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Synthesis, characterization, *in vitro* antiproliferative and cytotoxicity effects of a new class of 2-((1*R*,2*S*)-2-((*E*)-4-substitutedstyryl) cyclooctyl)benzo[*d*]thiazole derivatives

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Abstract: The novel 2-((1*R*,2*S*)-2-((*E*)-4-substitutedstyryl) cyclooctyl)benzo[*d*]thiazoles (**12-14**) were synthesized from the reaction of 10-(4-substitutedbenzylidene)bicyclo[6.2.0]decan-9-ones **(5a-8a)** with 2-aminothiophenol (**11**) in the presence of *p*-TsOH at reflux conditions in good yields. The antiproliferative activities of **12-14** were determined againts C6 (rat brain carcinoma) and HeLa (human cervical carcinoma) cell lines using BrdU cell proliferation ELISA assay. 5-Fu (5-fluorouracil) was used as standard. The antiproliferative activities of **12-14** and the control were investigated on eight concentrations (5, 10, 20, 30, 40, 50, 75 and 100 μM). The results showed that the synthesized compounds had significant antiproliferative activity and low cytotoxicity effects.

Keywords: Benzothiazole; anticancer; cytotoxicity; HeLa; C6.

1. Introduction

The chemistry of thiazoles has been studied for over a century due to their diverse biological activities. Thiazole ring found in many natural compounds such as vitamin B1 (thiamine), penicillin and carboxylase.[1,2] Terrestrial and marine compounds with different biological activities that represent a very important field in drug discovery.[3,4] Many drugs such as Sulfathiazole, Ritonavir, Abafungin, Bleomycin and Tiazofurin contain thiazol moiety.[5] Thiazole ring is an important pharmacophore and its coupling with other rings could furnish new biologically active compounds[6] which exhibit a wide range of biological properties, such as antitumor, anticonvulsant,[7] cardiotonic,[8] IMP dehydrogenase inhibiton,[10] analgesics,[10] anticancer.[11] Thiazole ring also finds applications in other fields, such as

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polymers,[12] liquid crystals,[13] photonucleases,[14] fluorescent dyes,[15,16] insecticides[17] and antioxidant [18]. Furthermore, 1,3-thiazole and 1,3-benzothiazoles possess important biological properties such as distinctive antifungal, antimicrobial, antitubercular, anticancer, anti-angiogenic, antiproliferative and cytotoxic activity.[19-22]

In view of the above mentioned facts, we report herein the synthesis, *in vitro* cytotoxic evaluation of some novel 2-strylcyclooctlyl substituted 1,3-benzothiazole derivatives.

2. Experimental

All reagents and solvents were purchased from Sigma-Aldrich and Merck and were used without further purification. The cell proliferation BrdU ELISA kits were obtained from Roche (Germany), 5-FU and others from Sigma and Merck. UV spectra were recorded on JASCO V 530. NMR spectra were recorded on a Bruker Avance III 400 spectrometer in chloroform-d ($CDCl_3$). Chemical shifts are reported in parts per million and were referenced to the residual solvent signal ($CDCl_3$: 7.28 and 77.00 ppm for ^1H and ^{13}C, respectively. Spin multiplicities are given as s (singlet), d (doublet), dd (doublet of doublets), dt (doublet of triplets), ddd (doublet of doublet of doublets), t (triplet), td (triplet of doublets) or m (multiplet). Melting points (mp) were measured with an Electrothermal 9100 apparatus and given as uncorrected. IR spectrums (KCl disc) were recorded on a Jasco FT/IR-430 spectrometer.

2.1. Synthesis of 10,10-dichlorobicyclo[6.2.0]decan-9-one (2)[23-27]

The mixture of cyclooctene (10 g, 0.09 mol) and zinc (11.6 g, 0.18 mol) powder in diethyl ether (150 mL) was cooled to 15 °C in an ice-water bath. To this suspension was added dropwise the solution of trichloroacetyl chloride (32.7 g,1.18 mol) in diethyl ether (100 mL) for 30 min. The reaction mixture was stirred for 4 hours, and extracted with chloroform (2x25 mL). The organic layer was dried over Na_2SO_4, and removing of the solvent gave the **7,7**-dichlorobicyclo[3.2.0]heptan-6-one as a pure product in a yield of 50% (10.4g).

^1H-NMR (400 MHz, $CDCl_3$, ppm): δ 3.62 (t, *J* = 10.4 Hz, 1H), 2.96 (t, *J* = 10.4 Hz, 1H), 2.09-1.99 (m, 2H), 1.81-1.73 (m, 2H), 1.66-1.28 (m, 8H). ^{13}C-NMR (100 MHz, $CDCl_3$, ppm): δ 198.1, 88.7, 59.2, 51.0, 28.9, 28.7, 25.6, 25.3, 24.8, 23.3. Anal. Calcd. for $C_{10}H_{14}Cl_2O$: C, 54.32; H, 6.38. Found: C, 54.25; H, 6.32.

2.2. Synthesis of (1S,8S)-) and (1S,8R)-bicyclo[6.2.0]decan-9-one (3a and 3b)[27,28]

The mixture of zinc powder (8.69 g, 1.36 mol) and acetic acid (75 mL) was heated to reflux temperature, and was added dropwise solution of **2** (10 g, 0.45 mol) in acetic acid (25 mL) for 30 min. The reaction mixture was refluxed for 20 hours and continued to stirring at room temperature for 6-7 hours. The reaction mixture was filtered for removing of inorganic materials. The filtrate was extracted with chloroform (2x25 mL) and the organic layer was dried over Na_2SO_4. After removing of the solvent in vacuum, the residue was distilled in 10^{-4} mmHg at 65-68 °C, and the mixture of (1*S*,8*S*)- / (1*S*,8*R*)-bicyclo[6.2.0]decan-9-one 3a and 3b was obtained in yield of 69% (4.75 g).

***3a and 3b*:** ^1H NMR (400 MHz, $CDCl_3$, ppm): δ 3.33-3.22 (m, 1H), 3.16-3.06 (m, 1H), 3.05-2.99 (m, 1H), 2.98-2.89 (m, 1H), 2.73-2.63 (m, 1H), 2.55-2.42 (m, 1H), 2.13-2.01 (m, 2H), 1.91-1.84 (m, 4H), 1.81-1.72 (m, 4H), 1.71-1.64 (m, 4H), 1.56-1.40 (m, 4H), 1.38-1.21 (m, 8H). ^{13}C NMR (100 MHz, $CDCl_3$, ppm): δ 213.0, 209.7, 65.0, 62.3, 52.3, 51.4, 37.1, 31.6, 30.2, 29.7, 29.6, 28.5, 28.2, 27.9, 27.4, 27.2, 26.3, 25.9, 25.3, 21.8.

2.3. General procedure for the synthesis of (E/Z)-10-arylidenebicyclo[6.2.0]decan-9-ones ***5-10*** [29-31]

The solution of equivalent amounts of bicyclo[6.2.0]decan-9-one (3a and 3b), corresponding benzaldehyde derivative and NaOH in ethanol (50 mL) was stirred at room temperature for 5 hours. The reaction mixture was extracted with chloroform (2x25 mL), and the organic layer was dried over Na_2SO_4. The solvent was removed in vacuum, and the crude product was purified on a silica gel column eluting with *n*-hexane: ethylacetate (9:1).

*2.3.1. (1R,8R,E)-10-(4-methylbenzylidene)bicyclo[6.2.0]decan-9-one (**5a**):* ^{1}H NMR (400 MHz, $CDCl_3$, ppm): δ 7.40 (d, *J* = 8.0 Hz, 2H), 7.23 (d, *J* = 8.0 Hz, 2H), 7.01 (d, *J* = 2.4 Hz, 1H), 3.40 (t, *J* = 9.6 Hz, 1H), 3.27 (t, *J* = 10.4 Hz, 1H), 2.40 (s, 3H), 2.15-2.11 (m, 1H), 2.02-1.98 (m, 1H), 1.78-1.34 (m, 10H). ^{13}C-NMR (100 MHz, $CDCl_3$, ppm): δ 204.2, 148.1, 140.2, 131.3, 130.5, 129.6, 126.6, 62.6, 41.8, 30.0, 29.0, 26.2, 25.6, 24.2, 22.4, 21.5. IR (KCl cm^{-1}): 3442, 2925, 2850, 1733, 1639, 1604, 1463, 1442, 1145, 1070, 815, 802, 532. Anal. Calcd. for $C_{18}H_{22}O$: C, 84.99; H, 8.72. Found: C, 84.81; H, 8.64.

*2.3.2. (1R,8R,E)-10-(4-methoxybenzylidene)bicyclo[6.2.0]decan-9-one (**6a**)***:** ^{1}H NMR (400 MHz, $CDCl_3$, ppm): δ 7.44 (d, *J* = 8.4 Hz, 2H), 6.99 (d, *J* = 2.0 Hz, 1H), 6.94 (d, *J* = 8.4 Hz, 2H), 3.86 (s, 3H), 3.38 (t, *J* = 9.6 Hz, 1H), 3.26 (t, *J* =10.0 Hz, 1H), 2.14-2.10 (m, 1H), 2.01-1.97 (m, 1H), 1.77-1.70 (m, 2H), 1.67-1.45 (m, 8H). ^{13}C-NMR (100 MHz, $CDCl_3$, ppm): δ 204.0, 160.9, 146.5, 132.2, 126.7, 126.4, 114.4, 62.4, 55.3. 41.6, 30.0, 29.0, 26.2, 25.6, 24.2, 22.3. IR (KCl, cm^{-1}): 3450, 3004, 2921, 2848, 1733, 1637, 1600, 1511, 1463, 1421, 1305, 1255, 1174, 1145, 1070, 1029, 829, 541. Anal. Calcd. for $C_{18}H_{22}O_2$: C, 79.96; H, 8.20. Found: C, 79.87; H, 8.11.

*2.3.3. (1R,8R,E)-10-(4-fluorobenzylidene)bicyclo[6.2.0]decan-9-one (**7a**):* ^{1}H NMR (400 MHz, $CDCl_3$, ppm): δ 7.45-7.42 (m, 2H), 7.12-7.04 (m, 2H), 6.92 (d, *J* = 2.0 Hz, 1H), 3.37 (t, *J* = 9.6 Hz, 1H), 3.25 (t, *J* = 11.6 Hz, 1H), 2.04-1.94 (m, 2H), 1.73-1.70 (m, 2H), 1.67-1.41 (m, 8H). ^{13}C-NMR (100 MHz, $CDCl_3$, ppm): δ 203.7, 164.5-162.0 (d, C-18, J_{CF} = 250.4 Hz), 148.6 (d, C-9, J_{CF} = 2.2 Hz), 132.2-132.1 (d, C-16,20, J_{CF} = 8.5 Hz), 130.3-130.2 (d, C-13, J_{CF} = 3.3 Hz),, 125.1, 116.1-115.9 (d, C-17,18, J_{CF} = 21.7 Hz), 62.7, 41.6, 29.9, 28.9, 26.1, 25.5, 24.2, 22.3. IR (KCl, cm^{-1}): 3463, 2923, 2852, 1743, 1641, 1598, 1508, 1238, 1147, 1068, 833, 831, 536. Anal. Calcd. for $C_{17}H_{19}FO$: C, 79.04; H, 7.41. Found: C, 78.96; H, 7.34.

*2.3.4. (1R,8R,E)-10-(4-bromobenzylidene)bicyclo[6.2.0]decan-9-one (**8a**)***:** ^{1}H NMR (400 MHz, $CDCl_3$, ppm): δ 7.54 (d, *J* = 8.4 Hz, 2H), 7.34 (d, *J* = 8.4 Hz, 2H), 6.94 (d, *J* = 2.4 Hz, 1H), 3.40 (t, *J* = 9.6 Hz, 1H), 3.28 (t, *J* = 12.4 Hz, 1H), 2.06-1.97 (m, 2H), 1.77-1.70 (m, 2H), 1.67-1.43 (m, 8H). ^{13}C-NMR (100 MHz, $CDCl_3$, ppm): δ 203.8, 149.8, 133.0, 132.1 (2C), 131.6 (2C), 125.1, 124.0, 62.9, 41.8, 29.9, 28.9, 26.1, 25.5, 24.2, 22.3. IR (KCl, cm^{-1}): 2919, 2848, 1737, 1637, 1486, 1145, 1064, 1006, 819, 742, 528. Anal. Calcd. for $C_{17}H_{19}BrO$: C, 63.96; H, 6.00. Found: C, 63.88; H, 5.91.

The *Z*-isomers (**b** and **d**) were separated as mixture almost in ratio of 3:1. Due to overlapping peaks between isomers, integration values could not be accurately determined.

*2.3.5. (Z)-10-(4-methoxybenzylidene)bicyclo[6.2.0]decan-9-ones (**6b** and **6d**):* ^{1}H NMR (400 MHz, $CDCl_3$, ppm): δ 8.01 (d, *J* = 8.4 Hz, 2H major isomer), 7.98 (d, *J* = 8.4 Hz, 2H minor isomer), 6.93-6.91 (d, *J* = 8.8 Hz, 4H), 6.26 (d, *J* = 2.0 Hz, 1H major isomer), 6.20 (d, *J* = 2.0 Hz, 1H minor isomer), 3.86 (s, 6H), 3.07-3.01 (m, 1H major isomer), 2.98-2.96 (m, 1H major isomer), 2.93-2.80 (m, 1H minor isomer), 2.78-2.61 (m, 1H minor isomer), 2.28-2.21 (m, 1H minor isomer), 2.19-2.01 (m, 1H minor isomer), 1.98-1.91 (m, 4H), 1.77-1.63 (m, 4H), 1.58-1.47 (m, 10H), 1.45-1.25 (m, 4H minor). ^{13}C-NMR (100 MHz, $CDCl_3$, ppm): δ 201.5, 200.1, 161.0 (2C), 146.3, 146.1, 132.5, 131.7 (4C), 131.5, 130.6, 128.2, 113.9 (4C), 62.6, 59.3. 55.3, 42.1, 39.8 (2C), 35.1, 29.9, 29.4, 29.1, 28.7, 28.3, 28.0, 27.8, 26.1 (2C), 25.9, 23.1. IR (KCl, cm^{-1}): 3016, 2917, 2844, 1714, 1594, 1513, 1454, 1359, 1303, 1265, 1182, 1139, 1054, 1025, 831, 563, 528.

*2.3.6. (Z)-10-(4-fluorobenzylidene)bicyclo[6.2.0]decan-9-ones (**7b** and **7d**):* ^{1}H NMR (400 MHz, $CDCl_3$, ppm): δ 8.04-7.97 (m, 4H), 7.09-7.05 (m, 4H), 6.27 (d, *J* = 2.4 Hz, 1H, major isomer),

6.21 (d, *J* = 2.4 Hz, 1H, minor isomer), 3.08-3.04 (m, 1H major isomer), 3.01-2.98 (m, 1H major isomer), 2.89-2.84 (m, 1H major isomer), 2.78-2.72 (m, 1H minor isomer), 2.28-2.22 (m, 1H minor isomer), 2.18-2.09 (m, 1H minor isomer) 1.99-1.91 (m, 4H), 1.76-1.70 (m, 5H), 1.66-1.22 (m, 9H). ^{13}C-NMR (100 MHz, $CDCl_3$, ppm): δ 201.5, 164.7, 162.5, 148.6, 132.0, 131.9 (4C), 131.7, 131.6, 131.3, 129.4, 115.6, 115.4 (4C), 62.7, 59.5, 42.2, 40.0, 34.9, 29.8, 29.3, 29.0, 28.6, 28.2, 28.0, 27.7, 26.1, 25.9, 23.1. IR (KCl, cm^{-1}): 2919, 2850, 1727, 1629, 1600, 1509, 1232, 1047, 840, 520.

*2.3.7. (Z)-10-(4-bromobenzylidene)bicyclo[6.2.0]decan-9-ones (**8b** and **8d**) mixture:* ^{1}H NMR (400 MHz, $CDCl_3$, ppm): δ 7.87-7.83 (m, 4H), 7.52-7.50 (m, 4H), 6.23 (d, *J* = 2.4 Hz, 1H, major isomer), 6.17 (d, *J* = 2.4 Hz, 1H, minor isomer), 3.11-3.02 (m, 1H, major isomer), 3.01-2.94 (m, 1H major isomer), 2.92-2.86 (m, 1H minor isomer), 2.78-2.72 (m, 1H minor isomer), 2.24-2.18 (m, 1H minor isomer) 2.16-2.06 (m, 1H minor isomer), 1.99-1.90 (m, 4H), 1.78-1.70 (m, 5H), 1.69-1.26 (m, 13H). ^{13}C-NMR (100 MHz, $CDCl_3$, ppm): δ 201.5, 200.0, 150.0, 149.9, 133.8, 133.7, 131.8, 131.7, 131.2 (4C), 131.1 (4C), 131.0, 129.2, 124.2, 62.8, 59.6, 42.3, 40.2, 34.8, 29.8, 29.3, 29.0, 28.5, 28.2, 27.9, 27.7, 26.1, 25.8, 23.1. IR (KCl, cm^{-1}): 2921, 2850, 1731, 1629, 1484, 1459, 1068, 1008, 813, 511.

The NMR spectrum **9a,c**, **9a,b,d** and **10a-d** were given in supporting information file.

*2.4. Synthesis of 2-((1R,2S)-2-((E)-4-substituestyryl)cyclooctyl)benzo[d]thiazoles **12-15**.* [30,31]

A solution of 10-arylidenebicyclo[6.2.0]decan-9-one (1 equiv.) and 2-aminothiophenol (**11**) (1.2 equiv.) in ethanol (50 mL) in the presence of catalytic amount of *p*-TsOH was refluxed for 10 hours. The reaction mixture was extracted with chloroform (2 X 20 mL), and the organic layer was dried over Na_2SO_4. The solvent was removed in vacuum, and the crude product was purified on a silica gel column eluting with *n*-hexane: ethylacetate (19:1).

*2.4.1. 2-((1R,2S)-2-((E)-4-methylstyryl)cyclooctyl)benzo[d]thiazole (**12**):* ^{1}H NMR (400 MHz, $CDCl_3$, ppm): δ 7.95 (d, *J* = 8.4 Hz, 1H), 7.79 (d, *J* = 8.0 Hz, 1H), 7.41 (t, *J* = 8.0, Hz, 1H), 7.31 (t, *J* = 8.0 Hz, 1H), 7.08 (d, *J* = 8.0 Hz, 2H), 7.00 (d, *J* = 8.0 Hz, 2H), 6.23 (d, *J* = 16.0 Hz 1H), 6.11 (dd, *J* = 16.0 Hz, 8.4 Hz, 1H), 3.49-3.44 (m, 1H), 2.97-2.95 (m, 1H), 2.26 (s, 3H), 2.14-2.09 (m, 2H), 1.83-1.80 (m, 5H), 1.64-1.58 (m, 5H). ^{13}C-NMR (100 MHz, $CDCl_3$, ppm): δ = 177.9, 152.8, 136.5, 134.8, 134.7, 133.0, 129.6, 128.9, 125.9, 125.6, 124.3, 122.5, 121.4, 48.5, 47.1, 32.6, 31.1, 27.5, 26.4, 25.8, 25.4, 21.0. IR (KCl, cm^{-1}): 3052, 3019, 2921, 2852, 1716, 1513, 1436, 1313, 1261, 1089, 1016, 964, 862, 794, 757, 728, 516. Anal. Calcd. for $C_{24}H_{27}NS$: C, 79.73; H, 7.53; N, 3.87. Found: C, 79.68; H, 7.48; N, 3.76.

*2.4.2. 2-((1R,2S)-2-((E)-4-methoxystyryl)cyclooctyl)benzo[d]thiazole (**13**):* ^{1}H NMR (400 MHz, $CDCl_3$, ppm): δ = 8.01 (d, *J* = 8.0 Hz, 1H), 7.83 (d, *J* = 8.0 Hz, 1H), 7.45 (t, *J* = 7.6 Hz, 1H), 7.34 (t, *J* = 7.6 Hz, 1H), 7.21 (d, *J* = 8.4 Hz, 2H), 6.81 (d, *J* = 8.4 Hz, 2H), 6.24 (d, *J* = 15.6 Hz 1H), 6.17 (dd, *J* = 15.6 Hz, 8.4 Hz, 1H), 3.83-3.79 (m, 1H), 3.82 (s, 3H), 3.04-3.03 (m, 1H), 2.28-2.20 (m, 1H), 2.15-2.09 (m, 1H), 1.93-1.89 (m, 2H), 1.82-1.75 (m, 5H), 1.68-1.60 (m, 3H). **^{13}C-NMR** (100 MHz, $CDCl_3$, ppm): δ 177.1, 158.7, 152.8, 134.9, 130.4, 129.3, 127.2, 125.6, 125.0, 124.4, 122.1, 121.4, 113.8, 55.2, 45.6, 44.9, 31.9, 31.6, 30.1, 26.8, 26.1, 25.2. IR (KCl, cm^{-1}): 3359, 2921, 2854, 1606, 1509, 1455, 1438, 1299, 1247, 1174, 1035, 966, 821, 759, 730, 530. Anal. Calcd. for $C_{24}H_{27}NOS$: C, 76.35; H, 7.21; N, 3.71. Found: C, 76.28; H, 7.09; N, 3.64.

*2.4.3. 2-((1R,2S)-2-((E)-4-fluorostyryl)cyclooctyl)benzo[d]thiazole (**14**):* ^{1}H NMR (400 MHz, $CDCl_3$, ppm): δ 8.01 (d, *J* = 8.0 Hz, 1H), 7.84 (d, *J* = 8.0 Hz, 1H), 7.46 (t, *J* = 8.0 Hz, 1H), 7.35 (t, *J* = 8.4 Hz, 1H), 7.23-7.20 (m, 2H), 6.97-6.92 (m, 2H), 6.25 (d, *J* = 16.0 Hz 1H), 6.13 (dd, *J* = 16.0 Hz, 8.0 Hz, 1H), 3.84-3.78 (m, 1H), 3.07-3.05 (m, 1H), 2.24-2.21 (m, 1H), 2.17-2.14 (m, 1H), 1.93-1.89 (m, 4H), 1.82-1.63 (m, 6H). ^{13}C-NMR (100 MHz, $CDCl_3$, ppm): δ 176.8, 163.2-160.7 (d, C-23, J_{CF} = 244.4 Hz), 152.8, 134.8, 133.7, 131.4, 129.5, 127.6-127.5 (d, C-21,25, J_{CF} = 7.9 Hz), 125.7, 124.5, 122.6, 121.4, 115.3-115.1 (d, C-22,24, J_{CF} = 21.5 Hz), 45.5, 44.9, 31.7, 30.0, 29.3, 26.8, 26.1, 25.2. Anal. Calcd. for $C_{23}H_{24}FNS$: C, 75.58; H, 6.62; N, 3.83. Found: C, 75.49; H, 6.54; N, 3.80.

2.5. Bioassays

2.5.1. Antiproliferative Activities

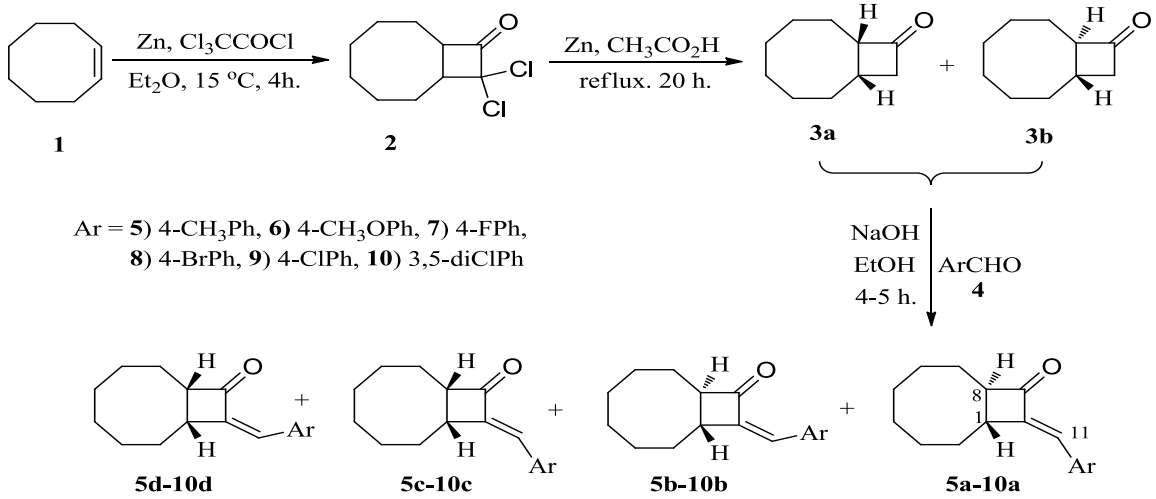
Antiproliferative activities of the compounds were investigated on HeLa and C6 cell lines using proliferation BrdU ELISA assay,[32-35] 5-FU was used as positive controls. The results are are presented as means ± SD of six values ($p < 0.01$). The IC_{50} and IC_{75} values of tested compounds were determined using ED50 plus v1.0.

2.5.2. Determined of Cytotoxicity effects: 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:
Cytotoxicity % = (Samples absorbance − low control)/(High control − low control) × 100.
Low control is the LDH activity released from the untreated the cells (= spontaneous LDH release).
High control is the maximum releasable LDH activity in the cell (= maximum LDH release).

3. Results and discussion

The target compounds **12-14**, 2-((1*R*,2*S*)-2-((*E*)-4-substituestyryl)cyclooctyl)benzo[*d*]thiazole, were synthesized in a four steps. Firstly, 10,10-dichlorobicyclo[6.2.0]decan-9-one (**2**) was prepared by addition of dichloroketene to cyclooctene (**1**). The reactions of cyclooctene (1) with trichloroacetyl chloride in presence of metallic zinc in Et_2O at 15 °C for 4 hours gave the dichloroketene adduct **2** in yield of 50% (Scheme 1). Secondly, reduction of 10,10-dichlorobicyclo[6.2.0]decan-9-one (**2**) with Zn in acetic acid afforded the mixture of isomers, (1*S*,8*S*)-bicyclo[6.2.0]decan-9-one (**3a**) and (1*S*,8*R*)-bicyclo[6.2.0]decan-9-one (**3b**) almost at a ratio of 1:1 in total yield of 69% (Scheme 1). Formed two isomers were determined by analysis of NMR spectrum. In the ^{13}C-NMR spectrum, two signals at 213.0 and 209.7 ppm clearly indicate formed two products which are isomers. Formation of two products can be explained by isomerization during the reaction. Despite all our efforts, the isomers could not be separated. Thirdly, the mixture of isomers (**3a** and **3b**) was reacted with the corresponding benzaldehyde derivatives in the presence of NaOH in ethanol at room temperature for 4-5 hours. After the completion of the reaction, the NMR and TLC studies show that at least four isomeric 10-arylidinebicyclo[6.2.0]decan-9-ones, **5a-10a**, **5b-10b**, **5c-10c** and **5d-10d**, were occurred (Scheme 1). From the ^{1}H NMR spectra of the mixture, the ratio of isomers **a**, **b**, **c** and **d** was determined as an approximately 5:1:1:3, respectively.



Scheme1. Synthesis compounds **5-10**

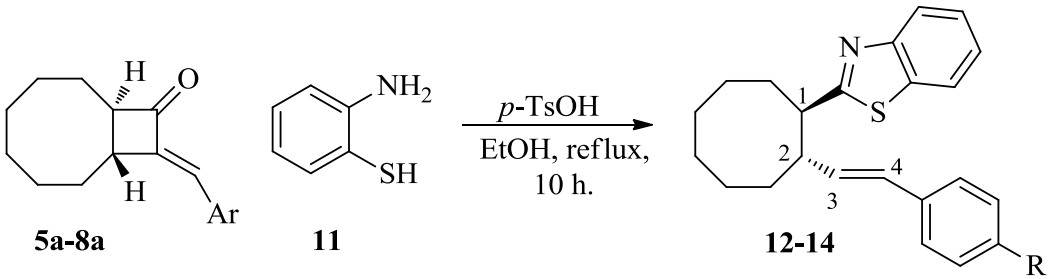
The isomers were separated on a silica gel column eluting with *n*-hexane: ethylacetate (9:1) and/or crystallization. From the column, the isomers **5a-8a** was separated almost as pure in good yields (60%-65%). The isomers **6b-8b** and **6d-8d** were isolated as a mixture almost at a ratio of 3:1, respectively (Table 1). The isomers **5b,d** and **5c-10c** were not isolated as an adequately amount for spectral analysis. Among the isomers **9a-d** (total yield 82%), isomers **9a,c** and **9a,b,d** could be separated as a mixture, separately. The other isomers **10a-d** was obtained in a 64% total yield but the isomers could not be separated despite all our efforts.

Table 1. Synthesized compounds **5-10**

Entry	Comp.	Ar	Yield (%)	M.p. (°C)	Comp.	Yield (%)
1	**5a**	4-CH_3Ph	65	89-92	-	-
2	**6a**	4-CH_3OPh	62	97-99	**6b,d**	20
3	**7a**	4-FPh	60	59-63	**7b,d**	25
4	**8a**	4-BrPh	68	114-117	**8b,d**	20
5	**9a,c**	4-ClPh	-	-	**9a,b,d**	-
6	**10a-d**	3,5-diClPh	64	-	-	-

The structures of the isomers were determined on the basis of spectral data and comparison with literature data. The *E* and *Z* forms of the isomers were determined using the chemical shift values of the protons in the phenyl ring. In the ^{1}H NMR spectra, while the phenyl protons of **6a** resonate at δ 7.44 and 6.94 that of **6b** resonate at δ 8.01 and 6.94. This difference in chemical shift values clearly indicates that the protons of **6b** are interacting with the carbonyl group. This requires the carbonyl group and the phenyl ring to be on the same side, so that the isomer **6b** has the *E* form. In addition, while the C11-H of **6a** resonates in a downfield region at δ 6.99 that of **6b** resonates in an up field region at δ 6.26 as evidenced by literature in similar structures.[36-41] Furthermore, the carbonyl carbon atoms of compounds **3a** and **3b** resonate at δ 213.1 and 209.1, respectively. Corner *et al*.[42] have synthesized the ketone **3a** as the single isomer and showed that the carbonyl carbon atom resonates at δ 213.3 ppm. In the ^{13}C-NMR spectra of the mixture, the carbonyl groups resonate at about δ 209, 206, 204-203 and 201, respectively. The *Z*-isomers **9a** and **9c** were isolated as a mixture and their C=O groups resonate at δ 209.9 and 206.4, respectively. From this result and above literature data, we assume that the isomer which resonates in a downfield region has the (1*R*,8*S*,*E*) and the isomer which resonates in an upfield region has the (1*R*,8*S*,*Z*) configuration. The carbonyl groups of all isomer isolated as a single product (**5a-8a**) resonate in an upfield region about at δ 204.2-203.8. So, we can say that the isomers **5a-8a** have the (1*R*,8*R*,*E*) configuration.

Finally, as a pure isolated 10-arylidinebicyclo [6.2.0] decan-9-ones **5a-8a** were reacted with 2-aminothiophenol (**11**) for synthesis of the target compounds, 2-(4-substitutedstyryl)-cyclooctyl)benzo[*d*]thiazoles, **12-14**. The reaction of **5a-8a** with 2-aminothiophenol (**11**) in the presence of catalytic amount of *p*-TsOH in ethanol at reflux conditions for 10 hours afforded the target compounds **12-14** in good yields 65-70% (Scheme 2). The compounds **12-14** were purified on a silica gel column eluting with *n*-hexane: ethylacetate (9:1).



5a-8a **11** **12-14**

R = **12**) 4-CH_3, **13)** 4-OCH_3, **14**) 4-F

Scheme 2. Synthesis of target compounds **12-14**

The structures of compounds **12-14** were determined by NMR studies. The ^{1}H NMR spectra of thiazoles (**12-14**), in each case, showed characteristic signals for thiazole ring at δ 8.00-7.95 (d, *J* = 8.0 Hz), 7.87-7.83 (d, *J* = 8.0 Hz), 7.49-7.43 (t, *J* = 7.6 Hz) and 7.41-7.33 (t, *J* = 7.6 Hz). The other decisive signals belong to olefinic protons. The olefinic proton C3-H resonates at δ 6.25-6.23 as a doublet (J = 16.0-15.6 Hz) and C4-H resonate at δ 6.17-6.11 as a doublet of doublet (J = 16.0-15.6 and 8.4-8.0 Hz). These coupling constants indicated that the C3-H and C4-H are the *trans* positions. All spectral data are in good agreement with the proposed structure.

The antiproliferative activities of the compounds **12-14** were determined against rat brain carcinoma (C6) and human cervical carcinoma (HeLa) cell lines using BrdU cell proliferation ELISA assay [32-35]. The antiproliferative activities of **12-14** and the controls were investigated on 5, 10, 20, 30, 40, 50, 75 and 100 µM concentrations and 5-Fluorouracil (5-FU) was chosen as a positive control due to its availability, and widespread using. The inhibitory potency of compounds against C6 and HeLa cell lines were showed in Figure 1 and Figure 2, respectively, and the IC_{50} and IC_{75} values were given in Table 2. According to the ELISA assay, the tested compounds showed more potent inhibitory effects on C6 cells than HeLa cells (Figure 1, Figure 2 and Table 2).

All compounds **12-14** showed an inhibitory effect at all concentration against C6 cells and inhibitory potency was dependent on dose increase (Figure 1). Moreover, compounds **12-14** showed higher activity than 5-FU at 40-100 µM concentrations. As IC_{50} values, compounds exhibited stronger activity with IC_{50} values (IC_{50} = < 5 µM for **12** and **13** followed by IC_{50} = 9.53 µM for **14**), against C6 cells when compared with 5-FU (IC_{50} = < 5 µM) (Table 2).

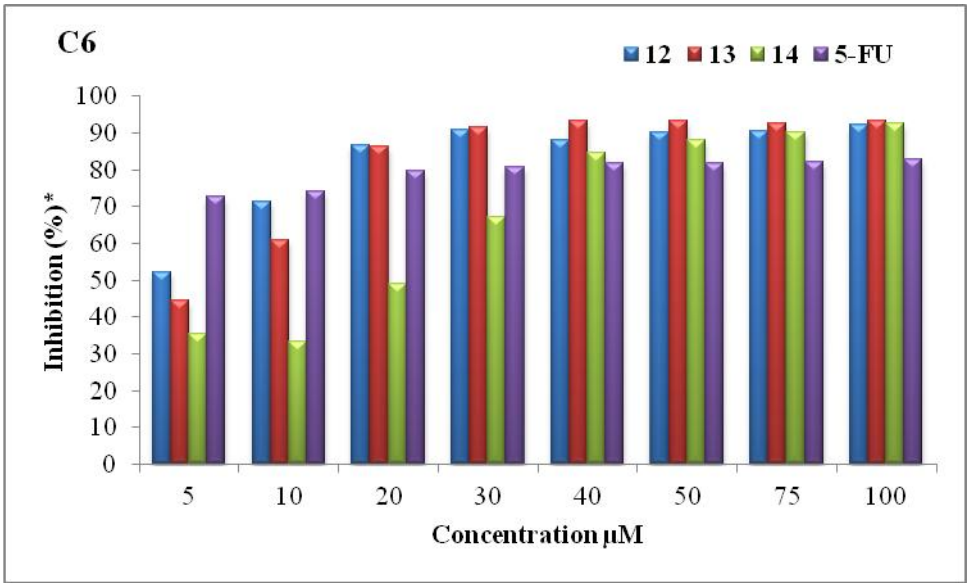


Figure 1. Antiproliferative activity of **12-14** and 5-FU against C6 cell lines, *each substance was tested twice in triplicates against cell lines. Data show an average of 2 individual experiments ($p<0.01$).

On the other hand, the inhibitory effects of **12-14** and 5-FU determined as generally increase activity with increasing depending to concentration against HeLa cell lines (Figure 2). Compound **13** was observed the higher activity than the standard 5-FU at all concentrations except 5 and 10 µM concentrations. In addition to, other samples were determined moderate activity compared to 5-FU at high concentrations (Figure 2). As IC_{50} values, the most active compound against Hela cell lines was found as compound **13** (IC_{50} = < 5 µM) followed by compound **12** (IC_{50} = 30.25 µM) and **14** (IC_{50} = 47.34 µM) when compared with 5-FU (IC_{50} = < 5 µM) (Table 2).

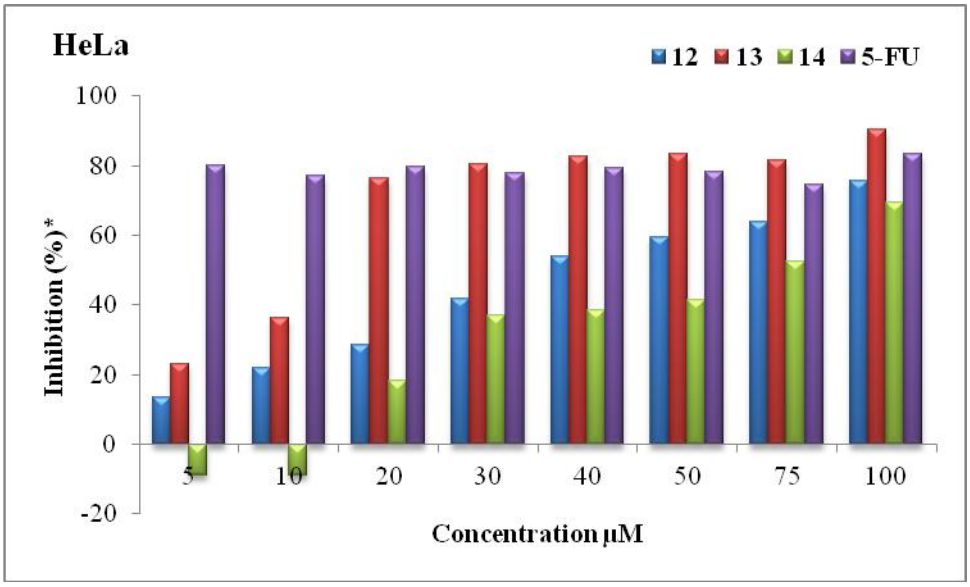


Figure 2. Antiproliferative activity of **12-14** and 5-FU against HeLa cell lines, *each substance was tested twice in triplicates against cell lines. Data show an average of 2 individual experiments ($p<0.01$).

When the activities of the compounds against C6 and HeLa cells were compared, it was determined that the compounds **12-14** exhibited selectively higher activity against C6 than HeLa. It was also observed that the methoxy group on the phenyl ring increased the anti-cancer activity on both cell lines.

Table 2. IC_{50} and IC_{75} values and Cytotoxicity (%) of **12-14**

Sample no	HeLa cell		C6 cell		Cytotoxicity (%)
	IC_{50}	IC_{75}	IC_{50}	IC_{75}	
12	30.25	59.89	<5	<5	5
13	<5	38.69	<5	10.36	1
14	47.39	69.32	9.53	44.17	10
5-FU	<5	31.86	<5	<5	24

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 **12-14** 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 Table 2. All compounds had lower cytotoxicity than 5-FU. The low cytotoxicity values of all the samples as well as their high antiproliferative activity were determined.

According to these results, compounds **12-14** showed potent and selective antiproliferative activity with low cytotoxicity values against C6 cell lines at almost all concentrations and HeLa cells particularly at high concentrations. These results are all encouraging, but further studies are required on the use of these molecules as anticancer drugs.

4. Conclusion

A series of a new class of 1,3-benzothiazole derivatives **(12-14)**, 2-((1*R*,2*S*)-2-((*E*)-4-substitutedstyryl) cyclooctyl)benzo[*d*]thiazole derivatives, were synthesized in a four-steps starting from cyclooctene in good yields. The antiproliferative activities of compounds **12-14** were evaluated against HeLa and C6 cell lines. Compounds **12-14** showed high activity with IC_{50} values ranging from IC_{50} = <5 µM to IC_{50} = 9.53 µM compared to 5-FU (IC_{50} = <5 µM). Furthermore, compound **13** (IC_{50} =

< 5 μM) exhibited at the same activity with 5-FU (IC_{50} = <5 μM) against HeLa cells. In addition, the compounds **12-14** showed a cell-selective effect against C6 cell lines.

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Supporting Information

Supporting information accompanies this paper on http://www.acgpubs.org/OC

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