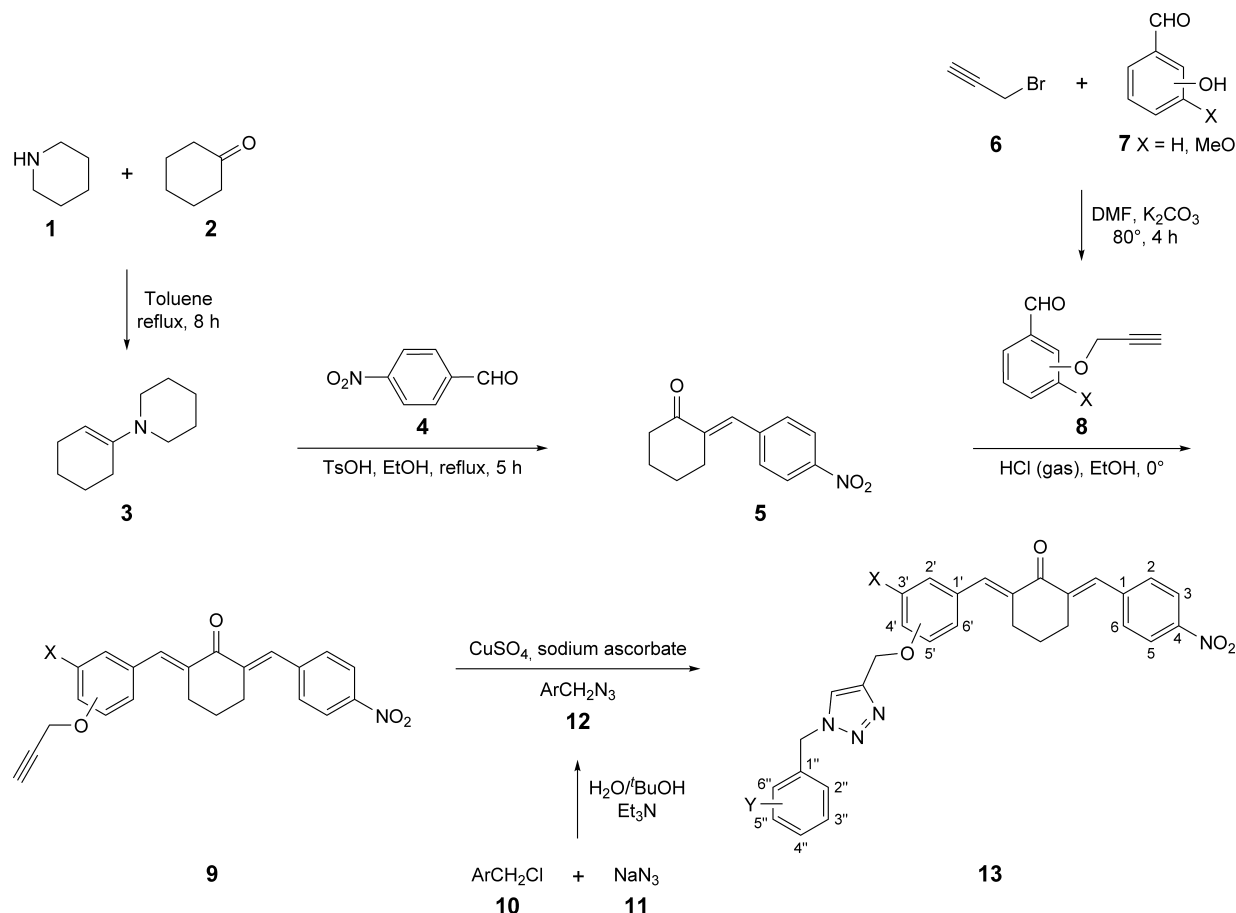
Introduction

1,3-Diarylprop-2-en-1-ones, known as chalcones are the main component of various natural products and important starting material for the synthesis of flavonoids and isoflavonoids [1]. The presence of the α,β -unsaturated moiety in chalcones leads to the important biological activities such as antimalarial [2], antibacterial [3], anti-tumor [4], antifungal [5], antiamebic [6], and antiplasmodial [7] activities. The promising biological properties of chalcones come back to the fact that they interact with thiols instead of amino and OH groups of nucleic acids avoiding the genotoxic problems [8]. Therefore, they have been the center of attention for preparing anticancer agents. It has been revealed that the electronic properties of substituents on the benzylidene moiety play an important role on the cytotoxicity and among them nitro group has displayed excellent activity [9 – 12]. In this respect, (*E,E*)-2-benzylidene-6-(4-nitrobenzylidene)cyclohexanones possessing α,β -unsaturated moiety as versatile chalcone analogs were found to have potential anticancer activity [10]. Also, anti-inflammatory activity of nitrobenzylidene chalcones has been successfully reported [13].

Among a large spectrum of *N*-heterocyclic compounds, 1,2,3-triazoles are largely attractive because of valuable biological properties such as cytotoxic [14], anti-HIV-1 [15], anti-influenza [16], antiplatelet [17], and anti-tuberculosis [18] activities. Considering the fact that hybridization of two or more biologically active scaffolds has been a versatile tool for advanced drug discovery research. Herein, in continuation of our research program on the synthesis of novel and bioactive heterocycles [19 – 26], we focused on the synthesis and cytotoxic evaluation of novel 1,2,3-triazole-4-linked (2*E*,6*E*)-2-benzylidene-6-(4-nitrobenzylidene)cyclohexanones **13** (Scheme).

Results and Discussion

Considering the efficiency of the NO₂ group on the bioactivity of chalcones, we specially focused on 1,2,3-triazole-4-linked 2-benzylidene-6-(4-nitrobenzylidene)cyclohexanones. Our investigation was started by the preparation of 2-(4-nitrobenzylidene)cyclohexanone (**5**). Usually, it was obtained by the reaction of cyclohexanone (**2**) and 4-nitrobenzaldehyde (**4**) [10]. In spite of the fact that we tried the similar procedure in the literature, the

Scheme. Synthesis of novel 1,2,3-triazole-4-linked (2*E*,6*E*)-2-benzylidene-6-(4-nitrobenzylidene)cyclohexanones **13**

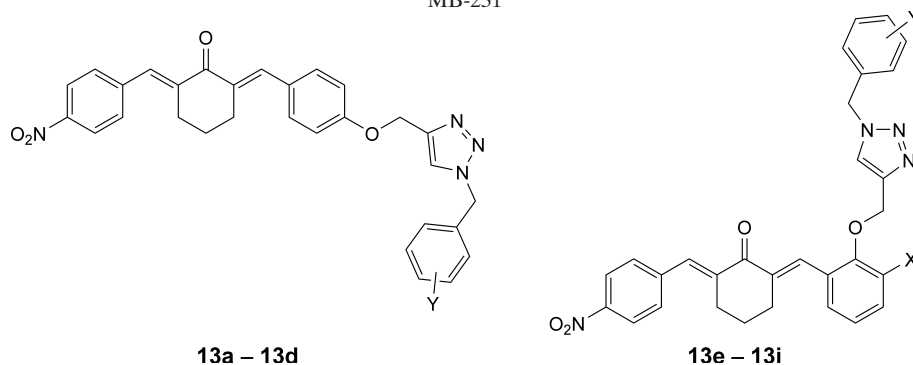
corresponding yield was low and compound **5** was prepared through the reaction of 1-(cyclohex-1-en-1-yl)piperidine (**3**) and 4-nitrobenzaldehyde (**4**) in the presence of TsOH in refluxing EtOH to give the compound **5** in good yield (70%). It should be noted that compound **3** was easily prepared from the reaction of piperidine (**1**) and cyclohexanone (**2**) in refluxing toluene [27].

As outlined in the *Scheme*, (prop-2-yn-1-yloxy)benzaldehyde derivatives **8** were prepared by the reaction of $\text{HC}\equiv\text{CCH}_2\text{Br}$ (**6**) and hydroxybenzaldehyde derivatives **7** in the presence of K_2CO_3 in DMF at 80 °C. Next, the reaction of compounds **5** and **8** in the presence of HCl (gas) led to the formation of products **9**, possessing a $\text{C}\equiv\text{C}$ bond, which were readily converted to the desired product **13** through ‘click reaction’ [28]. For this purpose, various organic azides **12** were prepared by the reaction of different benzyl chlorides/bromides **10** and NaN_3 (**11**) in the presence Et_3N in the mixture of $\text{H}_2\text{O}/\text{BuOH}$ at room temperature. Then, compound **9**, sodium ascorbate, and catalytic amount of CuSO_4 were added to the freshly prepared azides **12** leading to the formation of different 1,2,3-triazole-4-linked nitrochalcones **13**.

The scope of the reaction was studied by varying azide derivatives **12** (*Table*). The results show that all of

them, possessing electron donating or electron withdrawing groups as well as halogens, underwent ‘click reaction’ to afford product **13**. The structure of products **13** was confirmed using IR, ^1H - and ^{13}C -NMR spectroscopy as well as by chemical analysis. According to the ^1H -NMR spectra of the products, they were isomerically pure as the absorptions of the olefinic H-atoms were in the region of 7.00 – 8.00 ppm which is distinguishing for (*E*) isomers. It should be noted that the corresponding H-atoms related to (*Z*) configuration absorb at higher fields [29].

Then, the *in vitro* cytotoxic activity of all 1,2,3-triazole-4-linked nitrochalcones **13** were evaluated against MDA-MB-231, a human breast cancer cell line and compared with curcumin. All results were summarized in the *Table*. According to the IC_{50} values, compounds **13** showed good cytotoxic activity against MDA-MB-231. Among them, **13e** ($\text{IC}_{50} = 7.5 \mu\text{M}$), **13f** ($\text{IC}_{50} = 5.8 \mu\text{M}$), and **13i** ($\text{IC}_{50} = 8.0 \mu\text{M}$) exhibited higher activity in comparison to curcumin ($\text{IC}_{50} = 11.1 \mu\text{M}$). It is clear that the presence of a MeO group at 3-position of the benzylidene moiety plays an important role in the cytotoxicity. Comparing our results of cytotoxicity with those obtained by Dimmock *et al.* [10] revealed that the (2*E*,6*E*)-2-benzylidene-6-(4-nitrobenzylidene)cyclohexanone derivative

Table. Synthesis of 1,2,3-triazole-4-linked dibenzylidenecyclohexanones **13** and their *in vitro* cytotoxic activities (IC_{50} in μM) against MDA-MB-231

Entry	X	Y	Product 13	Yield [%] ^{a)}	MDA-MB-231 IC_{50} [μM]
1	–	4-F	13a	60	12.0 ± 0.3
2	–	4-Cl	13b	65	13.2 ± 0.1
3	–	2,3-Cl ₂	13c	60	12.6 ± 0.5
4	–	4-NO ₂	13d	70	13.5 ± 0.5
5	MeO	H	13e	65	7.5 ± 0.7
6	MeO	4-F	13f	60	5.8 ± 0.9
7	MeO	4-Cl	13g	62	14.0 ± 0.4
8	MeO	3,4-Cl ₂	13h	55	14.5 ± 0.1
9	MeO	4-MeO	13i	60	8.0 ± 0.4
10	Curcumin				11.1 ± 0.1

^{a)} Yield of isolated product.

possessing MeO groups showed higher activity as an effective factor in our study. However, it should be noted that their study was related to cytotoxicity on human Molt/C8 and CEM T-lymphocytes as well as murine L1210 cells.

Conclusions

In summary, we described an operational procedure for the synthesis of novel 1,2,3-triazole-4-linked nitrochalcones. Also, all compounds showed satisfactory cytotoxicity in MDA-MB-231, human breast cancer cell line. Good yields of products (55 – 70%) and user-friendly steps encourage organic and medicinal chemists to profit from both synthetic and biological aspects.

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Experimental Part

General

M.p.: Kofler hot stage apparatus; uncorrected. IR Spectra: Nicolet Magna FTIR 550 spectrophotometer; in KBr; $\tilde{\nu}$ in cm^{-1} . ¹H- and ¹³C-NMR spectra: Bruker FT-400 (400 and 100 MHz, resp.); δ in ppm rel. to Me₄Si as internal standard, J in Hz. Elemental analyses: VarioEL CHNS mode (Elementar Analysensysteme GmbH); in %. 1-(Cyclohex-1-en-1-yl)piperidine (**3**) was prepared according to [27].

Synthesis of (2E)-2-(4-Nitrobenzylidene)cyclohexanone (5) [10]. A mixture of 1-(cyclohex-1-en-1-yl)piperidine (**3**; 0.16 g, 1 mmol), 4-nitrobenzaldehyde (**4**; 0.15 g, 1 mmol), and TsOH (0.08 g, 0.5 mmol) in EtOH (15 ml) was heated at reflux for 5 h. After completion of the reaction, the mixture was cooled to r.t. and resulted crystals were filtered off and used for the subsequent reaction.

Synthesis of (Prop-2-yn-1-yloxy)benzaldehyde Derivatives 8. Typical Procedure. A mixture of HC $\equiv$ CCH₂Br (**6**; 0.12 g, 1 mmol), hydroxybenzaldehyde derivative **7** (1 mmol), and K₂CO₃ (0.14 g, 1 mmol) in DMF (15 ml) was stirred at 80° for 4 h. After completion of the reaction (checked by TLC), the mixture was cooled to r.t. and poured into crushed ice. The precipitated product was filtered off and used for the next reaction.

Synthesis of (2E,6E)-(4-Nitrobenzylidene)-[(prop-2-yn-1-yloxy)benzylidene]cyclohexanone Derivatives 9. Typical Procedure. Dry HCl gas was passed through an ice-cold soln. of (2E)-2-(4-nitrobenzylidene)cyclohexanone (**5**) (0.23 g, 1 mmol) and compound **8** (1 mmol) in abs. EtOH (3 ml) for 5 min. The mixture was allowed to stand at r.t. for further 5 min. The precipitated product was filtered off, washed with cold EtOH, and used for the next reaction.

Synthesis of 1,2,3-Triazole-4-Linked (2E,6E)-2-Benzylidene-6-(4-nitrobenzylidene)cyclohexanones 13. Typical Procedure. A soln. of benzyl chloride/bromide derivative **10** (1.1 mmol), NaN₃ (**11**; 0.06 g, 0.9 mmol), and Et₃N (0.13 g, 1.3 mmol) in a mixture of H₂O (4 ml)/^tBuOH (4 ml) was stirred at r.t. for 1 h to obtain azide **12**.

Subsequently, a mixture of **9** (1 mmol), sodium ascorbate (0.2 g, 0.1 mmol), and CuSO₄ (0.01 g, 7 mol %) was added to the freshly prepared azide derivative **12**, and the mixture was stirred at r.t. for 24 – 48 h. Upon completion of the reaction, monitored by TLC, the mixture was diluted with H₂O, poured into crushed ice, extracted using AcOEt (3 × 30 ml), and the solvent was evaporated under vacuum. Finally, all products were recrystallized from petroleum ether/AcOEt (1:1) to give pure products **13**.

(2E,6E)-2-(4-[[1-(4-Fluorobenzyl)-1H-1,2,3-triazol-4-yl]methoxy]benzylidene)-6-(4-nitrobenzylidene)cyclohexanone (13a). Pale-yellow solid. Yield: 0.32 g (60%). M.p. 121 – 123°. IR: 2970, 2850, 1665, 1583, 1575, 1350. ¹H-NMR: 1.71 – 1.98 (*m*, CH₂); 2.73 – 2.87 (*m*, 2 CH₂); 5.20 (*s*, CH₂); 5.68 (*s*, CH₂); 7.11 – 7.26 (*m*, 3 H, H-C(3',5'), CH); 7.35 – 7.65 (*m*, 7 H, H-C(2',6',2'',3'',5'',6''), CH); 7.76 (*d*, *J* = 8.0, H-C(2,6)); 8.25 (*d*, *J* = 8.0, H-C(3,5)); 8.29 (*s*, 1 H, triazole). ¹³C-NMR: 24.1; 28.3; 34.5; 47.4; 61.6; 115.3 (*d*, *J*(C,F) = 4.9); 116.0; 123.3; 125.3 (*d*, *J*(C,F) = 3.2); 125.4; 128.5; 130.4; 131.2 (*d*, *J*(C,F) = 8.1); 131.6; 132.8; 132.9; 133.1; 133.8; 134.3; 141.5; 147.2; 159.2; 160.5 (*d*, *J*(C,F) = 245.3); 189.0. Anal. calc. for C₃₀H₂₅FN₄O₄ (524.55): C 68.69, H 4.80, N 10.68; found: C 68.51, H 4.68, N 10.55.

(2E,6E)-2-(4-[[1-(4-Chlorobenzyl)-1H-1,2,3-triazol-4-yl]methoxy]benzylidene)-6-(4-nitrobenzylidene)cyclohexanone (13b). Pale-yellow solid. Yield: 0.35 g (65%). M.p. 61 – 163°. IR: 2925, 2855, 1660, 1585, 1575, 1350. ¹H-NMR: 1.70 – 1.95 (*m*, CH₂); 2.75 – 2.87 (*m*, 2 CH₂); 5.20 (*s*, CH₂); 5.65 (*s*, CH₂); 7.13 (*d*, *J* = 8.0, H-C(3',5')); 7.20 – 7.25 (*m*, 3 H, H-C(2'',6''), CH); 7.36 – 7.55 (*m*, 3 H, H-C(3'',5''), CH); 7.65 (*d*, *J* = 8.0, H-C(2',6')); 7.78 (*d*, *J* = 8.0, H-C(2,6)); 8.27 (*d*, *J* = 8.0, H-C(3,5)); 8.30 (*s*, 1 H, triazole). ¹³C-NMR: 24.1; 28.3; 34.5; 63.5; 61.6; 115.4; 124.0; 125.6; 128.4; 128.8; 129.2; 129.3; 130.4; 131.7; 132.9; 133.1; 133.8; 134.4; 138.8; 142.6; 142.9; 147.2; 159.1; 189.0. Anal. calc. for C₃₀H₂₅ClN₄O₄ (541.00): C 66.60, H 4.66, N 10.36; found: C 66.75, H 4.51, N 10.24.

(2E,6E)-2-(4-[[1-(2,3-Dichlorobenzyl)-1H-1,2,3-triazol-4-yl]methoxy]benzylidene)-6-(4-nitrobenzylidene)cyclohexanone (13c). Pale-yellow solid. Yield: 0.34 g (60%). M.p. 158 – 160°. IR: 2920, 2850, 1665, 1588, 1572, 1350. ¹H-NMR: 1.72 – 1.89 (*m*, CH₂); 2.85 – 2.90 (*m*, 2 CH₂); 5.22 (*s*, CH₂); 5.65 (*s*, CH₂); 7.10 – 7.14 (*m*, 3 H, H-C(3',5'), CH); 7.29 – 7.32 (*m*, H-C(5'',6'')); 7.50 – 7.67 (*m*, 4 H, H-C(2',6',4''), CH); 7.78 (*d*, *J* = 8.8, H-C(2,6)); 8.27 (*d*, *J* = 8.8, H-C(3,5)); 8.36 (*s*, 1 H, triazole). ¹³C-NMR: 24.1; 28.3; 34.5; 51.9; 61.6; 115.4; 123.4; 124.0; 125.5; 128.9; 130.5; 130.6; 131.5; 131.7; 132.8; 132.9; 133.1; 134.4; 136.4; 137.0; 137.4; 143.3; 144.4; 147.2; 159.1; 189.0. Anal. calc. for C₃₀H₂₄Cl₂N₄O₄ (575.45): C 62.62, H 4.20, N 9.74; found: C 62.79, H 4.38, N 9.58.

(2E,6E)-2-(4-Nitrobenzylidene)-6-(4-[[1-(4-nitrobenzyl)-1H-1,2,3-triazol-4-yl]methoxy]benzylidene)cyclohexanone (13d). Pale-yellow solid. Yield: 0.38 g (70%). M.p. 178 – 180°. IR: 2918, 2850, 1660, 1608, 1570, 1350. ¹H-NMR: 1.72 – 1.89 (*m*, CH₂); 2.85 – 2.92 (*m*, 2 CH₂);

5.22 (*s*, CH₂); 5.81 (*s*, CH₂); 7.10 – 7.15 (*m*, 3 H, H-C(3',5'), CH); 7.51 – 7.61 (*m*, 3 H, H-C(2',6'), CH); 7.65 (*d*, *J* = 8.8, H-C(2'',6'')); 7.82 (*d*, *J* = 8.8, H-C(2,6)); 8.13 (*d*, *J* = 8.8, H-C(3'',5'')); 8.24 (*d*, *J* = 8.8, H-C(3,5)); 8.29 (*s*, 1 H, triazole). ¹³C-NMR: 24.1; 29.2; 34.5; 52.4; 61.6; 115.3; 123.4; 124.4; 129.5; 130.5; 131.7; 132.8; 132.9; 136.2; 136.7; 137.0; 137.8; 141.5; 140.1; 143.3; 143.8; 144.4; 159.5; 189.0. Anal. calc. for C₃₀H₂₅N₅O₆ (551.56): C 65.33, H 4.57, N 12.70; found: C 65.52, H 4.42, N 12.81.

(2E,6E)-2-(2-[(1-Benzyl-1H-1,2,3-triazol-4-yl)methoxy]-3-methoxybenzylidene)-6-(4-nitrobenzylidene)cyclohexanone (13e). Pale-yellow solid. Yield: 0.35 g (65%). M.p. 168 – 170°. IR: 2925, 2850, 1660, 1620, 1575, 1350. ¹H-NMR: 1.75 – 1.88 (*m*, CH₂); 2.89 – 2.94 (*m*, 2 CH₂); 3.77 (*s*, MeO); 5.18 (*s*, CH₂); 5.62 (*s*, CH₂); 7.13 – 7.24 (*m*, 2 H, H-C(4'), CH); 7.33 – 7.38 (*m*, 6 H, Ph, CH); 7.60 – 7.66 (*m*, H-C(5',6')); 7.78 (*d*, *J* = 8.2, H-C(2,6)); 8.27 (*d*, *J* = 8.2, H-C(3,5)); 8.30 (*s*, 1 H, triazole). ¹³C-NMR: 22.7; 28.2; 34.5; 53.3; 55.9; 62.0; 113.6; 114.8; 124.0; 125.4; 128.4; 128.6; 128.9; 129.2; 130.4; 131.7; 133.1; 134.5; 136.4; 137.5; 140.1; 142.6; 143.1; 147.2; 148.8; 149.1; 189.0. MS: 536 (*M*⁺), 42, 404, 263, 215, 163, 135, 106, 88. Anal. calc. for C₃₁H₂₈N₄O₅ (536.59): C 69.39, H 5.26, N 10.44; found: C 69.50, H 5.38, N 10.58.

(2E,6E)-2-(2-[[1-(4-Fluorobenzyl)-1H-1,2,3-triazol-4-yl]methoxy]-3-methoxybenzylidene)-6-(4-nitrobenzylidene)cyclohexanone (13f). Pale-yellow solid. Yield: 0.33 g (60%). M.p. 144 – 146°. IR: 2925, 2850, 1660, 1625, 1575, 1350. ¹H-NMR: 1.75 – 1.97 (*m*, CH₂); 2.90 – 2.94 (*m*, 2 CH₂); 3.78 (*s*, MeO); 5.18 (*s*, CH₂); 5.62 (*s*, CH₂); 7.13 – 7.24 (*m*, 4 H, H-C(4',3'',5''), CH); 7.39 – 7.42 (*m*, 3 H, H-C(5',6'), CH); 7.5 (*d*, *J* = 7.5, H-C(2'',6'')); 7.79 (*d*, *J* = 8.4, H-C(2,6)); 8.28 (*d*, *J* = 8.4, H-C(3,5)); 8.30 (*s*, 1 H, triazole). ¹³C-NMR: 22.7; 28.3; 34.9; 52.5; 55.9; 60.0; 113.6; 114.8; 116.1 (*d*, *J*(C,F) = 21.3); 123.4; 124.0; 125.3; 128.9; 130.8 (*d*, *J*(C,F) = 8.3); 131.7; 133.1; 134.5; 137.5; 140.1; 143.2; 147.2; 148.8; 149.1; 160.1 (*d*, *J*(C,F) = 245.0); 189.0. Anal. calc. for C₃₁H₂₇FN₄O₅ (554.58): C 67.14, H 4.91, N 10.10; found: C 67.32, H 5.11, N 10.21.

(2E,6E)-2-(2-[[1-(4-Chlorobenzyl)-1H-1,2,3-triazol-4-yl]methoxy]-3-methoxybenzylidene)-6-(4-nitrobenzylidene)cyclohexanone (13g). Pale-yellow solid. Yield: 0.35 g (62%). M.p. 178 – 180°. IR: 2933, 2850, 1660, 1595, 1575, 1350. ¹H-NMR: 1.75 – 1.88 (*m*, CH₂); 2.90 – 2.92 (*m*, 2 CH₂); 3.75 (*s*, MeO); 5.20 (*s*, CH₂); 5.57 (*s*, CH₂); 7.11 – 7.25 (*m*, 4 H, H-C(4',2'',6''), CH); 7.37 – 7.41 (*m*, 3 H, H-C(5',6'), CH); 7.47 (*d*, *J* = 8.0, H-C(3'',5'')); 7.79 (*d*, *J* = 8.5, H-C(2,6)); 8.16 (*d*, *J* = 8.5, H-C(3,5)); 8.28 (*s*, 1 H, triazole). ¹³C-NMR: 22.7; 28.3; 34.5; 55.9; 62.0; 66.3; 113.9; 114.8; 123.4; 124.1; 125.7; 128.4; 128.8; 129.2; 129.3; 130.4; 131.7; 133.5; 138.1; 138.8; 141.6; 142.9; 146.7; 147.2; 148.8; 149.2; 189.0. Anal. calc. for C₃₁H₂₇ClN₄O₅ (571.03): C 65.21, H 4.77, N 9.81; found: C 65.36, H 4.61, N 9.66.

(2E,6E)-2-(2-[[1-(3,4-Dichlorobenzyl)-1H-1,2,3-triazol-4-yl]methoxy]-3-methoxybenzylidene)-6-(4-nitrobenzylidene)cyclohexanone (13h). Pale-yellow solid, Yield:

0.33 g (55%). M.p. 166 – 168°. IR: 2933, 2850, 1654, 1592, 1570, 1350. ¹H-NMR: 1.75 – 1.88 (*m*, CH₂); 2.75 – 2.92 (*m*, 2 CH₂); 3.77 (*s*, MeO); 5.16 (*s*, CH₂); 5.73 (*s*, CH₂); 7.12 – 7.22 (*m*, 2 H, H-C(4'), CH); 7.34 – 7.66 (*m*, 6 H, H-C(5',6',2'',5'',6''), CH); 7.80 (*d*, *J* = 8.2, H-C(2,6)); 8.15 (*d*, *J* = 8.2, H-C(3,5)); 8.28 (*s*, 1 H, triazole). ¹³C-NMR: 22.9; 28.3; 34.5; 55.9; 60.0; 62.3; 113.6; 114.8; 123.4; 124.0; 124.1; 125.4; 125.6; 128.5; 128.1; 129.1; 129.3; 130.1; 131.6; 132.5; 133.1; 138.1; 141.5; 142.7; 146.8; 147.1; 148.9; 149.0; 189.0. Anal. calc. for C₃₁H₂₆Cl₂N₄O₅ (605.47): C 61.50, H 4.33, N 9.25; found: C 61.35, H 4.41, N 9.39.

(2E,6E)-2-(3-Methoxy-2-[[1-(4-methoxybenzyl)-1H-1,2,3-triazol-4-yl]methoxy]benzylidene)-6-(4-nitrobenzylidene)-cyclohexanone (13i). Pale-yellow solid. Yield: 0.34 g (60%). M.p. 138 – 140°. IR: 2932, 2850, 1650, 1600, 1570, 1350. ¹H-NMR: 1.73 – 1.93 (*m*, CH₂); 2.90 – 2.94 (*m*, 2 CH₂); 3.74 (*s*, MeO); 3.77 (*s*, MeO); 5.17 (*s*, CH₂); 5.53 (*s*, CH₂); 6.93 (*d*, *J* = 8.4, H-C(3'',5'')); 7.15 – 7.17 (*m*, 2 H, H-C(4'), CH); 7.31 (*d*, *J* = 8.4, H-C(2'',6'')); 7.63 – 7.66 (*m*, 3 H, H-C(5',6'), CH); 7.79 (*d*, *J* = 8.8, H-C(2,6)); 8.24 – 8.28 (*m*, 3 H, H-C(3,5), triazole). ¹³C-NMR: 22.7; 28.3; 34.1; 52.8; 55.6; 55.9; 62.1; 113.7; 114.6; 114.8; 123.4; 124.0; 128.3; 128.9; 130.1; 130.5; 131.7; 133.1; 134.5; 137.7; 140.1; 142.7; 143.1; 147.2; 148.9; 149.2; 159.6; 189.0. Anal. calc. for C₃₂H₃₀N₄O₆ (566.61): C 67.83, H 5.34, N 9.89; found: C 67.74, H 5.48, N 9.71.

Biology

Reagents and Chemicals: RPMI 1640 and fetal bovine serum (FBS) were obtained from *Gibco BRL* (Grand Island, NY). 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide (MTT), trypsin-EDTA soln. and DMSO were purchased from *Sigma* (Saint Louis, MO, USA). Penicillin/streptomycin was purchased from *Invitrogen* (San Diego, CA, USA). MDA-MB-231 cells were obtained from the *National Cell Bank of Iran*, Pasteur Institute, Tehran, Iran. Cancer cell lines were grown in RPMI-1640 medium supplemented with 10% heat-inactivated fetal calf serum, 1% L-glutamine, 100 µg/ml streptomycin, and 100 U/ml penicillin and then incubated at 37 °C under a 5% concentration of CO₂.

The *in vitro* cytotoxic activity of all synthesized compounds was determined against MDA-MB-231 using MTT colorimetric assay as reported [30]. Cancer cell lines were grown in RPMI-1640 medium supplemented with 10% heat-inactivated fetal calf serum (*Gibco BRL*), 100 µg/ml streptomycin, and 100 U/ml penicillin at 37 °C in a humidified atmosphere with 5% CO₂ in air. Cells in the exponential growth phase were harvested by Trypsin-EDTA and diluted in complete growth medium to give a total cell count of 5 × 10⁴ cells/ml. 195 µl of the cell suspension was seeded into the wells of 96-well plates (*Nunc*, Denmark). The plates were incubated overnight in a humidified air atmosphere at 37 °C with 5% CO₂. After

overnight incubation, 5 µl of the media containing various concentrations of the compounds was added per well in triplicate (final concentration 1, 5, 10, and 20 µg/ml). The plates were incubated for further 72 h. The final concentration of DMSO was 0.1%. In each plate, there were three control wells (cells without test compounds) and three blank wells (the medium with 0.1% DMSO) for cell viability. Curcumin was used as positive control for cytotoxicity. After treatment, the medium was removed and 200 µl phenol red-free medium containing MTT (1 mg/ml) was added to the wells, followed by 4 h incubation. After incubation, the culture medium was replaced with 100 µl of DMSO and the absorbance of each well was measured by using a microplate reader at 492 nm. For each compound, the concentration causing 50% cell growth inhibition (IC₅₀) compared with the control was calculated from concentration response curves by nonlinear regression analysis.

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