Synthesis and *In Vitro* Cytotoxic Activity of Novel Triazole-Isoxazole Derivatives

Zahra Najafi,[a] Mohammad Mahdavi,[b] Maliheh Safavi,[c] Mina Saeedi,[b] Heshmatollah Alinezhad,[d] Mahboobeh Pordeli,[e] Sussan Kabudanian Ardestani,[e] Abbas Shafiee,[b] Alireza Foroumadi,[b] and Tahmineh Akbarzadeh[a,f]*

[a]Department of Medicinal Chemistry, Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran 14176, Iran
[b]Faculty of Pharmacy and Pharmaceutical Sciences Research Center, Tehran University of Medicinal Sciences, Tehran, Iran
[c]Department of Biotechnology, Iranian Research Organization for Science and Technology, Tehran, Iran
[d]Faculty of Chemistry, Mazandaran University, Babolsar, Iran
[e]Institute of Biochemistry and Biophysics, Department of Biochemistry, University of Tehran, Tehran, Iran
[f]Persian Medicine and Pharmacy Research Center, Tehran University of Medical Sciences, Tehran, Iran
*E-mail: akbarzad@tums.ac.ir

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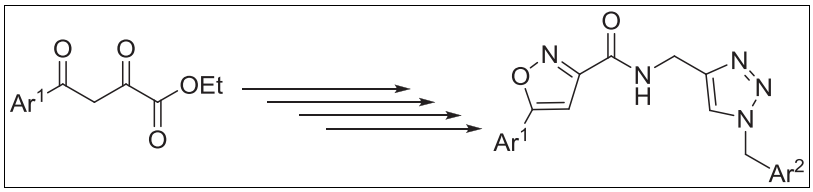

New derivatives of triazole-isoxazole were synthesized through a four-step reaction starting from various ethyl 4-aryl-2,4-dioxobutanoate derivatives. Finally, all compounds were examined by MTT assays for cytotoxic activity in two human breast cancer cell lines (MCF-7 and T-47D).

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INTRODUCTION

1,2,3-Triazole and isoxazole derivatives; two important five-membered heterocycles, have shown a significant class of privileged structures and absorbed a great deal of interest in drug discovery. 1,2,3-Triazoles have been highly recognized because of their cytotoxic [1], anti-HIV-1 [2], anti-influenza [3], anti-platelet [4], and anti-tuberculosis [5] activities. Likewise, isoxazole derivatives have attracted enormous attention for their anti-depression [6], antipyretics [7], anti-inflammatory [8], and also anti-tuberculosis [9] properties. It seems that the combination of 1,2,3-triazole and isoxazole scaffolds leads to the formation of compounds possessing promising biological activities.

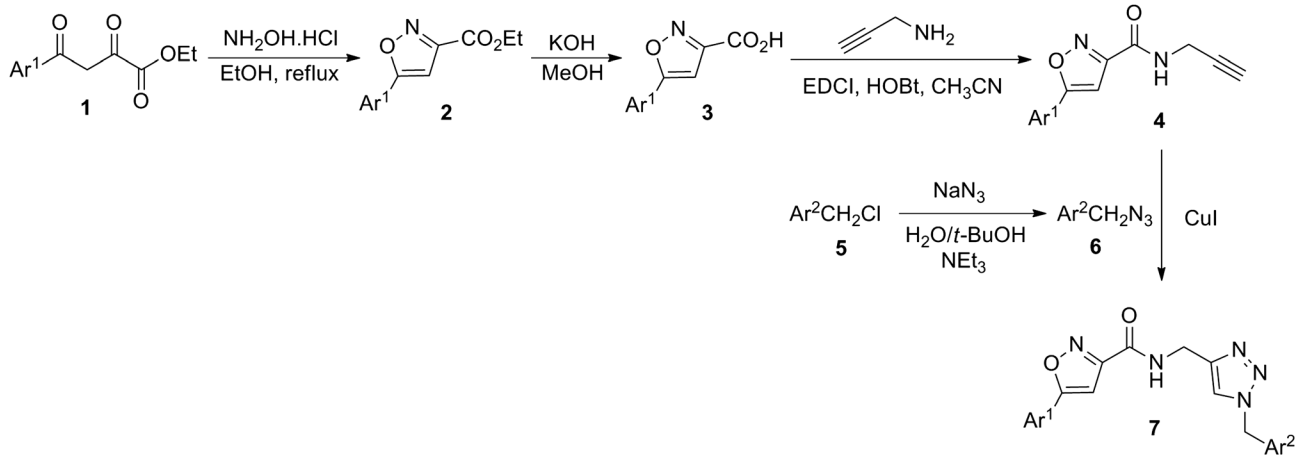
Up to now, a few efforts has been efficiently developed for the synthesis of compounds having both triazol and isoxazole skeletons [10–12]. Recently, a series of *N*-((1-benzyl-1*H*-1,2,3-triazol-4-yl)methyl)arylamides was synthesized by Stefely et al. as efficient inhibitors of cancer cell growth [13]. They exhibited *in vitro* antiproliferative activity against select cancer cell lines. Good results by this study and also lack of versatile reports in the literature encouraged us to prepare novel triazole-isoxazole derivatives and evaluate their cytotoxic activity. Herein, as a continuation of our work on the synthesis of novel heterocycles [14,15] and bioactive compounds [16] specially containing isoxazol skeleton [17], we have aimed to develop an effective protocol for the synthesis of triazole-isoxazole derivatives **7** as demonstrated in Scheme 1, starting from various ethyl 4-aryl-2,4-dioxobutanoate derivatives **1**.

RESULTS AND DISCUSSION

Chemistry. Our efforts to construct new triazole-isoxazole scaffold **7** was achieved using a four-step reaction starting from ethyl 4-aryl-2,4-dioxobutanoates **1** (Scheme 1). Various ethyl 5-arylisoxazole-3-carboxylate **2** was easily prepared according to our previous report through the reaction of **1** and hydroxylamine hydrochloride in ethanol at reflux [17]. Hydrolysis of ester group in the presence of potassium hydroxide in MeOH at reflux gave corresponding carboxylic acid **3**, which tolerated the reaction with propargyl amine in the presence of 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide and hydroxybenzotriazole in CH_3CN to obtain related amide derivative, 3-aryl-*N*-(prop-2-yn-1-yl)isoxazole-5-carboxamide derivatives **4**.

Presence of triple bond in compound **4** directed us toward click reaction to construct triazole ring. To achieve this goal, we reacted 5-phenyl-*N*-(prop-2-yn-1-yl)isoxazole-3-carboxamide **4a** with *in situ* prepared (azidomethyl)benzene **6a** under the name sharpless-type click reaction conditions [18]. It was perceived that **4a** reacted with **6a** in the presence of CuI (7 mol%) in H_2O/*t*-BuOH at room temperature for 24 h leading to the formation of corresponding product, *N*-((1-benzyl-1*H*-1,2,3-triazol-4-yl)methyl)-5-phenylisoxazole-3-carboxamide **7a** in good yields (68%). Next, using the developed method, various triazole-isoxazole derivatives involving different substituents on triazole and isoxazole rings were prepared (Table 1). It is worth to mention that all reactions were conducted either

Scheme 1. Synthesis of triazole-isoxazole derivatives **7**.



with electron-withdrawing or electron-donating groups and desired products were obtained in good yields.

Biological investigation. The *in vitro* cytotoxic activity of all triazole-isoxazole derivatives **7a–l** were evaluated against two human breast cancer cell lines MCF-7 and T-47D. All results have been summarized in Table 1. As can be seen in Table 1, according to IC_{50} values, most of compounds have no effect on the two cell lines. In MCF-7 cell line, all compounds except **7c** (Entry 3, Table 1) had IC_{50} higher than 100 µg/mL. In T-47D cell line, **7d** had IC_{50} lower than 100 µg/mL and was the most potent compound in this cell line.

CONCLUSIONS

In conclusion, we have developed a user-friendly and effectual method for the synthesis of novel triazole-isoxazole derivatives. The synthetic route for the synthesis of the title compounds was achieved through a four-step reaction starting from different ethyl 4-aryl-2,4-dioxobutanoate derivatives. In addition, all compounds were evaluated for their cytotoxic activity. Although most of compounds showed no activity against two breast cancer cell lines MCF-7 and T-47D, compounds **7c** and **7d** were found to be effective *in vitro* anti-cancer agents.

EXPERIMENTAL

Chemistry. Melting points were taken on a *Kofler* hot stage apparatus and are uncorrected. ^{1}H-NMR and ^{13}C-NMR spectra were recorded on *Bruker FT-500* and *FT-400* (*Germany*), using tetramethylslane as an internal standard. The infrared (IR) spectra were obtained on a *Nicolet Magna FTIR 550* spectrometer (KBr disks). The elemental analysis was performed with an Elementar Analysensystem GmbH *VarioEL* CHNS mode (*Germany*). Mass spectra were recorded on an Agilent Technology (HP) mass spectrometer operating at an ionization potential of 70 eV.

Ethyl 5-arylisoxazole-3-carboxylate **2** was prepared according to our previous report [17].

Procedure for the synthesis of 5-arylisoxazole-3-carboxylic acid (3). A solution of ethyl 5-arylisoxazole-3-carboxylate **2** (1 mmol) and potassium hydroxide (0.05 g, 1 mmol) in MeOH (5 mL) was heated at reflux for 3 h. After completion of reaction (checked by thin-layer chromatography), the reaction mixture was cooled to room temperature and acidified with concentrated hydrochloric acid (HCl). The precipitates were filtered off and washed with water to give the corresponding products without further purification.

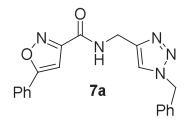
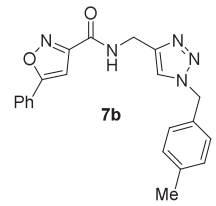
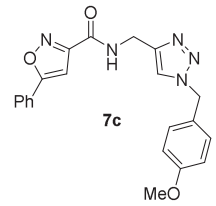
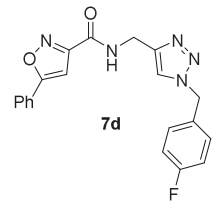
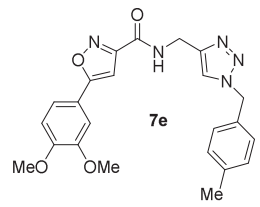
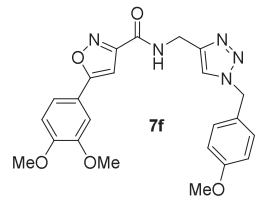
Procedure for the synthesis of 3-aryl-*N*-(prop-2-yn-1-yl) isoxazole-5-carboxamide (4). A solution of **3** (1 mmol), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (0.17 g, 1.1 mmol), and hydroxybenzotriazole (0.13 g, 1 mmol) in dry acetonitrile (5 mL) was stirred at room temperature for 30 min. Then, propargylamine (0.05 g, 1 mmol) was added to the mixture and the reaction was continued at room temperature for 24 h. After completion of reaction, the solvent was reduced under vacuum, and the residue was dissolved in dichloromethane and washed with sodium carbonate (10%). The organic phase was dried over Na_2SO_4, and the solvent was evaporated. The obtained compound was completely pure and used for further reaction.

Procedure for the synthesis of triazole-isoxazole derivatives (7). A solution of benzyl chloride derivative **5** (1.1 mmol), sodium azide (0.06 g, 0.9 mmol), and triethylamine (0.13 g, 1.3 mmol) in water (4 mL) and *tert*-butyl alcohol (4 mL) was stirred at room temperature for 30 min. The prepared compound **4** (0.5 mmol) and CuI (7 mol%) was added to reaction mixture, and it was continued for further 16–24 h. Upon completion of the reaction, monitored by thin-layer chromatography, the reaction mixture was diluted with water, poured in ice, and the precipitated product was filtered off, washed with cold water, and purified by flash chromatography on silica gel using petroleum ether/ethyl acetate/dichloromethane (3:1:1) as eluent.

***N*-((1-Benzyl-1H-1,2,3-triazol-4-yl)methyl)-5-phenylisoxazole-3-carboxamide (7a).** White crystals; yield: 68%; mp 201–204 °C. IR (KBr, cm^{-1}) υ: 3415, 3124, 2928, 1663, 1615, 1542; ^{1}H-NMR (500 MHz, $CDCl_3$) δ ppm: 4.75 (s, 2H, $NHCH_2$), 5.53 (s, 2H, CH_2), 6.96 (s, 1H, isoxazole), 7.28–7.30 (m, 2H, Ph), 7.37–7.40 (m, 3H, Ph), 7.45 (bs, 1H, NH), 7.49–7.50 (m, 3H, Ph), 7.57 (s, 1H, triazole), 7.79–7.80 (m, 2H, Ph); ^{13}C-NMR (100 MHz, DMSO-d_6) δ ppm: 35.0, 53.2, 100.3, 123.6, 126.2, 126.7, 128.5, 128.6, 129.2, 129.8, 131.3, 136.6, 145.1, 158.9, 159.9, 170.8; MS

Table 1

Chemical structures and *in vitro* cytotoxic activity (IC_{50}, μg/mL)[a] of triazole-isoxazole derivatives **7** (**a–l**) against breast cancer cell lines.

Entry	Product **7**	MCF-7	T47D
1	 **7a**	>100	—
2	 **7b**	>100	>100
3	 **7c**	85.7 ± 4.5	>100
4	 **7d**	>100	27.7 ± 0.1
5	 **7e**	>100	>100
6	 **7f**	>100	>100

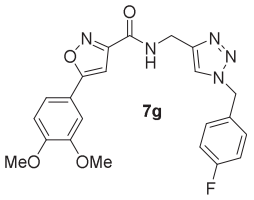
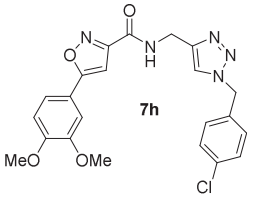
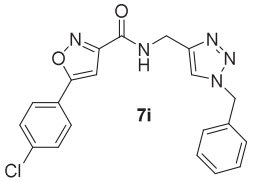
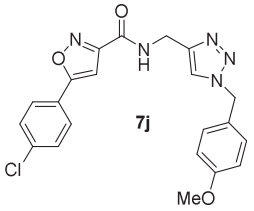
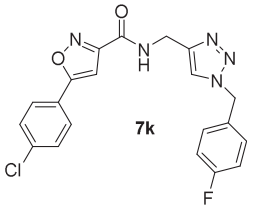
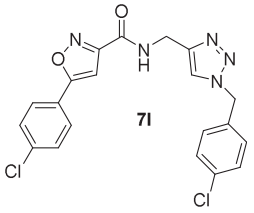
(*Continues*)

(*m/z*): 359 ([M]$^{+\cdot}$, 69), 240 (27), 187 (33), 172 (41), 158 (32), 143 (52), 105 (55), 91 (100), 77 (33); *Anal.* Calcd for $C_{20}H_{17}N_5O_2$: C, 66.84; H, 4.77; N, 19.49. Found: C, 66.94; H, 4.58; N, 19.23.

N-((1-(4-Methylbenzyl)-1H-1,2,3-triazol-4-yl)methyl)-5-phenylisoxazole-3-carboxamide (7b). White crystals; yield: 58%; mp 179–182 °C. IR (KBr, cm^{-1}) υ: 3390, 3127, 3072, 2925,

Table 1

(*Continued*)

Entry	Product **7**	MCF-7	T47D
7	 **7g**	>100	>100
8	 **7h**	>100	>100
9	 **7i**	>100	>100
10	 **7j**	>100	>100
11	 **7k**	>100	>100
12	 **7l**	>100	>100
13	Etoposide	7.5 ± 0.32	7.9 ± 0.45

[a]The IC_{50} values represent an average of three independent experiments (mean ± SD).

1665, 1611, 1541; ^{1}H-NMR (500 MHz, $CDCl_3$) δ ppm: 2.36 (s, 3H, CH_3), 4.73 (s, 2H, $NHCH_2$), 5.48 (s, 2H, CH_2), 6.95 (s, 1H, isoxazole), 7.18–7.20 (m, 4H, $H_{2'}$, $H_{3'}$, $H_{5'}$, $H_{6'}$), 7.40 (bs,

1H, NH), 7.48–7.51 (m, 4H, Ph, triazole), 7.79–7.81 (m, 2H, Ph); *Anal*. Calcd for $C_{21}H_{19}N_5O_2$: C, 67.55; H, 5.13; N, 18.76. Found: C, 67.39; H, 5.28; N, 18.91.

N-((1-(4-Methoxybenzyl)-1H-1,2,3-triazol-4-yl)methyl)-5-phenylisoxazole-3-carboxamide (7c). White crystals; yield: 65%; mp 180–183 °C. IR (KBr, cm^{-1}) υ: 3427, 3124, 2953, 1666, 1613, 1581; ^{1}H-NMR (500 MHz, $CDCl_3$) δ ppm: 3.82 (s, 3H, OCH_3), 4.76 (s, 2H, $NHCH_2$), 5.48 (s, 2H, CH_2), 6.91 (d, J = 8.2 Hz, 2H, $H_{3'}$, $H_{5'}$), 6.96 (s, 1H, isoxazole), 7.26 (d, J = 8.2 Hz, 2H, $H_{2'}$, $H_{6'}$), 7.48–7.50 (m, 3H, Ph), 7.58 (bs, 2H, triazole, NH), 7.78–7.80 (m, 2H, Ph); ^{13}C-NMR (100 MHz, $CDCl_3$) δ ppm: 35.0, 53.8, 55.3, 99.0, 114.5, 122.0, 125.9, 126.3, 126.7, 129.1, 129.8, 130.8, 144.3, 158.8, 159.0, 160.0, 171.6; MS (*m/z*): 389 ($[M]^{+\cdot}$, 45), 307 (45), 215 (18), 188 (34), 173 (40), 121 (100), 105 (34), 77 (37); *Anal*. Calcd for $C_{21}H_{19}N_5O_3$: C, 64.77; H, 4.92; N, 17.98. Found: C, 64.59; H, 5.17; N, 18.22.

N-((1-(4-Fluorobenzyl)-1H-1,2,3-triazol-4-yl)methyl)-5-phenylisoxazole-3-carboxamide (7d). White crystals; yield: 71%; mp 202–205 °C. IR (KBr, cm^{-1}) υ: 3360, 3113, 3065, 2923, 2853, 1669, 1606, 1537; ^{1}H-NMR (500 MHz, $CDCl_3$) δ ppm: 4.78 (s, 2H, $NHCH_2$), 5.51 (s, 2H, CH_2), 6.95 (s, 1H, isoxazole), 7.08 (t, J = 8.3 Hz, 2H, $H_{3'}$, $H_{5'}$), 7.28–7.31 (m, 2H, $H_{2'}$, $H_{6'}$), 7.49–7.50 (m, 4H, Ph, NH), 7.56 (s, 1H, triazole), 7.79–7.81 (m, 2H, Ph); *Anal*. Calcd for $C_{20}H_{16}FN_5O_2$: C, 63.65; H, 4.27; N, 18.56. Found: C, 63.50; H, 4.39; N, 18.73.

5-(3,4-Dimethoxyphenyl)-N-((1-(4-methylbenzyl)-1H-1,2,3-triazol-4-yl)methyl)isoxazole-3-carboxamide (7e). Off-white crystals; yield: 55%; mp 169–172 °C. IR (KBr, cm^{-1}) υ: 3375, 3134, 3061, 2960, 1668, 1609, 1541; ^{1}H-NMR (500 MHz, $CDCl_3$) δ ppm: 2.35 (s, 3H, CH_3), 3.95 (s, 3H, OCH_3), 3.96 (s, 3H, OCH_3) 4.71 (d, J = 5.8 Hz, 2H, $NHCH_2$), 5.47 (s, 2H, CH_2), 6.84 (s, 1H, isoxazole), 6.95 (d, J = 8.4 Hz, 1H, $H_{5''}$), 7.17–7.19 (m, 4H, $H_{2'}$, $H_{3'}$, $H_{5'}$, $H_{6'}$), 7.28 (d, J = 2.0 Hz, 1H, $H_{2''}$), 7.37 (dd, J = 8.4, 2.0 Hz, 1H, $H_{6''}$), 7.45 (t, J = 5.8 Hz, 1H, NH), 7.50 (s, 1H, triazole); *Anal*. Calcd for $C_{23}H_{23}N_5O_4$: C, 63.73; H, 5.35; N, 16.16. Found: C, 63.87; H, 5.14; N, 15.97.

5-(3,4-Dimethoxyphenyl)-N-((1-(4-methoxybenzyl)-1H-1,2,3-triazol-4-yl)methyl)isoxazole-3-carboxamide (7f). White crystals; yield: 63%; mp 159–161 °C. IR (KBr, cm^{-1}) υ: 3408, 3116, 2924, 1667, 1611, 1546; ^{1}H-NMR (500 MHz, $CDCl_3$) δ ppm: 3.81 (s, 3H, OCH_3), 3.95 (s, 3H, OCH_3), 3.97 (s, 3H, OCH_3), 4.73 (d, J = 4.9 Hz, 2H, $NHCH_2$), 5.46 (s, 2H, CH_2), 6.84 (s, 1H, isoxazole), 6.91 (d, J = 8.6 Hz, 2H, $H_{3'}$, $H_{5'}$), 6.96 (d, J = 8.4 Hz, 1H, $H_{5''}$), 725 (d, J = 8.6 Hz, 2H, $H_{2'}$, $H_{6'}$), 7.29 (d, J = 1.9 Hz, 1H, $H_{2''}$), 7.38 (dd, J = 8.4 Hz, 1H, $H_{6''}$), 7.44 (bs, 1H, NH), 7.51(s, 1H, triazole); *Anal*. Calcd for $C_{23}H_{23}N_5O_5$: C, 61.46; H, 5.16; N, 15.58. Found: C, 61.22; H, 4.91; N, 15.32.

5-(3,4-Dimethoxyphenyl)-N-((1-(4-fluorobenzyl)-1H-1,2,3-triazol-4-yl)methyl)isoxazole-3-carboxamide (7g). Off-white crystals; yield: 68%; mp 159–162 °C. IR (KBr, cm^{-1}) υ: 3456, 3129, 2927, 2844, 1668, 1607, 1577; ^{1}H-NMR (500 MHz, $CDCl_3$) δ ppm: 3.95 (s, 3H, OCH_3), 3.97 (s, 3H, OCH_3), 4.73 (d, J = 5.9 Hz, 2H, $NHCH_2$), 5.50 (s, 2H, CH_2), 6.84 (s, 1H, isoxazole), 6.95 (d, J = 8.4 Hz, 1H, $H_{5''}$), 7.08 (t, J = 8.2 Hz, 2H, $H_{3'}$, $H_{5'}$), 7.28–7.30 (m, 3H, $H_{2'}$, $H_{6'}$, $H_{2''}$), 7.38 (d, J = 8.4 Hz, 1H, $H_{6''}$), 7.40 (bs, 1H, NH), 7.53 (s, 1H, triazole); *Anal*. Calcd for $C_{22}H_{20}FN_5O_4$: C, 60.41; H, 4.61; N, 16.01. Found: C, 60.23; H, 4.80; N, 15.86.

N-((1-(4-Chlorobenzyl)-1H-1,2,3-triazol-4-yl)methyl)-5-(3,4-dimethoxyphenyl)isoxazole-3-carboxamide (7h). Off-white crystals; yield: 68%; mp 202–205 °C. IR (KBr, cm^{-1}) υ: 3319, 2956, 2837, 1669, 1609, 1551; ^{1}H-NMR (500 MHz, $CDCl_3$) δ ppm: 3.95 (s, 3H, OCH_3), 3.96 (s, 3H, OCH_3), 4.71 (d, J = 5.9 Hz, 2H, $NHCH_2$), 5.49 (s, 2H, CH_2), 6.84 (s, 1H, isoxazole), 6.95 (d, J = 8.4 Hz, 1H, $H_{5''}$), 7.22 (d, J = 8.4 Hz, 2H, $H_{2'}$, $H_{6'}$), 7.28 (d, J = 1.9 Hz, 1H, $H_{2''}$), 7.34–7.37 (m, 3H, $H_{3'}$, $H_{5'}$, $H_{6''}$), 7.40 (bs, 1H, NH), 7.53 (s, 1H, triazole); MS (*m/z*): 455 ($[M]^{+\cdot}$ + 2, 7), 453 ($[M]^{+\cdot}$, 21), 165 (57), 125 (100), 89 (84), 63 (36); *Anal*. Calcd for $C_{22}H_{20}ClN_5O_4$: C, 58.22; H, 4.44; N, 15.43. Found: C, 58.45; H, 4.23; N, 15.29.

N-((1-Benzyl-1H-1,2,3-triazol-4-yl)methyl)-5-(4-chlorophenyl) isoxazole-3-carboxamide (7i). Off-white crystals; yield: 75%; mp 225–227 °C. IR (KBr, cm^{-1}) υ: 3371, 3111, 3064, 2923, 2853, 1667, 1612, 1538; ^{1}H-NMR (500 MHz, $CDCl_3$) δ ppm: 4.71 (d, J = 4.6 Hz, 2H, $NHCH_2$), 5.53 (s, 2H, CH_2), 6.94 (s, 1H, isoxazole), 7.28–7.30 (dd, J = 7.4, 1.9 Hz, 2H, Ph), 7.37–7.41 (m, 3H, Ph), 7.43 (bs, 1H, NH), 7.47 (d, J = 8.5 Hz, 2H, $H_{2'}$, $H_{6'}$), 7.54 (s, 1H, triazole), 7.73 (d, J = 8.5 Hz, 2H, $H_{3'}$, $H_{5'}$); ^{13}C-NMR (100 MHz, DMSO-d_6) δ ppm: 35.0, 53.2, 100.3, 123.6, 126.2, 126.7, 128.5, 128.6, 129.2, 129.8, 131.3, 136.6, 145.1, 158.9, 159.9, 170.8; MS (*m/z*): 395 ($[M]^{+\cdot}$ + 2, 15), 393 ($[M]^{+\cdot}$, 46), 268 (10), 240 (25), 221 (17), 192 (28), 172 (41), 125 (100), 105 (51), 89 (35); *Anal*. Calcd for $C_{20}H_{16}ClN_5O_2$: C, 61.00; H, 4.10; N, 17.78. Found: C, 61.17; H, 4.29; N, 17.90.

5-(4-Chlorophenyl)-N-((1-(4-methoxybenzyl)-1H-1,2,3-triazol-4-yl)methyl)isoxazole-3-carboxamide (7j). Off-white crystals; yield: 61%; mp 224–227 °C. IR (KBr, cm^{-1}) υ: 3416, 3120, 2925, 2854, 1669, 1612, 1543; ^{1}H-NMR (500 MHz, $CDCl_3$) δ ppm: 3.81 (s, 3H, OCH_3), 4.72 (d, J = 5.9 Hz, 2H, $NHCH_2$), 5.46 (s, 2H, CH_2), 6.90 (d, J = 8.4 Hz, 2H, $H_{3'}$, $H_{5'}$), 6.94 (s, 1H, isoxazole), 7.25 (d, J = 8.4 Hz, 2H, $H_{2'}$, $H_{6'}$), 7.37 (bs, 1H, NH), 7.47–7.49 (m, 3H, triazole, $H_{2''}$, $H_{6''}$), 7.73 (d, J = 8.5 Hz, 2H, $H_{3''}$, $H_{5''}$); *Anal*. Calcd for $C_{21}H_{18}ClN_5O_3$: C, 59.51; H, 4.28; N, 16.52. Found: C, 59.23; H, 4.55; N, 16.33.

5-(4-Chlorophenyl)-N-((1-(4-fluorobenzyl)-1H-1,2,3-triazol-4-yl)methyl)isoxazole-3-carboxamide (7k). White crystals; yield: 71%; mp 219–221 °C. IR (KBr, cm^{-1}) υ: 3458, 3109, 1668, 1608, 1539; ^{1}H-NMR (500 MHz, $CDCl_3$) δ ppm: 4.73 (d, J = 5.9 Hz, 2H, $NHCH_2$), 5.50 (s, 2H, CH_2), 6.94 (s, 1H, isoxazole), 7.08 (t, J = 8.6 Hz, 2H, $H_{3'}$, $H_{5'}$), 7.28–7.30 (m, 2H, $H_{2'}$, $H_{6'}$), 7.40 (t, J = 5.9 Hz, 1H, NH), 7.48 (d, J = 8.5 Hz, 2H, $H_{2''}$, $H_{6''}$), 7.52 (s, 1H, triazole), 7.73 (d, J = 8.5 Hz, 2H, $H_{3''}$, $H_{5''}$); *Anal*. Calcd for $C_{20}H_{15}ClFN_5O_2$: C, 58.33; H, 3.67; N, 17.01. Found: C, 58.14; H, 3.84; N, 16.87.

N-((1-(4-Chlorobenzyl)-1H-1,2,3-triazol-4-yl)methyl)-5-(4-chlorophenyl)isoxazole-3-carboxamide (7l). Cream crystals; yield: 78%; mp 217–219 °C. IR (KBr, cm^{-1}) υ: 3369, 3116, 3064, 2946, 2854, 1668, 1608, 1542; ^{1}H-NMR (500 MHz, $CDCl_3$) δ ppm: 4.73 (s, 2H, $NHCH_2$), 5.49 (s, 2H, CH_2), 6.93 (s, 1H, isoxazole), 7.22 (d, J = 7.90 Hz, 2H, $H_{2'}$, $H_{6'}$), 7.35 (d, J = 7.90 Hz, 2H, $H_{3'}$, $H_{5'}$), 7.41 (bs, 1H, NH), 7.47 (d, J = 8.4 Hz, 2H, $H_{2''}$, $H_{6''}$), 7.53 (s, 1H, triazole), 7.72 (d, J = 8.4 Hz, 2H, $H_{3''}$, $H_{5''}$); *Anal*. Calcd for $C_{20}H_{15}Cl_2N_5O_2$: C, 56.09; H, 3.53; N, 16.35. Found: C, 55.86; H, 3.29; N, 16.18.

Biology. ***Reagents and chemicals.*** RPMI 1640 and fetal bovine serum were purchased from Gibco BRL (Grand Island, NY). 3-(4,5-Dimethylthiazol-2-yl)-2,5 diphenyltetrazolium bromide (MTT), trypsin-EDTA solution, and dimethyl sulfoxide (DMSO) were obtained from Sigma (Saint Louis, MO, USA). Penicillin/streptomycin was purchased from Invitrogen (San Diego, CA, USA).

Cell lines and cell culture. Human breast cancer cell lines including MCF-7 and T-47D 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 CO_2.

Cytotoxicity assay. The *in vitro* cytotoxic activity of all synthesized compounds was determined against two human breast cancer cell lines including MCF-7 and T-47D using MTT colorimetric assay according to the literature method. MTT assay is based on reduction of the tetrazolium salt to blue-colored formazan by mitochondrial dehydrogenases in viable cells.[19]. 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% CO_2 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 5×10^4 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% CO_2. 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. Etoposide 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 wells, followed by 4 h incubation. After incubation, the culture medium was then 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 (IC_{50}) compared with the control was calculated from concentration response curves by non-linear regression analysis.

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