Turkish Journal of Chemistry

http://journals.tubitak.gov.tr/chem/

Research Article

Turk J Chem
(2017) 41: 125 – 134

doi:10.3906/kim-1512-80

Synthesis and cytotoxicity of novel thioxo-quinazolino[3,4-*a*]quinazolinones

Negar MOHAMMADHOSSEINI[1], Mina SAEEDI[2,3], Shahram MORADI[1], Mohammad MAHDAVI[5], Omidreza FIRUZI[4], Alireza FOROUMADI[5], Abbas SHAFIEE[5,*]

[1]Faculty of Chemistry, Tehran-North Branch, Islamic Azad University, Tehran, Iran
[2]Medicinal Plants Research Center, Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran, Iran
[3]Persian Medicine and Pharmacy Research Center, Tehran University of Medical Sciences, Tehran, Iran
[4]Medicinal and Natural Product Chemistry Research Center, Shiraz University of Medical Sciences, Shiraz, Iran
[5]Department of Medicinal Chemistry, Faculty of Pharmacy and Pharmaceutical Sciences Research Center, Tehran University of Medical Sciences, Tehran, Iran

Received: 26.12.2015 • **Accepted/Published Online:** 05.04.2016 • **Final Version:** 22.02.2017

Abstract: Various thioxo-quinazolino[3,4-*a*]quinazolinones were prepared and evaluated for their cytotoxicity in MOLT-4 (lymphoblastic leukemia) and MCF-7 (breast adenocarcinoma) cell lines. Synthesis of the target compounds was started from isatoic anhydride. Successive reaction of isatoic anhydride with benzylamine and 2-nitrobenzaldehyde, reduction of the nitro group, and reaction with CS_2 gave 12-benzyl-6-thioxo-6,7,11b,12-tetrahydro-13*H*-quinazolino[3,4-*a*]quinazolin-13-one. The latter compound reacted with various 2-chloro-*N*-substituted acetamides to afford the corresponding fused quinazolinone derivatives.

Key words: Quinazolinones, cytotoxicity, thioxo-quinazolino[3,4-*a*]quinazoline, 2-nitrobenzaldehyde

1. Introduction

Quinazoline and its derivatives, a large and important class of heterocyclic compounds, have attracted lots of attention due to their efficient and versatile biological activities[1] such as antimicrobial,[2] anticancer,[3] anticholinesterase,[4] and antihypertensive.[5] Among the versatile and efficient medicinal properties of quinazolines, their anticancer activity has occupied significant attention since raltitrexed[6] and thymitaq[7] (Figure) have been used in cancer chemotherapy. Hence, synthesis of quinazolinones has been the focus of attention and various methods have been reported for their synthesis in the literature.[8]

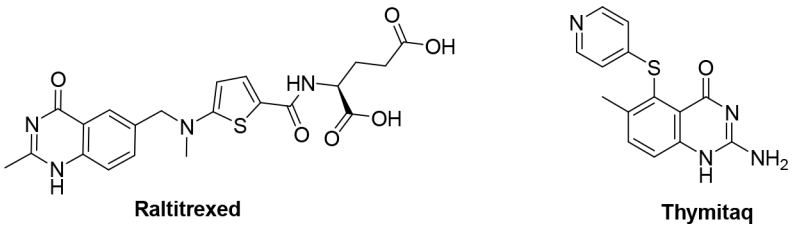


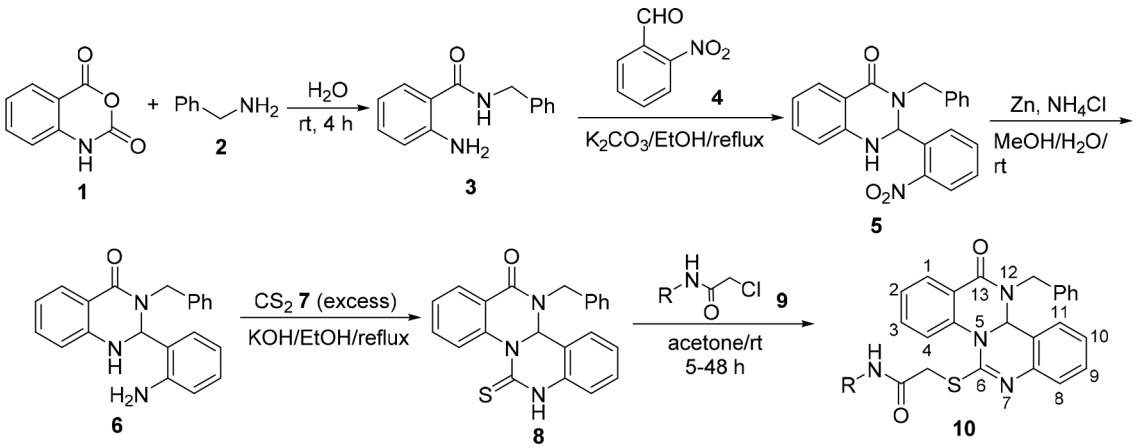
Figure. The structure of raltitrexed and thymitaq.

*Correspondence: shafieea@tums.ac.ir

Quinazolinones are usually prepared by the reaction of 2-aminobenzonitrile and 3-phenyl-acryloyl chloride followed by oxidative ring closure under basic conditions.[9] Cyclization reaction of 2-aminobenzoic acids and the corresponding modified protocols have been versatile and efficient procedures for the synthesis of qinazolinones.[10,11] Palladium-mediated N-heterocyclisation of 2-nitrophenyl ketones with formamide[12] as well aspalladium-mediated cyclocarbonylation of o-iodoanilines with heterocumulenes in the presence of CO (g) are newly reported protocols in the field of quinazolinones.[13]

Recently, isatoic anhydride has been reported as a versatile starting material,[14,15] and in this regard various procedures have been developed by our research group leading to the synthesis of a wide spectrum of functionalized quinazolinones. Obviously, 2-aminobenzamides obtained from reaction of isatoic anhydride and amines are efficient bident nucleophiles to react with various electrophiles such as dimethyl acetylenedicarboxylate,[16] aldehydes,[17–19] Vilsmeier reagent,[20] boronic acids,[21] carbon disulfide and anthranilic acids,[22] and N,N'-dialkylcarbodiimides.[23]

In continuation of our research program for the synthesis of novel heterocycles[24–27] using isatoic anhydride,[16–23] herein we report the synthesis of a wide range of novel thioxo-quinazolino[3,4-a]quinazolinones **10** to investigate their cytotoxicity (Scheme).



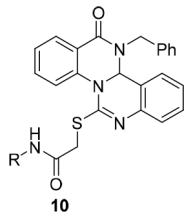
Scheme. Synthesis of novel thioxo-quinazolino[3,4-a]quinazolinones **10**.

2. Results and discussion

2.1. Chemistry

The synthesis of novel desired thioxo-quinazolino[3,4-a]quinazoline derivatives **10** is depicted in the Scheme. Compounds **3**, **5**, **6**, and **8** were prepared according to our previous report.[19] Reaction of isatoic anhydride **1** and benzylamine **2** in water at ambient temperature for 4 h gave 2-amino-N-benzylbenzamide **3**, which reacted with 2-nitrobenzaldehyde **4** in refluxing EtOH in the presence of K_2CO_3 followed by reduction of the nitro group in the presence of Zn/NH_4Cl to give 2-(2-aminophenyl)-3-benzyl-2,3-dihydroquinazolin-4(1H)-one **6**. Next, reaction of the latter compound with an excess amount of carbon disulfide (CS_2) **7** in refluxing EtOH in the presence of KOH afforded 12-benzyl-6-thioxo-6,7,11b,12-tetrahydro-13H-quinazolino[3,4-a]quinazolin-13-one **8**. Finally, reaction of compound **8** and 2-chloro-N-substituted acetamides **9** (obtained from chloroacetyl chloride and different amines in DMF at room temperature) in acetone at room temperature led to the formation of the desired products **10** (Table).

Table. Synthesis and cytotoxicity of thioxo-quinazolino[3,4-*a*]quinazolinones **10**.



10

Entry	R	Product **10**	MOLT-4 IC_{50} (μM)	MCF-7 cells IC_{50} (μM)
1	Cyclohexyl	**10a**	14.9 ± 0.8	21.8 ± 1.9
2	C_6H_5	**10b**	> 100	> 100
3	2-MeO-C_6H_4	**10c**	> 100	> 100
4	3-MeO-C_6H_4	**10d**	> 100	> 100
5	4-MeO-C_6H_4	**10e**	> 100	> 100
6	2,5-diMeO-C_6H_3	**10f**	> 100	> 100
7	3,4-diMeO-C_6H_3	**10g**	> 100	> 100
8	3,4,5-triMeO-C_6H_2	**10h**	> 100	> 100
9	2-Cl-C_6H_4	**10i**	> 100	> 100
10	3-Cl-C_6H_4	**10j**	> 100	> 100
11	4-Cl-C_6H_4	**10k**	54.1 ± 1.3	> 100
12	2,5-diCl-C_6H_3	**10l**	> 100	> 100
13	3,4-diCl-C_6H_3	**10m**	> 100	> 100
14	2-Me-C_6H_4	**10n**	> 100	> 100
15	3-Me-C_6H_4	**10o**	> 100	> 100
16	4-Me-C_6H_4	**10p**	> 100	> 100
17	Cisplatin		3.3 ± 0.3	12.3 ± 3.0

The structures of all products derived from **10** were elucidated by IR, ^{1}H NMR, and ^{13}C NMR spectroscopy as well as chemical analysis. For example, in the ^{1}H NMR spectrum of compound **10a**, cyclohexyl protons were detected in the range of 1.09–3.54 ppm. Methylene protons were observed at 3.70 and 4.49 ppm. Moreover, a CH singlet signal was distinguished at 6.29 ppm. Aromatic protons (H_1, H_2, H_3, H_4, H_8, H_9, H_{10}, H_{11}, and phenyl as depicted in the Scheme) were detected in the range of 6.79–8.27 ppm according to the expected chemical shifts and coupling patterns. Furthermore, amide NH proton was observed at 10.30 ppm. The ^{13}C NMR spectrum showed the expected 28 signals including 19 aromatic (between 115.8 and 168.0 ppm) and 9 aliphatic signals (between 24.5 and 69.7 ppm).

2.2. Biological investigation

The effects of sixteen molecules derived from compound **10** on cell viability were evaluated in two cancer cell lines, namely MOLT-4 (lymphoblastic leukemia) and MCF-7 (breast adenocarcinoma). Cisplatin was used as a positive control (Table). Based on calculated IC_{50} values, most of compounds showed no cytotoxicity and, among them, compounds **10a** and **10k** were found to be cytotoxic active derivatives. Compound **10a** depicted activity against MOLT-4 and MCF-7 with IC_{50} values = 14.9 and 21.8 μM, respectively, compared with cisplatin (IC_{50} values = 3.3 and 12.3). It is obvious that the cyclohexyl group induced higher activity compared with the other aryl group connected to acetamide moiety. Compound **10k** showed cytotoxicity against MOLT-4 (IC_{50} = 54.1 μM) and no activity was observed against MCF-7. It is clear that introduction of methoxy, chlorine, and

methyl groups led to the elimination of cytotoxic activity and only the presence of chlorine at the 4-position of aryl connected to acetamide moiety induced cytotoxicity against MOLT-4.

3. Experimental

3.1. Apparatus and chemicals

Melting points were determined on a Kofler hot stage apparatus and are uncorrected. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker 500-MHz instrument using TMS as an internal standard. IR spectra were acquired on a Nicolet Magna 550-FT spectrometer. All reagents were obtained from Merck and Aldrich.

3.2. Preparation of fused quinazolinone derivatives 10

Compounds **3**, **5**, **6**, and **8** were prepared according to our previous report.[19]

A mixture of 12-benzyl-6-thioxo-6,7,11b,12-tetrahydro-13*H*-quinazolino[3,4-*a*]quinazolin-13-one **8** (1 mmol), 2-chloro-*N*-substituted acetamide **9** (1 mmol), and potassium iodide (1 mmol) in acetone (8 mL) was heated at room temperature for 5–48 h. After completion of the reaction, water (8 mL) was added to the reaction mixture, and the precipitates were filtered off and recrystallized from EtOH /H_2O (90/10) to give the corresponding product **10**.

3.2.1. N-Cyclohexyl-2-((13-oxo-12-benzyl-11b,12-dihydro-13H-quinazolino[3,4-a]quinazolin-6-yl) thio)acetamide (10a)

Yield: 32%, mp 173–175 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 1.09–1.22 (m, 2H, Cyclohexyl), 1.28–1.39 (m, 2H, Cyclohexyl), 1.53–1.60 (m, 2H, Cyclohexyl), 1.62–1.69 (m, 2H, Cyclohexyl), 1.85–1.97 (m, 2H, Cyclohexyl), 3.48–3.54 (m, 1H, Cyclohexyl), 3.70 (s, 2H, CH_2), 4.49 (s, 2H, SCH_2), 6.29 (s, 1H, CH), 6.79 (d, J= 7.5 Hz, 1H, H_4), 6.90 (d, J = 7.5 Hz, 1H, H_8), 7.13–7.21 (m, 5H, Ph), 7.45 (t, J= 7.5 Hz, 1H, H_9), 7.53–7.63 (m, 4H, H_2, H_3, H_{10}, H_{11}), 8.27 (d, J= 7.5 Hz, 1H, H_1), 10.30 (s, 1H, NH); ^{13}C NMR (125 MHz, $CDCl_3$) δ: 24.5, 25.5, 32.6, 32.7, 34.8, 44.8, 48.1, 48.2, 69.7, 115.8, 123.3, 124.7, 125.2, 126.7, 126.9, 127.0, 128.2, 128.4, 128.6, 129.5, 131.2, 132.1, 135.1, 137.2, 139.5, 156.4, 162.8, 168.0; IR (cm^{-1}) υ: 3478, 1664, 1551; Anal. Calcd for $C_{30}H_{30}N_4O_2S$: C, 70.56; H, 5.92; N, 10.97; Found: C, 70.79; H, 6.10; N, 10.69.

3.2.2. 2-((13-Oxo-12-benzyl-11b,12-dihydro-13H-quinazolino[3,4-a]quinazolin-6-yl)thio)-N-phenylacetamide (10b)

Yield: 25%, mp 172–174 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 3.96 (d, J= 18.5 Hz, 1H, CH_2), 4.07 (d, J= 18.5 Hz, 1H, CH_2), 4.17 (d, J= 20.0 Hz, 1H, CH_2), 4.50 (d, J= 20.0 Hz, 1H, CH_2), 6.57 (s, 1H, CH), 6.71 (d, J= 9.0 Hz, 1H, H_4), 6.77 (t, J= 9.0 Hz, 1H, H_2), 6.97 (d, J= 9.0 Hz, 1H, H_8), 7.00–7.11 (m, 4H, Ph), 7.13 (t, J= 9.0 Hz, 1H, H_9), 7.19 (t, J= 9.0 Hz, 1H, H_{10}), 7.29–7.38 (m, 3H, Ph), 7.56–7.63 (m, 3H, Ph), 7.66–7.75 (m, 2H, H_3, H_{11}), 8.07 (d, J = 9.0 Hz, 1H, H_1), 10.29 (s, 1H, NH); ^{13}C NMR (125 MHz, $CDCl_3$) δ: 37.0, 44.5, 68.9, 117.0, 119.6, 123.9, 125.1, 125.6, 126.3, 126.8, 127.0, 128.4, 128.9, 129.2, 129.3, 130.0, 131.2, 132.7, 137.9, 139.4, 140.1, 141.4, 155.2, 162.6, 164.0, 166.3; IR (cm^{-1}) υ: 3396, 1655, 1543; Anal. Calcd for $C_{30}H_{24}N_4O_2S$: C, 71.41; H, 4.79; N, 11.10; Found: C, 71.22; H, 4.58; N, 11.23.

3.2.3. 2-((13-Oxo-12-benzyl-11b,12-dihydro-13H-quinazolino[3,4-a]quinazolin-6-yl)thio)-N-(2-methoxyphenyl)acetamide (10c)

Yield: 79%, mp 179–181 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 3.63 (s, 3H, OCH_3), 3.97 (d, J= 19.0 Hz, 1H, CH_2), 4.01 (d, J= 19.0 Hz, 1H, CH_2), 4.18 (d, J= 20.0 Hz, 1H, CH_2), 4.48 (d, J= 20.0 Hz, 1H, CH_2), 6.60 (s, 1H, CH), 6.74–6.79 (m, 2H, H_4, H_3'), 6.91 (t, J= 9.5 Hz, 1H, H_2), 6.97–6.77 (m, 5H, Ph), 7.10 (d, J= 9.2 Hz, 1H, H_8), 7.14–7.22 (m, 2H, H_9, H_{11}), 7.41 (t, J= 9.2 Hz, 1H, H_{10}), 7.57–7.74 (m, 4H, H_3, H_4', H_5', H_6'), 8.08 (d, J= 9.5 Hz, 1H, H_1), 9.55 (s, 1H, NH); ^{13}C NMR (125 MHz, $CDCl_3$) δ: 36.1, 44.5, 55.9, 69.0, 111.4, 113.3, 117.0, 120.8, 120.9, 122.1, 125.4, 125.6, 126.3, 126.8, 127.0, 127.7, 128.3, 128.7, 128.9, 129.2, 131.2, 132.7, 137.9, 139.9, 141.3, 149.2, 155.4, 162.6, 166.8; IR (cm^{-1}) υ: 3251, 1684, 1657; Anal. Calcd for $C_{31}H_{26}N_4O_3S$: C, 69.64; H, 4.90; N, 10.48; Found: C, 69.36; H, 5.06; N, 10.73.

3.2.4. 2-((13-Oxo-12-benzyl-11b,12-dihydro-13H-quinazolino[3,4-a]quinazolin-6-yl)thio)-N-(3-methoxyphenyl)acetamide (10d)

Yield: 70%, mp 178–180 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 3.71 (s, 3H, OCH_3), 3.96 (d, J= 15.0 Hz, 1H, CH_2), 4.06 (d, J= 15.0 Hz, 1H, CH_2), 4.18 (d, J= 16.2 Hz, 1H, CH_2), 4.51 (d, J= 16.2 Hz, 1H, CH_2), 6.56 (s, 1H, CH), 6.64 (d, J= 7.0 Hz, 1H, $H_{4'}$), 6.76–6.78 (m, 2H, H_8, H_4), 6.88 (d, J= 7.0 Hz, 1H, $H_{6'}$), 7.03 (d, J= 7.2 Hz, 1H, H_{11}), 7.06–7.15 (m, 4H, Ph), 7.16–7.24 (m, 2H, H_2, Ph), 7.03 (s, 1H, $H_{2'}$), 7.35 (t, J= 7.2 Hz, 1H, H_3), 7.59 (t, J= 7.2 Hz, 1H, H_{10}), 7.65–7.75 (m, 2H, H_9, $H_{5'}$), 8.08 (d, J= 7.2 Hz, 1H, H_1), 10.28 (s, 1H, NH); ^{13}C NMR (125 MHz, $CDCl_3$) δ: 37.0, 44.5, 55.4, 68.9, 104.1, 105.4, 109.3, 111.9, 117.0, 123.9, 125.1, 125.6, 126.4, 128.4, 128.6, 128.9, 129.3, 130.0, 131.2, 132.7, 137.9, 140.1, 140.6, 140.7, 141.4, 155.2, 156.0, 162.6, 166.3; IR (cm^{-1}) υ: 3507, 1699, 1650; Anal. Calcd for $C_{31}H_{26}N_4O_3S$: C, 69.64; H, 4.90; N, 10.48; Found: C, 69.88; H, 5.13; N, 10.59.

3.2.5. 2-((13-Oxo-12-benzyl-11b,12-dihydro-13H-quinazolino[3,4-a]quinazolin-6-yl)thio)-N-(4-methoxyphenyl)acetamide (10e)

Yield: 96%, mp 150–152 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 3.71 (s, 3H, OCH_3), 3.93 (d, J= 15.0 Hz, 1H, CH_2), 4.04 (d, J= 15.0 Hz, 1H, CH_2), 4.18 (d, J= 16.2 Hz, 1H, CH_2), 4.51 (d, J= 16.2 Hz, 1H, CH_2), 6.56 (s, 1H, CH), 6.77–6.78 (m, 2H, H_4, H_{11}), 6.89 (d, J= 8.8 Hz, 2H, $H_{3'}$, $H_{5'}$), 7.01 (t, J= 7.6 Hz, 1H, Ph), 7.03–7.22 (m, 5H, H_2, Ph), 7.35 (t, J= 7.6 Hz, 1H, H_{10}), 7.49 (d, J= 8.8 Hz, 2H, $H_{2'}$, $H_{6'}$), 7.61 (t, J= 7.6 Hz, 1H, H_3), 7.70 (d, J= 7.6 Hz, 1H, H_8), 7.71–7.75 (t, J = 7.6 Hz, 1H, H_9), 8.07 (d, J= 7.6 Hz, 1H, H_1), 10.16 (s, 1H, NH); ^{13}C NMR (125 MHz, $CDCl_3$) δ: 36.9, 44.5, 55.6, 68.9, 114.2, 114.3, 117.0, 120.9, 121.2, 123.9, 124.0, 125.1, 125.6, 126.4, 126.8, 127.0, 128.4, 128.9, 131.2, 132.6, 132.7, 137.9, 140.1, 141.5, 155.8, 162.6, 165.7; IR (cm^{-1}) υ: 3359, 1672, 1643; Anal. Calcd for $C_{31}H_{26}N_4O_3S$: C, 69.64; H, 4.90; N, 10.48; Found: C, 69.48; H, 5.11; N, 10.77.

3.2.6. 2-((13-Oxo-12-benzyl-11b,12-dihydro-13H-quinazolino[3,4-a]quinazolin-6-yl)thio)-N-(2,5-dimethoxyphenyl)acetamide (10f)

Yield: 41%, mp 187–188 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 3.58 (s, 3H, OCH_3), 3.69 (s, 3H, OCH_3), 3.97 (d, J= 14.8 Hz, 1H, CH_2), 4.10 (d, J= 14.8 Hz, 1H, CH_2), 4.19 (d, J= 16.4 Hz, 1H, CH_2), 4.45 (d, J= 16.4 Hz, 1H, CH_2), 6.60 (d, J= 2.2 Hz, 1H, $H_{6'}$), 6.62 (s, 1H, CH), 6.76 (d, J= 8.8 Hz, 1H, H_4), 6.90 (d, J= 8.8

Hz, 1H, H_8), 6.98–7.05 (m, 3H, H_2, H_9, $H_{3'}$), 7.09 (d, J= 8.8 Hz, 1H, H_{11}), 7.14–7.22 (m, 3H, Ph), 7.41 (td, J= 2.4, 8.8 Hz, 1H, H_{10}), 7.60 (t, J= 8.8 Hz, 1H, H_3), 7.65–7.74 (m, 3H, Ph, $H_{4'}$), 8.07 (d, J= 8.8 Hz, 1H, H_1), 9.06 (s, 1H, NH); ^{13}C NMR (125 MHz, $CDCl_3$) δ: 36.2, 44.5, 55.8, 56.4, 69.5, 106.2, 107.5, 108.3, 112.0, 117.0, 124.1, 125.4, 125.6, 126.3, 126.8, 127.0, 128.4, 128.7, 128.9, 130.8, 131.2, 132.8, 137.9, 139.9, 141.2, 143.2, 153.4, 155.4, 162.7, 167.0; IR (cm^{-1}) υ: 3257, 1686, 1655; Anal. Calcd for $C_{32}H_{28}N_4O_4S$: C, 68.07; H, 5.00; N, 9.92; Found: C, 68.25; H, 4.83; N, 10.03.

3.2.7. 2-((13-Oxo-12-benzyl-11b,12-dihydro-13H-quinazolino[3,4-a]quinazolin-6-yl)thio)-N-(3,4-dimethoxyphenyl)acetamide (10g)

Yield: 54%, mp 192–193 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 3.52 (d, J= 14.5 Hz, 1H, CH_2), 3.80 (s, 3H, OCH_3), 3.84 (s, 3H, OCH_3), 3.96 (d, J= 14.5 Hz, 1H, CH_2), 4.32 (d, J= 15.7 Hz, 1H, CH_2), 4.70 (d, J= 15.7 Hz, 1H, CH_2), 6.46 (s, 1H, CH), 6.74–6.78 (m, 3H, H_2, $H_{5'}$, H_4), 6.87 (d, J= 6.7 Hz, 1H, $H_{6'}$), 7.00 (d, J= 7.1 Hz, 1H, H_8), 7.04–7.07 (m, 3H, $H_{2'}$, H_3, H_9), 7.18–7.28 (m, 3H, Ph), 7.45 (td, J= 1.0, 7.1 Hz, 1H, H_{10}), 7.52–7.60 (m, 3H, Ph, H_{11}), 8.26 (d, J= 7.1 Hz, 1H, H_1), 10.04 (s, 1H, NH); ^{13}C NMR (125 MHz, $CDCl_3$) δ: 35.6, 44.7, 55.8, 56.1, 69.5, 104.3, 111.2, 111.4, 115.9, 123.1, 124.5, 125.5, 126.5, 126.8, 126.9, 128.1, 128.4, 128.8, 129.5, 131.2, 132.1, 137.1, 139.3, 140.6, 145.5, 146.1, 149.0, 157.1, 162.7, 166.8; IR (cm^{-1}) υ: 3253, 1662, 1606; Anal. Calcd for $C_{32}H_{28}N_4O_4S$: C, 68.07; H, 5.00; N, 9.92; Found: C, 68.32; H, 4.82; N, 9.70.

3.2.8. 2-((13-Oxo-12-benzyl-11b,12-dihydro-13H-quinazolino[3,4-a]quinazolin-6-yl)thio)-N-(3,4,5-trimethoxyphenyl)acetamide (10h)

Yield: 20%, mp 200–202 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 3.49 (d, J= 13.8 Hz, 1H, CH_2), 3.77 (s, 3H, OCH_3), 3.79 (s, 6H, 2 × OCH_3), 3.94 (d, J= 13.8 Hz, 1H, CH_2), 4.32 (d, J= 15.7 Hz, 1H, CH_2), 4.69 (d, J= 15.7 Hz, 1H, CH_2), 6.30 (s, 1H, CH), 6.73–6.77 (m, 4H, $H_{2'}$, $H_{6'}$, H_2, H_{11}), 7.01 (d, J= 7.4 Hz, 1H, H_8), 7.05–7.08 (m, 3H, H_4, H_9, H_{10}), 7.18–7.26 (m, 2H, Ph), 7.44 (t, J= 7.4 Hz, 1H, H_3), 7.52–7.61 (m, 3H, Ph), 7.26 (d, J= 7.4 Hz, 1H, H_1), 10.24 (s, 1H, NH); ^{13}C NMR (125 MHz, $CDCl_3$) δ: 35.6, 44.7, 55.9, 60.9, 69.5, 96.9, 113.2, 116.0, 123.0, 123.10, 125.5, 126.4, 126.8, 126.9, 127.1, 128.9, 129.5, 131.0, 131.3, 132.2, 134.4, 137.0, 139.3, 140.7, 153.2, 157.2, 162.6, 167.0; IR (cm^{-1}) υ: 3434, 1689, 1662; Anal. Calcd for $C_{33}H_{30}N_4O_5S$: C, 66.65; H, 5.08; N, 9.42; Found: C, 67.42; H, 5.40; N, 9.23.

3.2.9. 2-((13-Oxo-12-benzyl-11b,12-dihydro-13H-quinazolino[3,4-a]quinazolin-6-yl)thio)-N-(2-chlorophenyl)acetamide (10i)

Yield: 23%, mp 154–156 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 3.88 (d, J= 14.4 Hz, 1H, CH_2), 3.96 (d, J= 14.4 Hz, 1H, CH_2), 3.38 (d, J= 15.8 Hz, 1H, CH_2), 4.55 (d, J= 15.8 Hz, 1H, CH_2), 6.27 (s, 1H, CH), 6.86–6.98 (m, 3H, H_2, H_4, H_{11}), 7.01–7.14 (m, 5H, Ph), 7.25–7.33 (m, 3H, $H_{4'}$, $H_{5'}$, H_8), 7.42 (t, J= 8.3 Hz, 1H, H_{10}), 7.52–7.62 (m, 3H, $H_{3'}$, H_3, H_9), 8.28 (m, 2H, H_1, $H_{6'}$), 9.49 (s, 1H, NH); ^{13}C NMR (125 MHz, $CDCl_3$) δ: 35.6, 44.8, 69.7, 113.1, 116., 122.5, 123.5, 124.5, 124.6, 125.2, 126.6, 126.9, 127.5, 128.1, 128.3, 128.4, 129.0, 129.4, 130.9, 132.0, 134.9, 137.2, 138.2, 139.4, 141.0, 155.6, 162.8, 167.5; IR (cm^{-1}) υ: 3450, 1691, 1650; Anal. Calcd for $C_{30}H_{23}ClN_4O_2S$: C, 66.85; H, 4.30; N, 10.39. Found: C, 66.56; H, 4.09; N, 10.58.

3.2.10. 2-((13-Oxo-12-benzyl-11b,12-dihydro-13H-quinazolino[3,4-a]quinazolin-6-yl)thio)-N-(3-chlorophenyl)acetamide (10j)

Yield: 17%, mp 199–201 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 3.53 (d, J= 14.2 Hz, 1H, CH_2), 3.90 (d, J= 14.2 Hz, 1H, CH_2), 4.32 (d, J= 17.7 Hz, 1H, CH_2), 4.72 (d, J= 17.7 Hz, 1H, CH_2), 6.30 (s, 1H, CH), 6.75–6.77 (m, 2H, H_4, H_{11}), 7.00–7.10 (m, 3H, H_2, H_9, $H_{4'}$), 7.18-7.28 (m, 5H, H_3, H_8, H_{10}, $H_{5'}$, $H_{6'}$), 7.48–7.62 (m, 6H, H2', Ph), 7.27 (d, J= 7.5 Hz, 1H, H_1), 10.24 (s, 1H, NH); ^{13}C NMR (125 MHz, $CDCl_3$) δ: 35.7, 44.7, 69.6, 116.0, 117.5, 119.7, 123.2, 124.5, 125.6, 126.6, 126.9, 127.0, 128.2, 128.5, 128.8, 129.6, 129.9, 130.9, 131.4, 132.2, 134.6, 139.4, 139.7, 140.8, 149.6, 157.1, 162.7, 167.4; IR (cm^{-1}) υ: 3322, 1683, 1661; Anal. Calcd for $C_{30}H_{23}ClN_4O_2S$: C, 66.85; H, 4.30; N, 10.39. Found: C, 66.62; H, 4.58; N, 10.21.

3.2.11. 2-((13-Oxo-12-benzyl-11b,12-dihydro-13H-quinazolino[3,4-a]quinazolin-6-yl)thio)-N-(4-chlorophenyl)acetamide (10k)

Yield: 22%, mp 212–214 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 3.53 (d, J= 14.2 Hz, 1H, CH_2), 3.91 (d, J= 14.2 Hz, 1H, CH_2), 4.31 (d, J= 14.2 Hz, 1H, CH_2), 4.72 (d, J= 14.2 Hz, 1H, CH_2), 6.30 (s, 1H, CH), 6.73–6.77 (m, 2H, H_2, H_4), 7.02–7.08 (m, 5H, Ph), 7.16 (d, J= 7.5 Hz, 1H, H_8), 7.24 (d, J= 8.7 Hz, 2H, $H_{3'}$, $H_{5'}$), 7.38 (d, J= 8.7 Hz, 2H, $H_{2'}$, $H_{6'}$), 7.49 (t, J= 7.5 Hz, 1H, H_{10}), 7.52–7.62 (m, 3H, H_3, H_9, H_{11}), 8.26 (d, J= 7.6 Hz, 1H, H_1), 10.19 (s, 1H, NH); ^{13}C NMR (125 MHz, $CDCl_3$) δ: 35.7, 44.7, 69.6, 113.3, 116.0, 116.6, 120.7, 123.1, 124.4, 125.5, 126.5, 126.8, 128.1, 128.4, 128.8, 128.9, 129.1, 129.5, 131.4, 132.2, 136.8, 139.3, 140.8, 157.1, 162.7, 167.3; IR (cm^{-1}) υ: 3242, 1662, 1556; Anal. Calcd for $C_{30}H_{23}ClN_4O_2S$: C, 66.85; H, 4.30; N, 10.39. Found: C, 66.97; H, 4.48; N, 10.11.

3.2.12. 2-((13-Oxo-12-benzyl-11b,12-dihydro-13H-quinazolino[3,4-a]quinazolin-6-yl)thio)-N-(2,5-dichlorophenyl)acetamide (10l)

Yield: 23%, mp 197–199 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 3.84 (d, J= 14.4 Hz, 1H, CH_2), 3.97 (d, J= 14.4 Hz, 1H, CH_2), 4.33 (d, 1H, CH_2, J= 15.8 Hz), 4.58 (d, J= 15.8 Hz, 1H, CH_2), 6.27 (s, 1H, CH), 6.79–6.84 (m, 3H, H_2, H_4, H_{11}), 7.00 (d, J= 8.0 Hz, 1H, H_8), 7.07–7.14 (m, 4H, Ph), 7.20 (d, J= 8.1 Hz, 1H, $H_{3'}$), 7.26 (d, J= 8.1 Hz, 1H, $H_{4'}$), 7.42 (t, J= 8.0 Hz, 1H, H_{10}), 7.51–7.56 (m, 2H, Ph, H_9), 7.59 (t, J= 7.5 Hz, 1H, H_3), 8.27 (d, J= 7.5 Hz, 1H, H_1), 8.41 (d, J= 2.1 Hz, 1H, $H_{6'}$), 9.57 (s, 1H, NH); ^{13}C NMR (125 MHz, $CDCl_3$) δ: 35.6, 44.6, 69.7, 114.0, 116.1, 121.3, 121.1, 122.1, 124.4, 124.6, 125.2, 126.6, 126.8, 126.9, 128.1, 128.4, 128.4, 129.5, 129.6, 130.9, 132.1, 135.9, 137.2, 139.3, 141.0, 155.6, 162.7, 167.6; IR (cm^{-1}) υ: 3233, 1687, 1660; Anal. Calcd for $C_{30}H_{22}Cl_2N_4O_2S$: C, 62.83; H, 3.87; N, 9.77. Found: C, 62.74; H, 3.69; N, 10.14.

3.2.13. 2-((13-Oxo-12-benzyl-11b,12-dihydro-13H-quinazolino[3,4-a]quinazolin-6-yl)thio)-N-(3,4-dichlorophenyl)acetamide (10m)

Yield: 20%, mp 185–187 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 3.54 (d, J= 14.2 Hz, 1H, CH_2), 3.87 (d, J= 14.2 Hz, 1H, CH_2), 4.31 (d, J= 15.7 Hz, 1H, CH_2), 4.74 (d, J= 15.7 Hz, 1H, CH_2), 6.31 (s, 1H, CH), 6.67–6.75 (m, 2H, H_2, H_4), 7.02–7.09 (m, 3H, H_9, H_{10}, H_{11}), 7.15 (d, J= 7.8 Hz, 1H, H_8), 7.21–7.26 (m, 2H, H_3, $H_{5'}$), 7.31 (d, J= 7.8 Hz, 1H, $H_{6'}$), 7.48–7.68 (m, 5H, Ph), 7.64 (d, J= 2.4 Hz, 1H $H_{2'}$), 8.26 (dd, J= 1.5, 7.8 Hz, 1H, H_1), 10.37 (s, 1H, NH); ^{13}C NMR (125 MHz, $CDCl_3$) δ: 35.7, 44.7, 69.6, 116.0, 118.2, 118.7, 121.2, 123.0, 124.4, 125.7, 126.5, 126.9, 127.0, 128.2, 128.3, 128.5, 128.9, 130.4, 131.4, 132.2, 132.7, 137.1, 137.7, 139.3, 140.7,

157.2, 162.7, 167.4; IR (cm^{-1}) υ: 3306, 1685, 1661; Anal. Calcd for $C_{30}H_{22}Cl_2N_4O_2S$: C, 62.83; H, 3.87; N, 9.77. Found: C, 62.60; H, 3.69; N, 9.90.

3.2.14. 2-((13-Oxo-12-benzyl-11b,12-dihydro-13H-quinazolino[3,4-a]quinazolin-6-yl)thio)-N-(2-methylphenyl)acetamide (10n)

Yield: 18%, mp 210–212 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 2.08 (s, 3H, CH_3), 3.85 (d, J= 14.4 Hz, 1H, CH_2), 3.89 (d, J= 14.4 Hz, 1H, CH_2), 4.47–4.49 (m, 2H, CH_2), 6.28 (s, 1H, CH), 6.72–6.80 (m, 2H, H_2, H_4), 6.90 (t, J= 8.0 Hz, 1H, H_9), 7.0–7.05 (m, 4H, Ph), 7.13–7.18 (m, 4H, Ph, H_8, H_{11}, $H_{4'}$), 7.21 (t, J= 8.0 Hz, 1H, H_{10}), 7.41 (t, J= 8.0 Hz, 1H, H_3), 7.56–7.64 (m, 3H, $H_{3'}$, $H_{5'}$, $H_{6'}$), 7.77 (d, J= 8.0 Hz, 1H, H_1), 9.02 (s, 1H, NH); ^{13}C NMR (125 MHz, $CDCl_3$) δ: 18.0, 35.4, 44.7, 69.7, 113.4, 116.2, 123.4, 124.7, 125.1, 125.3, 126.5, 126.6, 126.9, 127.2, 127.6, 128.2, 128.4, 128.6, 129.5, 130.3, 131.1, 132.1, 135.8, 137.1, 139.3, 140.9, 156.6, 162.8, 167.5; IR (cm^{-1}) υ: 3252, 1690, 1670; Anal. Calcd for $C_{31}H_{26}N_4O_2S$: C, 71.79; H, 5.05; N, 10.80. Found: C, 71.61; H, 5.26; N, 10.63.

3.2.15. 2-((13-Oxo-12-benzyl-11b,12-dihydro-13H-quinazolino[3,4-a]quinazolin-6-yl)thio)-N-(3-methylphenyl)acetamide (10o)

Yield: 24%, mp 191–193 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 2.60 (s, 3H, CH_3), 3.50 (d, J= 14.1 Hz, 1H, CH_2), 3.95 (d, J= 14.1 Hz, 1H, CH_2), 4.34 (d, J= 15.7 Hz, 1H, CH_2), 4.69 (d, J= 15.7 Hz, 1H, CH_2), 6.29 (s, 1H, CH), 6.75–6.78 (m, 2H, H_2, H_4), 6.89 (d, J= 7.0 Hz, 1H, $H_{4'}$), 6.99 (m, 2H, H_8, H_{10}), 7.03–7.13 (m, 3H, H_9, H_{11}, Ph), 7.14–7.23 (m, 4H, Ph), 7.33 (s, 1H, $H_{2'}$), 7.48 (t, J= 7.4 Hz, 1H, H_3), 7.54 (t, J= 7.0 Hz, 1H, $H_{5'}$), 7.58 (d, J= 7.0 Hz, 1H, $H_{6'}$), 8.27 (d, J= 7.4 Hz, 1H, H_1), 9.96 (s, 1H, NH); ^{13}C NMR (125 MHz, $CDCl_3$) δ: 21.5, 35.7, 44.7, 69.6, 113.4, 116.0, 116.6, 120.3, 123.4, 124.5, 124.8, 125.4, 126.3, 126.6, 126.9, 127.0, 127.1, 128.2, 128.7, 129.5, 131.3, 137.2, 138.1, 138.8, 139.5, 140.9, 156.9, 162.7, 167.2; IR (cm^{-1}): 3252, 1697, 1661; Anal. Calcd for $C_{31}H_{26}N_4O_2S$: C, 71.79; H, 5.05; N, 10.80. Found: C, 71.58; H, 5.29; N, 10.66.

3.2.16. 2-((13-Oxo-12-benzyl-11b,12-dihydro-13H-quinazolino[3,4-a]quinazolin-6-yl)thio)-N-(4-methylphenyl)acetamide (10p)

Yield: 24%, mp 185–187 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 2.30 (s, 3H, CH_3), 3.50 (d, J= 14.1 Hz, 1H, CH_2), 3.95 (d, J= 14.1 Hz, 1H, CH_2), 4.33 (d, J= 15.7 Hz, 1H, CH_2), 4.70 (d, J= 15.7 Hz, 1H, CH_2), 6.29 (s, 1H, CH), 6.99–7.0 (m, 2H, H_4, H_{11}), 7.05–7.12 (m, 5H, Ph), 7.19–7.21 (m, 2H, H_2, H_9), 7.33 (d, J= 8.2 Hz, 2H, $H_{3'}$, $H_{5'}$), 7.45–7.48 (m, 2H, H_8, H_{10}), 7.52–7.62 (m, 3H, H_3, H_2, $H_{6'}$), 8.26 (d, J= 7.6 Hz, 1H, H_1), 9.93 (s, 1H, NH); ^{13}C NMR (125 MHz, $CDCl_3$) δ: 20.8, 35.7, 44.7, 69.6, 116.0, 116.7, 119.6, 123.3, 124.5, 125.6, 126.3, 126.8, 127.0, 127.5, 128.2, 128.3, 129.4, 129.5, 131.3, 132.1, 135.6, 137.2, 139.4, 140.9, 156.9, 162.7, 167.1; IR (cm^{-1}) υ: 3515, 1683, 1656; Anal. Calcd for $C_{31}H_{26}N_4O_2S$: C, 71.79; H, 5.05; N, 10.80. Found: C, 71.465; H, 5.31; N, 10.41.

3.3. Cytotoxicity assay

Reagents and chemicals. Fetal bovine serum (FBS), phosphate buffered saline (PBS), RPMI 1640, and trypsin were purchased from Biosera (Ringmer, UK). 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) and penicillin/streptomycin were obtained from Sigma-Aldrich (St Louis, MO, USA) and Invit-

rogen (San Diego, CA, USA), respectively. Cisplatin was obtained from EBEWE Pharma (Unterach, Austria) and dimethyl sulfoxide was purchased from Merck (Darmstadt, Germany).

Cell lines and cell culture. MOLT-4 (human lymphoblastic leukemia) and MCF-7 (human breast adenocarcinoma) cells were obtained from the National Cell Bank of Iran, Pasteur Institute, Tehran, Iran. Cells were kept at 37 °C in humidified air containing 5% CO_2 and were grown in suspension (MOLT-4) or monolayer cultures (MCF-7). They were maintained in RPMI 1640 supplemented with 10% FBS, and 100 units/mL penicillin-G and 100 μg/mL streptomycin.

The in vitro cytotoxicity of compounds **10** was determined against two MOLT-4 and MCF-7 cell lines. The inhibitory effect of the synthesized compounds on cancer cells' growth was assessed by the MTT reduction assay.[28] The synthesized compounds were dissolved in dimethyl sulfoxide, and diluted in growth medium at least 400 times. Maximum concentration of DMSO in the wells was 0.5%. Cells were seeded in 96-well plates (5000 cells/well) and incubated at 37 °C for 24 h. Different concentrations of test compounds in the range of 10–100 μM were added and, after 72 h of further incubation at 37 °C, 0.5 mg/mL 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) dissolved in growth medium without phenol red was added to the wells. After 4 h, formazan crystals were solubilized in 200 μL of dimethyl sulfoxide and the optical density was measured at 570 nm, with background correction at 655 nm, using a microplate reader.

Acknowledgments

This work was supported by Tehran University of Medical Sciences and Iran National Science Foundation (INSF).

References

1. Khan, I.; Ibrar, A.; Abbas, N.; Saeed, A. *Eur. J. Med. Chem.* **2014**, *76*, 193-244.
2. Kuyper, L. F.; Baccanari, D. P.; Jones, M. L.; Hunter, R. N.; Tansik, R. L.; Joyner, S. S.; Boytos, C.; Rudolph, S. K.; Knick, V.; Wilson, H. R.; et al. *J. Med. Chem.* **1996**, *39*, 892-903.
3. Giardin, D.; Martarelli, D.; Sagratini, G.; Angeli, P.; Ballinari, D.; Gulini, U.; Melchiorre, C.; Poggesi, E.; Pompei, P. *J. Med. Chem.* **2009**, *52*, 4951-4954.
4. Decker, M. *Eur. J. Med. Chem.* **2005**, *40*, 305-313.
5. Jain, K. S.; Bariwal, J. B.; Kathiravan, M. K.; Phoujdar, M. S.; Sahne, R. S.; Chauhan, B. S.; Shah, A. K.; Yadav, M. R. *Bioorg. Med. Chem.* **2008**, *16*, 4759-4762.
6. Widemann, B. C.; Balis, F. M.; Godwin, K. S.; McCully, C.; Adamson, P. C. *Cancer Chemother. Pharmacol.* **1999**, *44*, 439-443.
7. Niculescu-Duvaz, I. *Curr. Opin. Investig. Drugs* **2001**, *2*, 693-705.
8. Connolly, D. J.; Cusack, D.; O'Sullivan T. P.; Guiry, P. J. *Tetrahedron* **2005**, *61*, 10153-10202.
9. Bandgar, B. P. *Synth. Commun.* **1997**, *27*, 2065-2068.
10. Jiang, J. B.; Hesson, D. P.; Dusak, B. A.; Dexter, D. L.; Kang, G. L.; Hamel, E. *J. Med. Chem.* **1990**, *33*, 1721-1728.
11. Dai, X.; Wong, A.; Virgil, S. *J. Org. Chem.* **1998**, *63*, 2597-2600.
12. Akazome, M.; Yamamoto, J.; Kondo, T.; Watanabe, Y. *J. Organomet. Chem.* **1995**, *494*, 229-234.
13. Larksarp, C.; Alper, H. *J. Org. Chem.* **2000**, *65*, 2773-2777.
14. Gopalsamy, A.; Yang, H. *J. Comb. Chem.* **2000**, *2*, 378-381.
15. Yang, R. Y.; Kaplan, A. *Tetrahedron Lett.* **2000**, *41*, 7005-7008.

16. Nahavandian, S.; Allameh, S.; Saeedi, M.; Ansari, S.; Mahdavi, M.; Foroumadi, A.; Shafiee, A. *Helv. Chim. Acta* **2015**, *98*, 1028-1033.

17. Mahdavi, M.; Pedrood, K.; Safavi, M.; Saeedi, M.; Pordeli, M.; Ardestani, S. K.; Emami, S.; Adib, M.; Foroumadi, A.; Shafiee, A. *Eur. J. Med. Chem.* **2015**, *95*, 492-499.

18. Esmaeili-Marandi, F.; Yavari, I.; Saeedi, M.; Mahdavi, M.; Shafiee, A. *Helv. Chim. Acta* **2016**, *99*, 37-40.

19. Shafii, B.; Saeedi, M.; Mahdavi, M.; Foroumadi, A.; Shafiee, A. *Synth. Commun.* **2014**, *44*, 215-221.

20. Farzipour, S.; Mahdavi, M.; Saeedi, M.; Yavari, H.; Mirzahekmati, M.; Ghaemi, N.; Foroumadi, A.; Shafiee, A. *Synth. Commun.* **2014**, *44*, 481-487.

21. Mahdavi, M.; Asadi, M.; Saeedi, M.; Tehrani, M. H., Mirfazli, S. S.; Shafiee, A.; Foroumadi, A. *Synth. Commun.* **2013**, *43*, 2936-2942.

22. Asadi, M.; Masoomi, S.; Ebrahimi, S. M.; Mahdavi, M.; Saeedi, M.; Ranjbar, P. R.;Shafiee, A.; Foroumadi, A. *Monatsh. Chem.* **2014**, *145*, 497-504.

23. Asadi, M.; Ebrahimi, M.; Mahdavi, M.; Saeedi, M.; Ranjbar, P. R.; Yazdani,F.; Shafiee, A.; Foroumadi, A. *Synth. Commun.* **2013**, *43*, 2385-2392.

24. Saeedi, M.; Mahdavi, M.; Foroumadi, A.; Shafiee, A. *Tetrahedron* **2013**, *69*, 3506-3510.

25. Mahdavi, M.; Hariri, R.; Saeedi, M.; Foroumadi, A.; Shafiee, A.; Akbarzadeh, T. *Tetrahedron Lett.* **2015**, *56*, 7082-7084.

26. Goli-Garmroodi, F.; Omidi, M.; Saeedi, M.; Sarrafzadeh, F.; Rafinejad, A.; Mahdavi, M.; Bardajee, G. R.; Akbarzadeh, T.; Firoozpour, L.; Shafiee, A.; et al. *Tetrahedron Lett.* **2015**, *56*, 743-746.

27. Mohammadhosseini, N.; Moradi, Sh.; Khoobi, M.; Shafiee, A. *J. Heterocycl. Het.* **2016**, *53*, 1595-1602.

28. Shekari, F.; Sadeghpour, H.; Javidnia, K.; Saso, L.; Nazari, F.; Firuzi, O.; Miri, R. *Eur. J. Pharmacol.* **2015**, *746*, 233-244.