TÜBİTAK

Turkish Journal of Chemistry

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

Research Article

Turk J Chem
(2017) 41: 179 – 189

doi:10.3906/kim-1604-84

Synthesis and anticancer and cytotoxic effects of novel 1,4-phenylene-bis-N-thiocarbamoylpyrazole and 1,4-phenylene-bis-pyrazolylthiazole derivatives

Meliha Burcu GÜRDERE[1,*], Erdoğan KAMO[1], Yakup BUDAK[1], Ayşe ŞAHİN YAĞLIOĞLU[2], Mustafa CEYLAN[1]

[1]Department of Chemistry, Faculty of Arts and Sciences, Gaziosmanpaşa University, Tokat, Turkey
[2]Department of Chemistry, Faculty of Sciences, Çankırı Karatekin University, Çankırı, Turkey

Received: 27.04.2016 • **Accepted/Published Online:** 10.09.2016 • **Final Version:** 19.04.2017

Abstract: Thiazolylpyrazoline derivatives were recently reported as potent anticancer agents. In this study, novel 1,4-phenylene-bis-**N**-thiocarbamoylpyrazoles (**3a**–**h**) and 1,4-phenylene-bis-pyrazolylthiazoles (**5a**–**h**) were prepared and screened for their anticancer activities against C6 (rat brain tumor cells) and HeLa (human uterus carcinoma). Anticancer activity studies were performed as a dose-dependent assay starting with eight concentrations. 5-Fluorouracil (5-FU) was used as a positive control. Compounds **3c**, **3d**, and **3h** were examined and they revealed almost the same activities compared with 5-FU in terms of cell selectivity against C6 cells. Moreover, compounds **3a**–**h** had lower cytotoxicity than 5-FU. The low cytotoxicity values of **3a**–**h** as well as their high antiproliferative activity were encouraging, but further studies are required on the use of these molecules as anticancer drugs.

Key words: Bis-chalcone, bis-N-thiocarbamoylpyrazole, bis-pyrazolylthiazole, HeLa, C6, anticancer activity, cytotoxicity

1. Introduction

Cancer is rated among the topmost causes of death worldwide,[1–3] and one of the anticancer treatment methods is chemotherapy. However, finding the best way to handle the side effects of chemotherapeutic drugs is still a great problem in clinical medicine. Therefore, hundreds of studies have been carried out in a bid to discover new anticancer agents that have high efficacy and minimal side effects.[4] Over the past three decades, pyrazole derivatives have been identified as an active molecule for drug agents,[5] and pyrazole derivatives have intensely been studied[6–9] as anticancer agents and found to be potent in various types of cancers.[10,11]

Moreover, thiazole derivatives are useful compounds for synthesis of biologically active agents like antimicrobial,[12] anticancer,[13,14] analgesic, and anti-inflammatory agents.[15] A large number of thiazole derivatives have potent anticancer activity and show selective activity against HeLa cancer cells.[16,17] In addition, a series of novel thiazolylpyrazoline derivatives were recently reported as potent anticancer agents.[18]

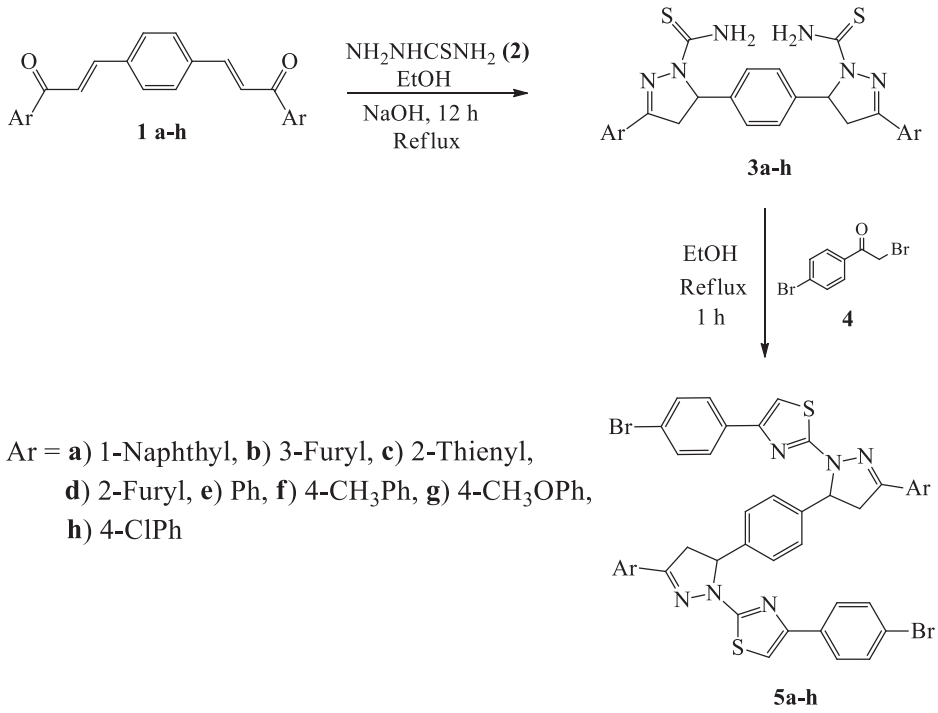
Encouraged by these results, we decided to examine the synthesis and anticancer and cytotoxic activity of a series of novel 1,4-phenylene-bis-N-thiocarbamoylpyrazole and 1,4-phenylene-bis-pyrazolylthiazole derivatives.

*Correspondence: burcugurdere@gmail.com

2. Results and discussion

2.1. Chemistry

The aim of this study was to undertake the design, preparation, and screening of the anticancer activity of 1,4-phenylene-bis-N-thiocarbamoylpyrazoles **3a**–**h** and 1,4-phenylene-bis-pyrazolylthiazoles **5a**–**h** from 1,4-phenylene-bis-chalcones **1a**–**h**. For this, firstly, the bis-chalcones **1a**–**h** were obtained by condensation of terephthalaldehyde with substituted arylketones in the presence of 20% aqueous NaOH in ethanol at r.t.[19–21] The bis-chalcones **1a**–**h** were made to react with thiosemicarbazide in EtOH in the presence of NaOH at reflux for 12 h to get 1,4-phenylene-bis-N-thiocarbamoylpyrazole **3a**–**h** in moderate to good yields. The structures of **3a**–**h** were elucidated using spectral data (IR and NMR) and elemental analysis. Furthermore, the compounds **3a**–**h** on condensation with 2,4'-bromoacetophenone, followed by cyclization in ethanolic KOH, gave 1,4-phenylene-bis-pyrazolylthiazoles **5a**–**h** (Scheme). The structures of **5a**–**h** were confirmed on the basis of spectral data (IR and NMR) and elemental analysis. All spectral data are in good agreement with proposed structures.



Scheme. The synthetic route for preparation of **3a**–**h** and **5a**–**h**.

2.2. Anticancer and cytotoxic activity results

All the compounds (**3a**–**h** and **5a**–**h)** were investigated for their potential growth inhibitory activity against C6 and HeLa cancer cells. The tests were performed by BrdU ELISA assay in vitro. The tests were performed at 5, 10, 20, 30, 40, 50, 75, and 100 μM concentrations, and 5-fluorouracil (5-FU), which is one of the most effective anticancer agents, was used as the reference drug for standard. Molecules showed high activity at high doses.

The activities of the compounds **3a**–**h** against C6 are shown in Figure 1A and the Table. All the tested compounds (**3a**–**h**) showed anticancer activity with IC_{50} values (IC_{50} represents the concentration of an inhibitor that is required for 50% inhibition of its target cells) ranging from <5 to 33.02 μM. Compounds **3c**, **3d**, and **3h** showed almost the same activity as 5-FU at high concentration. While compounds **3b**, **3e**, and **3f**

exhibited very good activity, compounds **3a** and **3g** demonstrated moderate anticancer activity when compared to 5-FU. The most active compounds were **3c**, **3d**, and **3g** with IC_{50} values of <5 μM.

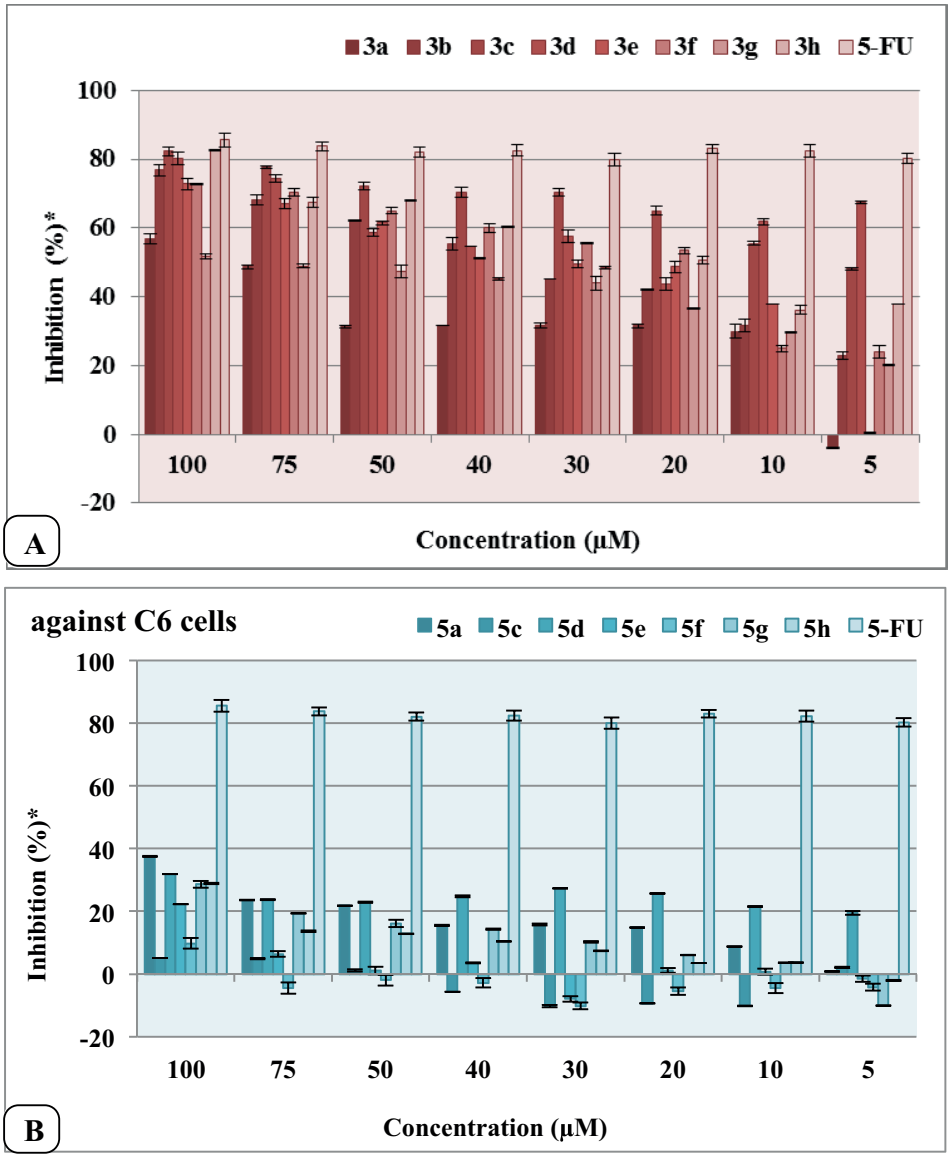
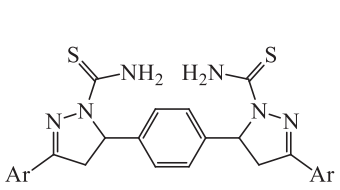


Figure 1. The anticancer activities of **3a**–**h** (A) and **5a** and **5c**–**h** (B) against C6 cells. *Each substance was tested twice in triplicate against cell lines. Data show average of two individual experiments ($P < 0.01$).

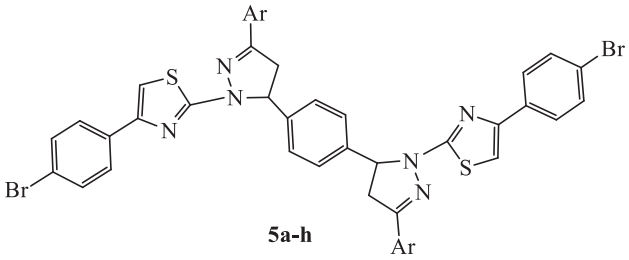
Anticancer activities of **5a** and **5c**–**5h** against C6 cells are given in Figure 1B and the Table. While compounds **5a** and **5c**–**5h** demonstrated very low activity at 100 μM, the other concentrations were almost inactive when compared to 5-FU.

Anticancer activities of **3a**–**h** and **5a** and **5c**–**h** were determined against HeLa cells and the results are given in Figures 2A and 2B, respectively. The activities of **3a**–**h** were shown to be much weaker than that of 5-FU (Figure 2A). In addition, compounds **5a** and **5c**–**5h** were obtained in the absence of significant activity when compared with 5-FU (Figure 2B).

Table. IC_{50} and IC_{75} values of **3a**–**h** and **5a** and **5c**–**5h** against C6 and HeLa cells.



3a-h



5a-h

Compounds	Ar	HeLa	C6	Cytotoxicity (%)
		IC50	IC50	
3a	1-Naphthyl	96.65	33.02	8
3b	3-Thienyl	54.08	18.82	16
3c	2-Thienyl	> 100	< 5	14
3d	2-Furyl	81.54	< 5	12
3e	Ph	> 100	19.39	8
3f	4-CH_3Ph	< 5	7.06	12
3g	4-CH_3OPh	> 100	< 5	12
3h	4-ClPH	67.59	9.04	24
5a	1-Naphthyl	> 100	45.81	nd
5c	3-Thienyl	93.24	80.06	nd
5d	2-Thienyl	> 100	< 5	nd
5e	2-Furyl	96.51	78.07	nd
5f	Ph	> 100	> 100	nd
5g	4-CH_3Ph	97.09	51.29	nd
5h	4-CH_3OPh	> 100	58.21	nd
5-FU		16.32	5.8	20

nd: no detection

Cytotoxicity (%) is a term used for substances to describe how toxic or poisonous to cells they can potentially be. Exposure to cytotoxic substances can result in permanent cellular damage or even death. For this reason, the cytotoxic activity of **3a**–**h** was determined on C6 cell lines. The cytotoxicity experiments were performed at 100 μM concentration, because the concentration was the highest dose that was studied in the antiproliferative test. 5-FU was regarded as a standard drug. The test results are given in the Table. All compounds (except **3h**) had lower cytotoxicity than 5-FU. In particular, **3a** and **3e** samples was less toxic than 5-FU. The low cytotoxicity values of all the samples as well as their high antiproliferative activity were determined. Furthermore, because compounds **5a** and **5c**–**5h** have low anticancer activities, their cytotoxic activities were not investigated. These results are all encouraging, but further studies are required on the use of these molecules as anticancer drugs.

According to these results, compounds **3a**–**h** showed potent and selective anticancer activity with low cytotoxicity values against C6 cell lines. Among compounds **3a**–**h**, the most active compounds were **3c**, **3d**, and **3h**. The high activities of **3c**, **3d**, and **3g** can be explained by the influence of the groups (thienyl and furyl ring and *p*-chloro group, respectively) in their structures.

In addition, compounds **5a**–**h** are almost inactive against both cell lines as compared to 5-FU. This result can be explained by their structures. We assumed that they cannot achieve sufficient interaction with cells due to their large volume and so they did not show any activity.

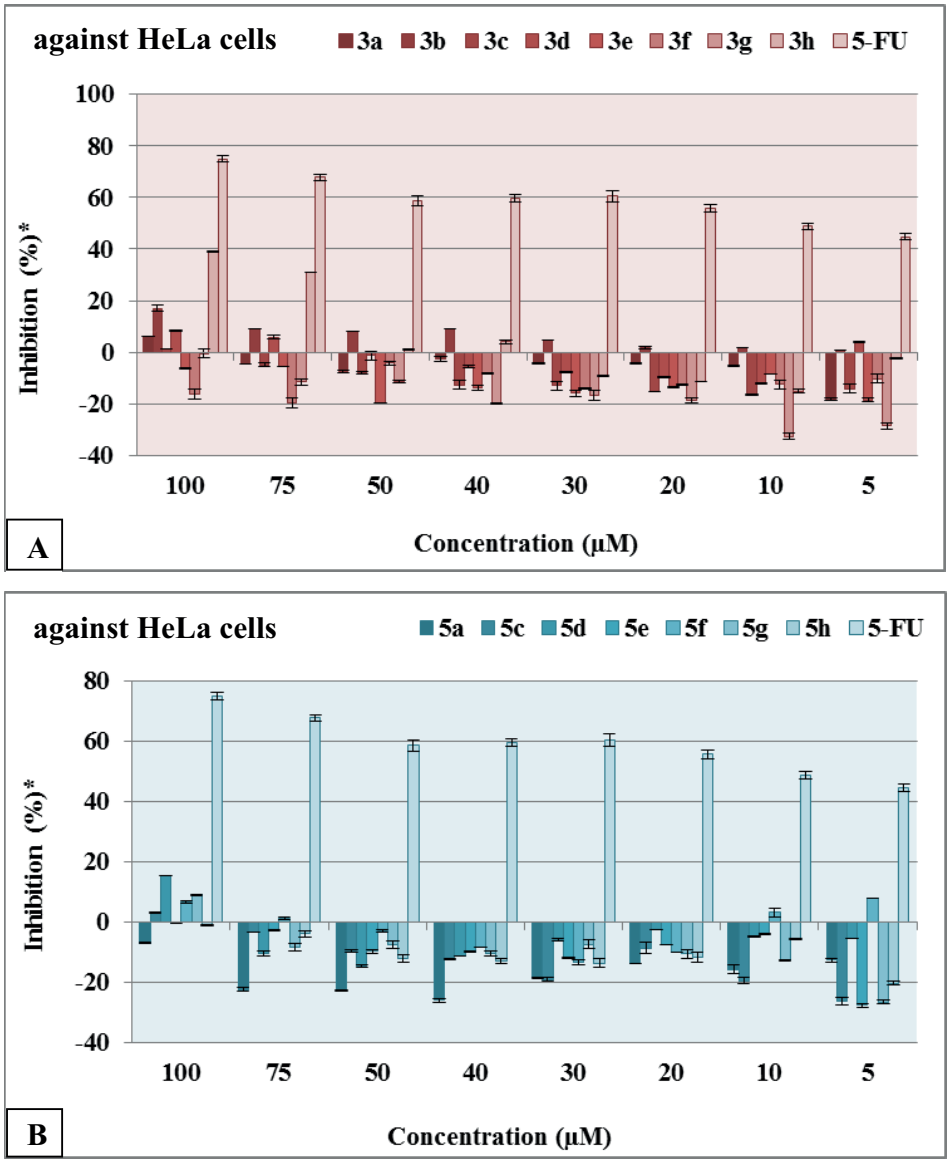


Figure 2. The anticancer activities of **3a**–**h** (A) and **5a** and **5c**–**h** (B) against HeLa cells. *Each substance was tested twice in triplicate against cell lines. Data show average of two individual experiments ($P < 0.01$).

In summary, a series of novel 1,4-phenylene-bis-**N-**thiocarbamoylpyrazole (**3a**–**h**) and 1,4-phenylene-bis-pyrazolylthiazole derivatives (**5a**–**h**) were synthesized and evaluated for their anticancer and cytotoxicity effects against C6 and HeLa cell lines. While compounds **3a**–**h** showed potent and selective anticancer activity with low cytotoxicity values against C6 cell lines, they exhibited very low activity against HeLa cell lines. The most active compound was **3f** (containing 4-methyl group) with 5.5 μM IC_{50} values against HeLa cell lines (IC_{50} of 5-FU = 16.58 μM). Furthermore, the most active compounds were **3c**, **3d**, and **3g** (containing thenyl and furyl ring and 4-methoxy group, respectively) with <5 μM IC_{50} value against C6 cell lines (IC_{50} of 5-FU = 5.8 μM). Among the compounds **5a**–**h**, only one compound, **5d**, (containing 2-thenyl ring) showed very high activity with <5 μM IC_{50} value against both cell lines. In addition, compounds **5a**–**h** were almost inactive against both cell lines when compared to 5-FU. These results are regarded as very encouraging, but further studies are required

to evaluate the mechanism of action for the anticancer activity of compounds **3a**–**h**, particularly against C6 cell lines.

3. Experimental

3.1. General

IR spectra (KBr disc or $CHCl_3$) were saved by Jasco FT/IR-430 spectrometer. ^{1}H and ^{13}C NMR spectra were measured on a Bruker Avance DPX-400 instrument. As internal δ (0.00) for standards served TMS ^{1}H NMR and $CDCl_3$ δ (77.0) for ^{13}C NMR spectroscopy. J values are given in Hz. Melting points were measured on an Electrothermal 9100 apparatus. Elemental analyses were obtained from a LECO CHNS 932 Elemental Analyzer. The major chemicals were purchased from Sigma-Aldrich and Fluka.

3.1.1. General procedure for the synthesis of bis-pyrazole derivatives (3a–h)

To a solution of chalcones (**1a**–**h**) (2 mmol) in ethanol was added NaOH (2 mmol, 2.5 M) and thiosemicarbazide (**2**) (4 mmol). The mixture was heated at reflux temperature for 12 h. The reaction was follow up with TLC. The products were transferred into acidic ice-water ($\sim$ pH 2, adjusted by HCl), and the solid mass that separated out was filtered, dried, and crystallized from ethanol.

5,5'-(1,4-phenylene)bis(3-(naphthalen-1-yl)-4,5-dihydro-1*H*-pyrazole-1-carbo-thioamide) (3a). White solid. Yield: 78%; mp 277–279 °C. IR (KCl, cm^{-1}): 3444, 3399, 3253, 3133, 1587, 1511, 1473, 1457, 1398, 1359, 1108, 1078, 831, 800, 775. ^{1}H NMR (400 MHz, DMSO, ppm): δ 9.25 (d, J = 8.8 Hz, 2H), 8.20 (s, 2H), 8.03–7.98 (dd, J = 11.6 Hz, 8.4 Hz 4H), 7.80 (s, 2H), 7.77–7.72 (dd, J = 13.0 Hz, 7.2 Hz, 2H), 7.70–7.66 (m, 2H), 7.60 (t, J = 7.4 Hz, 2H), 7.53–7.48 (dd, J = 13.4 Hz, 7.2 Hz, 2H), 7.19 (s, 2H), 7.18 (s, 2H), 5.96 (d, J = 11.2 Hz, 2H), 4.20–4.11 (m, 2H), 3.31–3.23 (m, 2H). ^{13}C NMR (100 MHz, DMSO, ppm): δ 176.6 (2C), 156.6 (2C), 141.9 (2C), 133.9 (4C), 131.7 (2C), 130.2, 130.1, 129.0 (2C), 128.5 (4C), 127.5, 127.4, 126.8 (2C), 126.1 (2C), 125.6 (4C), 61.8 (2C), 45.3 (2C). Anal. calc. for $C_{34}H_{28}N_6S_2$: C, 69.83; H, 4.83; N, 14.37. Found: C, 69.78; H, 4.72; N, 14.21.

5,5'-(1,4-phenylene)bis(3-(thiophen-3-yl)-4,5-dihydro-1*H*-pyrazole-1-carbothio-amide) (3b). White solid, Yield: 63%; mp 253–256 °C. IR (KCl, cm^{-1}): 3430, 3282, 3147, 1579, 1523, 1477, 1419, 1351 1085, 836, 784, 634. ^{1}H NMR (400 MHz, DMSO, ppm): δ 8.02 (s, 2H), 7.94 (d, J = 10.8 Hz, 2H) 7.78 (s, 2H), 7.66 (s, 4H), 7.07 (s, 2H), 7.05 (s, 2H), 5.87 (d, J = 11.2 Hz, 2H), 3.87–3.78 (m, 2H), 3.11–3.02 (m, 2H). ^{13}C NMR (100 MHz, DMSO, ppm): δ 176.3 (2C), 152.0 (2C), 141.9 (2C), 133.9 (2C), 128.9 (2C), 128.0 (2C), 126.2 (2C), 125.9 (4C), 62.7 (2C), 43.5 (2C). Anal. calc. for $C_{22}H_{20}N_6S_4$: C, 53.20; H, 4.06; N, 16.92. Found: C, 53.08; H, 4.02; N, 16.81.

5,5'-(1,4-phenylene)bis(3-(thiophen-2-yl)-4,5-dihydro-1*H*-pyrazole-1-carbothio-amide) (3c). White solid, Yield: 61%; mp 263–265 °C. IR (KCl, cm^{-1}): 3430, 3272, 3139, 1579, 1473, 1427, 1349, 1328, 1087, 836, 717. ^{1}H NMR (400 MHz, DMSO, ppm): δ 8.01 (s, 2H), 7.76 (d, J = 4.8 Hz, 2H), 7.58 (s, 2H), 7.48–7.46 (m, 2H), 7.16–7.08 (m, 6H), 5.93 (d, J = 11.2 Hz, 2H), 3.94–3.87 (m, 2H), 3.16–3.09 (m, 2H). ^{13}C NMR (100 MHz, DMSO, ppm): δ 176.1 (2C), 151.6 (2C), 141.8 (2C), 134.1 (2C), 131.6 (2C), 130.8 (2C), 128.5 (2C), 125.9 (4C), 63.0 (2C), 43.5 (2C). Anal. calc. for $C_{22}H_{20}N_6S_4$: C, 53.20; H, 4.06; N, 16.92. Found: C, 53.01; H, 3.98; N, 16.77.

5,5'-(1,4-phenylene)bis(3-(furan-2-yl)-4,5-dihydro-1*H*-pyrazole-1-carbothio-amide) (3d). White solid, Yield: 45%; mp 273–275 °C. IR (KCl, cm^{-1}): 3473, 3432, 3361, 3293, 3141, 1614, 1585, 1490, 1463, 1355, 1083, 1008, 836, 819, 761. ^{1}H NMR (400 MHz, DMSO, ppm): δ 8.04 (s, 2H), 7.91 (d, J = 6.8 Hz, 2H), 7.59 (s, 2H), 7.08–7.02 (m, 6H), 6.67–6.65 (dd, J = 3.2 Hz, 1.6 Hz, 2H), 5.93–5.90 (dd, J = 11.2 Hz, 2.8 Hz, 2H), 3.88–3.80 (m, 2H), 3.02–2.96 (m, 2H). ^{13}C NMR (100 MHz, DMSO, ppm): δ 176.3 (2C), 147.1 (2C), 146.4 (2C), 146.2 (2C), 141.6 (2C), 125.9 (4C), 115.5 (2C), 112.9 (2C), 62.5 (2C), 42.6 (2C). Anal. calc. for $C_{22}H_{20}O_2N_6S_2$: C, 56.88; H, 4.34; N, 18.09. Found: C, 56.76; H, 4.28; N, 17.98.

5,5'-(1,4-phenylene)bis(3-phenyl-4,5-dihydro-1*H*-pyrazole-1-carbothioamide) (3e). White solid, Yield: 42%; mp 276–278 °C. IR (KCl, cm^{-1}): 3426, 3251, 3141, 1589, 1569, 1465, 1444, 1367, 1344, 1095, 827, 765, 690. ^{1}H NMR (400 MHz, DMSO, ppm): δ 8.06 (s, 2H), 7.91 (s, 2H), 7.85 (t, J = 7.2 Hz, 4H), 7.46–7.43 (m, 6H), 7.08 (d, J = 8.0 Hz, 4H), 5.93–5.90 (dd, J = 11.2 Hz, 2.8 Hz, 2H), 3.91–3.82 (m, 2H), 3.15–3.06 (m, 2H). ^{13}C NMR (100 MHz, DMSO, ppm): δ 176.5 (2C), 154.4 (2C), 141.9 (2C), 135.6 (2C), 130.1 (2C), 129.2 (2C), 129.1 (4C), 127.5 (2C), 125.9 (4C), 63.1 (2C), 42.6 (2C). Anal. calc. for $C_{26}H_{24}N_6S_2$: C, 64.44; H, 4.99; N, 17.34. Found: C, 64.36; H, 4.88; N, 17.30.

5,5'-(1,4-phenylene)bis(3-(p-tolyl)-4,5-dihydro-1*H*-pyrazole-1-carbothioamide) (3f). White solid, Yield: 53%; mp 283–285 °C. IR (KCl, cm^{-1}): 3434, 3262, 3153, 1590, 1467, 1371, 1342, 1095, 815. ^{1}H NMR (400 MHz, DMSO, ppm): δ 8.01 (s, 2H), 7.86 (s, 2H), 7.77–7.74 (dd, J = 8.0 H, 4.4 Hz, 4H), 7.25 (d, J = 8.0 Hz, 4H), 7.07 (d, J = 2.8 Hz, 4H), 5.91–5.88 (m, 2H), 3.87–3.80 (m, 2H), 3.13–3.10 (m, 2H), 2.33 (s, 6H). ^{13}C NMR (100 MHz, DMSO, ppm): δ 176.3 (2C), 155.6 (2C), 142.0 (2C), 141.0 (2C), 129.7 (4C), 128.5 (2C), 127.5 (4C), 125.9 (4C), 62.8 (2C), 42.7 (2C), 21.5 (2C). Anal. calc. for $C_{28}H_{28}N_6S_2$: C, 65.59; H, 5.50; N, 16.39. Found: C, 65.46; H, 5.48; N, 16.30.

5,5'-(1,4-phenylene)bis(3-(4-methoxyphenyl)-4,5-dihydro-1*H*-pyrazole-1-carbothioamide) (3g). White solid, Yield: 61%; mp 287–288 °C. IR (KCl, cm^{-1}): 3446, 3245, 3133, 1606, 1585, 1481, 1373, 1307, 1245, 1174, 1035, 829. ^{1}H NMR (400 MHz, DMSO, ppm): δ 7.96 (s, 2H), 7.82–7.78 (m, 6H), 7.07 (d, J = 7.6 Hz, 4H), 6.98 (d, J = 8.4 Hz, 4H), 5.89 (d, J = 10.8 Hz, 2H), 3.87–3.79 (m, 8H), 3.12–3.04 (m, 2H). ^{13}C NMR (100 MHz, DMSO, ppm): δ 176.1 (2C), 161.6 (2C), 155.4 (2C), 141.9 (2C), 129.3 (4C), 125.9 (4C), 123.7 (2C), 114.6 (4C), 62.8 (2C), 55.8 (2C), 42.8 (2C). Anal. calc. for $C_{28}H_{28}O_2N_6S_2$: C, 61.74; H, 5.18; N, 15.43. Found: C, 61.67; H, 5.08; N, 15.35.

5,5'-(1,4-phenylene)bis(3-(4-chlorophenyl)-4,5-dihydro-1*H*-pyrazole-1-carbothio-amide) (3h). White solid, Yield: 48%; mp 295–296 °C. IR (KCl, cm^{-1}): 3434, 3419, 3237, 3127, 1585, 1467, 1375, 1091, 821. ^{1}H NMR (400 MHz, DMSO, ppm): δ 8.15 (s, 2H), 8.09 (s, 2H), 7.88 (d, 4H), 7.50 (d, J =8.4 Hz, 4H), 7.06 (d, J =9.2 Hz, 4H), 5.93–5.90 (m, 2H), 3.90–3.80 (m, 2H), 3.13–3.04 (m, 2H). ^{13}C NMR (100 MHz, DMSO, ppm): δ 176.4 (2C), 155.6 (2C), 141.9 (2C), 135.6 (2C), 131.1 (2C), 129.1 (4C), 127.5 (4C), 125.9 (4C), 62.9 (2C), 42.7 (2C). Anal. calc. for $C_{26}H_{22}Cl_2N_6S_2$: C, 56.42; H, 4.01; N, 15.18. Found: C, 56.33; H, 3.98; N, 15.05.

3.1.2. General method for the synthesis of derivatives 1,4-bis (1- (4- (4-bromophenyl) thiazol-2-yl) -4,5-dihydro-1*H*-pyrazol-5-yl) benzene (5a–h)

A mixture of **3a**–**h** (1 mmol) derivatives and 2,4'-dibromoacetophenone (**4**) (2 mmol) in ethanol (20 mL) was refluxed for 1 h. After cooling, the solid product was collected by filtration and the pure products obtained by washing with ethanol.

1,4-bis(1-(4-(4-bromophenyl)thiazol-2-yl)-3-(naphthalen-1-yl)-4,5-dihydro-1*H*-pyrazol-5-yl) benzene (5a). Orange solid, Yield: 85%; mp 256–258 °C. IR (KCl, cm^{-1}): 1542, 1508, 1471, 1396, 1340, 1290, 1047, 1008, 829, 798, 771. ^{1}H NMR (400 MHz, DMSO, ppm): δ 9.30 (d, J = 8.4 Hz, 2H), 8.03–7.97 (m, 4H), 7.75–7.68 (m, 4H), 7.63–7.51 (m, 12H), 7.42–7.33 (m, 6H), 5.68–5.63 (dd, J = 11.6, 6.8 Hz, 2H), 4.27–4.18 (m, 2H), 3.59–3.51 (m, 2H). ^{13}C NMR (100 MHz, DMSO, ppm): δ 165.2 (2C), 165.0 (2C), 153.9 (2C), 149.9 (2C), 149.8 (2C), 141.4 (2C), 134.1 (2C), 134.0 (2C), 131.8 (2C), 131.7 (2C), 131.2 (2C), 130.2 (2C), 129.3 (2C), 128.1 (2C), 127.8 (2C), 127.6 (2C), 127.3 (2C), 126.7 (2C), 125.7 (2C), 121.0 (2C), 120.9 (2C), 105.8 (2C), 105.6 (2C), 63.4 (2C), 46.2, 46.1. Anal. calc. for $C_{50}H_{34}Br_2N_6S_2$: C, 63.70; H, 3.63; N, 8.91. Found: C, 63.64; H, 3.59; N, 8.86.

1,4-bis(1-(4-(4-bromophenyl)thiazol-2-yl)-3-(thiophen-3-yl)-4,5-dihydro-1*H*-pyrazol-5-yl) benzene (5b). Yellow solid, Yield: 65%; mp 304–306 °C. IR (KCl, cm^{-1}): 1542, 1513, 1471, 1396, 1349, 1290, 1068, 1049, 1008, 827, 782, 728. ^{1}H NMR (400 MHz, DMSO, ppm): δ 7.90–7.87 (dd, J = 8.4, 2.4 Hz, 2H), 7.60 (d, J = 8.4, 4H), 7.55–7.51 (m, 4H) 7.43 (s, 4H), 7.38 (d, J = 8.0 Hz, 4H), 7.34 (s, 2H), 5.63–5.60 (dd, J = 11.6, 6.8 Hz, 2H), 4.04–3.99 (dd, J = 17.6, 12.0 Hz, 2H), 3.28–3.20 (m, 2H). ^{13}C NMR (100 MHz, DMSO, ppm): δ 165.0 (2C), 149.7 (2C), 149.5 (2C), 141.5 (2C), 134.0 (2C), 133.6 (2C), 131.8 (4C), 128.3 (2C), 127.8 (4C), 127.5 (2C), 127.3 (4C), 125.6 (2C), 120.9 (2C), 105.6 (2C), 64.1 (2C), 40.6, 40.4. Anal. calc. for $C_{38}H_{26}Br_2N_6S_4$: C, 53.40; H, 3.07; N, 9.83. Found: C, 53.34; H, 3.01; N, 9.76.

1,4-bis(1-(4-(4-bromophenyl)thiazol-2-yl)-3-(thiophen-2-yl)-4,5-dihydro-1*H*-pyrazol-5-yl) benzene (5c). Yellow solid, Yield: 40%; mp 291–293 °C. IR (KCl, cm^{-1}): 1540, 1513, 1471, 1396, 1317, 1070, 1047, 1008, 825, 728, 709. ^{1}H NMR (400 MHz, DMSO, ppm): δ 7.76–7.74 (m, 2H), 7.60 (d, J = 8.8, 4H), 7.54 (d, J = 8.4, 2H), 7.44 (s, 4H), 7.42–7.41 (m, 4H), 7.38–7.33 (m, 2H), 7.33 (s, 2H), 5.69–5.63 (dd, J = 11.6, 6.8 Hz, 2H), 4.07–3.99 (m, 2H), 3.28–3.21 (m, 2H). ^{13}C NMR (100 MHz, DMSO, ppm): δ 164.7 (2C), 149.7 (2C), 149.4 (2C), 141.3 (2C), 134.3 (2C), 134.0 (2C), 131.8 (2C), 131.7 (2C), 130.2 (2C), 129.9 (2C), 128.5 (2C), 127.8 (4C), 127.4 (4C), 121.0 (2C), 105.7 (2C), 64.5 (2C), 44.4, 44.3. Anal. calc. for $C_{38}H_{26}Br_2N_6S_4$: C, 53.40; H, 3.07; N, 9.83. Found: C, 53.38; H, 2.99; N, 9.80.

1,4-bis(1-(4-(4-bromophenyl)thiazol-2-yl)-3-(furan-2-yl)-4,5-dihydro-1*H*-pyrazol-5-yl)benzene (5d). Orange solid, Yield: 69%; mp 306–308 °C. IR (KCl, cm^{-1}): 1536, 1513, 1471, 1396, 1355, 1317, 1068, 1054, 1043, 1006, 823, 728. ^{1}H NMR (400 MHz, DMSO, ppm): δ 7.88 (s, 2H), 7.60 (d, J = 8.4 Hz, 4H), 7.42 (s, 4H), 7.38 (d, J = 8.4 Hz, 4H), 7.34 (s, 2H), 6.94 (d, J = 3.6 Hz, 2H), 6.67–6.65 (m, 2H), 5.65–5.61 (dd, J =11.8 Hz, 6.8 Hz, 2H), 3.99–3.92 (m, 2H), 3.27–3.19 (m, 2H). ^{13}C NMR (100 MHz, DMSO, ppm): δ 164.7 (2C), 149.7 (2C), 146.5 (2C), 145.9 (2C), 141.2 (2C), 133.9 (2C), 132.5 (2C), 131.6 (4C), 127.8 (4C), 127.4 (4C), 120.9 (2C), 114.0 (2C), 112.6 (2C), 105.7 (2C), 63.9 (2C), 43.3 (2C). Anal. calc. for $C_{38}H_{26}Br_2N_6O_2S_2$: C, 55.48; H, 3.19; N, 10.22. Found: C, 55.41; H, 3.09; N, 10.18.

1,4-bis(1-(4-(4-bromophenyl)thiazol-2-yl)-3-phenyl-4,5-dihydro-1*H*-pyrazol-5-yl)benzene (5e). White solid, Yield: 66%; mp 307–310 °C. IR (KCl, cm^{-1}): 1542, 1515, 1494, 1417, 1398, 1319, 1133, 1051, 1008, 829, 759, 688. ^{1}H NMR (400 MHz, DMSO, ppm): δ 7.76–7.75 (m, 4H), 7.60 (d, J =8.4 Hz 2H), 7.54 (d, J =8.4 Hz 2H), 7.46–7.36 (m, 14H), 7.32 (s, 2H), 5.68–5.63 (dd, J =11.8 Hz, 6.8 Hz 2H), 4.07–3.99 (m, 2H), 3.35–3.28 (m, 2H). ^{13}C NMR (100 MHz, DMSO, ppm): δ 165.0, 164.8 (2C), 153.5 (2C), 150.5 (2C), 141.5 (2C), 133.9 (3C), 131.8, 131.6 (2C), 131.3, 130.4 (2C), 129.3 (4C), 127.8 (4C), 127.4 (4C), 126.8 (4C),

120.9 (2C), 105.5 (2C), 64.5 (2C), 43.5 (2C). Anal. calc. for $C_{42}H_{30}Br_2N_6S_2$: C, 59.86; H, 3.59; N, 9.97. Found: C, 59.73; H, 3.50; N, 9.86.

1,4-bis(1-(4-(4-bromophenyl)thiazol-2-yl)-3-(*p*-tolyl)-4,5-dihydro-1*H*-pyrazol-5-yl)benzene (5f). Orange solid, Yield: 64%; mp 314–317 °C. IR (KCl, cm^{-1}): 1544, 1511, 1473, 1398, 1319, 1290, 1130, 1068, 1051, 1008, 815, 728. ^{1}H NMR (400 MHz, DMSO, ppm): δ 7.67–7.63 (m, 4H), 7.60 (d, J =8.4 Hz 2H), 7.53 (d, J =8.4 Hz 2H), 7.42–7.34 (m, 8H), 7.28–7.26 (m, 6H), 5.65–5.60 (dd, J =11.6 Hz, 6.8 Hz 2H), 4.00 (q, J =12.0 Hz, 2H), 3.31–3.21 (m, 2H), 2.35 (s, 6H). ^{13}C NMR (100 MHz, DMSO, ppm): δ 164.9 (2C), 153.5 (2C), 149.7 (2C), 141.5 (2C), 140.3 (2C), 134.0 (2C), 131.7 (2C), 131.6 (2C), 130.2 (4C), 128.5 (2C), 127.8 (4C), 127.4 (4C), 126.8 (4C), 120.9 (2C), 105.6 (2C), 64.4 (2C), 43.5 (2C), 21.5 (2C). Anal. calc. for $C_{44}H_{34}Br_2N_6S_2$: C, 60.69; H, 3.94; N, 9.65. Found: C, 60.53; H, 3.87; N, 9.56.

1,4-bis(1-(4-(4-bromophenyl)thiazol-2-yl)-3-(4-methoxyphenyl)-4,5-dihydro-1*H*-pyrazol-5-yl)benzene (5g). Yellow solid, Yield: 62%; mp 300–304 °C. IR (KCl, cm^{-1}): 1608, 1546, 1509, 1471, 1396, 1315, 1249, 1176, 1049, 1008, 831, 728. ^{1}H NMR (400 MHz, DMSO, ppm): δ 7.70 (t, J = 6.8 Hz, 4H), 7.60 (d, J = 8.4 Hz, 2H), 7.56 (d, J = 8.4 Hz, 2H), 7.42–7.31 (m, 10H), 7.01 (t, J = 6.8 Hz, 4H), 5.60 (m, 2H), 4.02–3.96 (m, 2H), 3.81 (s, 6H), 3.38–326 (m, 2H). ^{13}C NMR (100 MHz, DMSO, ppm): δ 165.0 (2C), 161.1 (2C), 153.3 (2C), 149.7 (2C), 141.5 (2C), 134.0 (2C), 131.7 (4C), 128.5 (4C), 127.8 (4C), 127.4 (4C), 123.8 (2C), 120.8 (2C), 114.7 (4C), 105.3 (2C), 64.3 (2C), 55.8 (2C), 43.7 (2C). Anal. calc. for $C_{44}H_{34}Br_2N_6O_2S_2$: C, 58.54; H, 3.80; N, 9.31. Found: C, 58.49; H, 3.77; N, 9.24.

1,4-bis(1-(4-(4-bromophenyl)thiazol-2-yl)-3-(4-chlorophenyl)-4,5-dihydro-1*H*-pyrazol-5-yl) benzene (5h). Orange solid, Yield: 61%; mp 321–325 °C. IR (KCl, cm^{-1}): 1585, 1540, 1513, 1492, 1469, 1396, 1375, 1317, 1133, 1089, 1049, 1008, 821, 728. ^{1}H NMR (400 MHz, DMSO, ppm): δ 7.79–7.53 (m, 4H), 7.61–7.49 (m, 8H), 7.44–7.38 (m, 8H), 7.33 (s, 2H), 5.69–5.64 (dd, J =11.8 Hz, 6.8 Hz 2H), 4.06–3.97 (m, 2H), 3.36–3.21 (m, 2H). ^{13}C NMR (100 MHz, DMSO, ppm): δ 164.6 (2C), 152.4 (2C), 149.7 (2C), 141.3 (2C), 134.9 (2C), 133.9 (2C), 131.7 (4C), 130.2 (2C), 129.3 (4C), 128.5 (4C), 127.8 (4C), 127.4 (4C), 120.9 (2C), 105.6 (2C), 64.6 (2C), 43.3 (2C). Anal. calc. for $C_{42}H_{28}Br_2Cl_2N_6S_2$: C, 55.34; H, 3.10; N, 9.22. Found: C, 55.29; H, 3.02; N, 9.19.

3.2. Biological evaluation

3.2.1. Bioassays

BrdU ELISA colorimetric kits were provided from Roche (Germany). 5-FU was provided by Sigma.

3.2.2. Cell lines and cell culture

The tested compounds and 5-FU were dissolved in dimethyl sulfoxide (DMSO). Then the stock solution was diluted with DMEM. DMSO concentration was below 0.1% in stock solutions. The tests were used for C6 and HeLa cells. The antiproliferative activity tests and cell culture study were performed according to the literature.[22,23]

3.2.3. Lactate dehydrogenase (LDH) leakage assay

The LDH leakage assay was performed using an LDH cytotoxicity detection kit (Roche, Germany) with respect to the manufacturer's protocol. The cytotoxicities (%) were determined on 100 μM concentrations against C6 cells and calculated according to the formula

$$\text{Cytotoxicity \%} = (\text{Samples absorbance} - \text{low control})/(\text{High control} - \text{low control}) \times 100.$$

3.2.4. Statistical analysis

The results are means ± SD of nine values. Differences between groups were determined by ANOVA ($P < 0.01$).

3.2.5. Determination of IC_{50} and IC_{75} values

IC_{50} and IC_{75} values were determined using ED50 plus v1.0.

Acknowledgment

The authors are thanks to the Gaziosmanpaşa University Scientific Research Projects Commission (Project No: BAP2011/95) for financial support.

References

1. Gouhar, R. S.; Fathalla, O. A.; Abd El-Karim, S. S. *Der. Pharma. Chemica.* **2013**, *5*, 225- 233.
2. Sharma, V.; Sharma, V.; Kumar, P.; Kumar, V. *Pak. J. Pharm. Sci.* **2014**, *27*, 1851-1855.
3. Meenalosini, S.; Janet, J.; Kannan, E. *Am. J. Appl. Sci.* **2012**, *9*, 1020-1029.
4. Reshma, J. N. *Der Pharmacia Lettre* **2014**, *6*, 285-295.
5. Chou, L. C.; Huang, L. J.; Hsu, M. H.; Fang, M. C.; Yang, J. S.; Zhuang, S. H. *Eur. J. Med. Chem.* **2010**, *45*, 1395-1402.
6. Koçyiğit-Kaymakçıoğlu. B.; Beyhan, N.; Tabanca, N.; Abbas, A.; Wedge, D. E.; Duke, S. O.; Bernier, U. R.; Khan, I. A. *Med. Chem. Res.* **2015**, *24*, 3632-3644.
7. Patel, V. M.; Desai, K. R. *Arkivoc.* **2004**, (*i*) 123-129.
8. Beyhan, N.; Kocyigit-Kaymakcioglu, B.; Gümrü, S., Aricioglu, F. *Arabian J. Chem.* **2013**, (in press).
9. Kocyigit-Kaymakcıoglu, B.; Oruç-Emre E. E.; Beyhan, N.; Toklu, H. Z.; Gümrü, S.; Arıcıoglu, F. *AUJST-C.* **2011**, *2*, 137-144.
10. Cheng, L. P.; Li, H. Q.; Sun, J.; Zhou, Y.; Zhu, H. L. *Bioorg. Med. Chem.* **2010**, *18*, 4606- 4614.
11. Insuasty, B.; Chamizo, L.; Munoz, J.; Tigreros, A.; Aboni, R.; Nogueras, M.; Cobo, J. *Arch. Pharm. Chem. Life Sci.* **2012**, *345*, 275-286.
12. Argyropoulou, I.; Geronikaki, A.; Vicini, P.; Zanib, F. *Arkivoc* **2009**, *6*, 89-102.
13. Li, W.; Lu, Y.; Wang, Z.; Dalton, J. T.; Miller, D. D. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 4113-4117.
14. Al-Saadi, M. S.; Faidallah, H. M.; Rostom, S. A. F. *Arch. Pharm. Chem. Life Sci.* **2008**, *341*, 424-434.
15. Rostom, S. A.; El-Ashmawy, I. M.; Abdel Razik, H. A.; Badr, M. H.; Ashour, M. A. *Bioorg. Med. Chem.* **2009**, *17*, 882-895.
16. Hutchinson, I.; Jennings, S. A.; Vishnuvajjala, B. R.; Westwell, A. D.; Stevens, M. F. G. *J. Med. Chem.* **2002**, *45*, 744-747.
17. Kashiyama, E.; Hutchinson, I.; Chua, M. S.; Stinson, S. F.; Phillipes, L. R.; Kaur, G.; Sausville, E. A.; Bradshaw, T. D.; Westwell, A. D.; Stevens, M. F. G. *J. Med. Chem.* **1999**, *42*, 4172-4184.
18. Lu, P. C.; Li, D. D.; Li, Q. S.; Lu, X.; Xiao, Z. P.; Zhu, H. L. *Bioorg. Med. Chem. Lett.* **2011**, *21*, 5374-5377.
19. Budak, Y.; *Chinese. J. Chem.* **2012**, *30*, 341-344.
20. Ceylan, M.; Gürdere, M. B.; Gezegen, H.; Budak, Y. *Synth. Commun.* **2010**, *40*, 2598-2606.

21. Karaman, I.; Gezegen, H.; Gürdere, M. B.; Dingil, A.; Ceylan, M. *Chem. Biodivers.* **2010**, *7*, 400-408.

22. Ceyhan, G.; Köse, M.; Tümer, M.; Demirtas, I.; Sahin Yaglioglu, A.; McKee, V. *J. Lumin.* **2013**, *143*, 623-634.

23. Karakus, G.; Polat, Z. A.; Sahin Yaglioglu, A.; Karahan, M.; Yenidunya, A. F. *J. Biomat. Sci-Polym. E.* **2013**, *24*, 1260-1276.