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Short communication

Synthesis, structure elucidation and antitumour activity of *N*-substituted amides of 3-(3-ethylthio-1,2,4-triazol-5-yl)propenoic acid

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ABSTRACT

New *N*-substituted amides of 3-(3-ethylthio-1,2,4-triazol-5-yl)propenoic acid (**2**–**12**) were designed and prepared by the condensation reaction of *exo*-*S*-ethyl-7-oxabicyclo-[2.2.1]-hept-5-ene-2,3-dicarbonyl isothiosemicarbazide (**1**) with primary amines. The chemical structure of all compounds was confirmed by IR, ^{1}H NMR, ^{13}C NMR spectra, the X-ray crystallography (for compounds **8**, **11**, **12**) and elemental analysis.

Moreover, compounds **9**–**11** were screened for their anticancer activity. Compounds **9** (in concentrations of 0.32 mM and 0.16 mM), **10** (in concentrations of 0.28 mM and 0.14 mM), and **11** (in concentrations of 0.35 mM and 0.17 mM) were found to be evidently effective *in vitro* against lung cell line (IC50). The distinctly marked antiproliferative effect of compounds **9** and **10** in breast carcinoma cells *in vitro* was ascertained. Moreover, the lowest cytotoxicity of compound **9** in concentrations of 0.16 mM and 0.03 mM against the normal skin fibroblast cell line and breast carcinoma cell *in vitro* after 24- and 48-h periods of incubation was noticed in this study.

1. Introduction

1,2,4-Triazole and its derivatives are an important group of compounds in modern heterocyclic chemistry. From scientific literature it is known that depending on the type of substituents derivatives of 1,2,4-triazole show a wide range of pharmacological activities. Some of them were found to possess antifungal [1,2], antimicrobial [3,4], anti-inflammatory [5–7], antidepressant [8] and antiviral [9,10] properties. Certain compounds containing a 1,2,4-triazole skeleton have shown anticonvulsant [11,12] and antitumour activity [13–19].

In addition they have a wide range of therapeutic properties and are used as drugs in modern medicine. Vorozole, Letrozole and Anastrozole, having triazole moieties, are very effective nonsteroidal aromatase inhibitors. They are useful for preventing breast cancer [20–22]. Therefore triazoles, particularly 1,2,4-triazoles, are perspective scaffolds for designing anticancer drugs.

Prompted by these biological data we synthesized some unknown derivatives of 1,2,4-triazole, containing the amide and ethylthio group, to explore their possible biological activity. In this paper we present the discoveries, synthesis and testing results of possible anticancer activity of *N*-substituted amides of 3-(3-ethylthio-1,2,4-triazol-5-yl)propenoic acid.

2. Chemical part

In the present work *exo*-*S*-ethyl-7-oxabicyclo-[2.2.1]-hept-5-ene-2,3-dicarbonyl isothiosemicarbazide (**1**) was used as the starting material. This compound was prepared by a previously reported method [23] by the direct condensation of *exo*-7-oxabicyclo-[2.2.1]-hept-5-ene-2,3-dicarboxylic anhydride with *S*-ethyl isothiosemicarbazide hydrobromide in glacial acetic acid at room temperature. *N*-Substituted amides of 3-(3-ethylthio-1,2,4-triazol-5-yl)propenoic acid (**2**–**12**) were obtained by heating of starting compound with aliphatic and aromatic primary amines in boiling glacial acetic acid. The reaction conditions were established experimentally. The general synthetic pathway is presented in Scheme 1.

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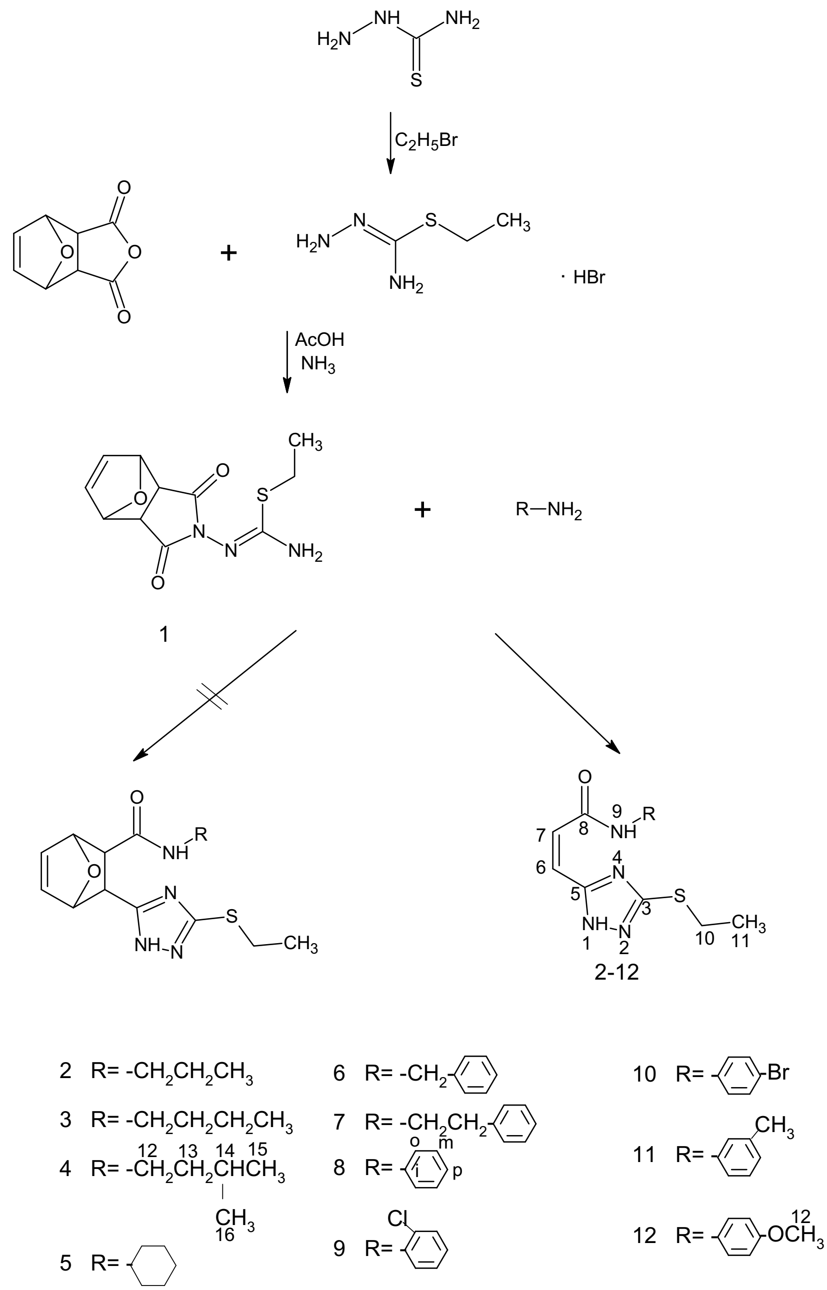

Scheme 1.

3. Results and discussion

3.1. Chemistry

3.1.1. Synthesis

During our investigations we expected to obtain *N*-substituted amides of 3-(3-ethylthio-1,2,4-triazol-5-yl)bicyclo[2.2.1]hept-5-ene-2-carboxylic acid. To our surprise nucleophilic ring opened and decomposed. The reaction of compound **1** with aliphatic and aromatic primary amines in boiling glacial acetic acid produced unexpected *N*-substituted amides of 3-(3-ethylthio-1,2,4-triazol-5-yl)propenoic acid (**2**–**12**). The chemical structure of obtained compounds was elucidated on the basis of ^{1}H and ^{13}C NMR.

3.1.2. Crystal structure analysis

Molecular structures of compounds **8**, **11** and **12**, together with the atom-labelling schemes are shown in Figs. 1–3, respectively. An X-ray analysis elucidates that compounds **8**, **11** and **12** adopt the *cis* (*Z*) configuration of the olefin C8═C9 atoms. The bond distances of the triazole and arylamide fragments are within typical ranges [24], moreover, the main structural parameters in all three molecules are very similar. As indicated from the torsion angles (Table 1) the central propenoamide part in all molecules is almost perfectly planar and essentially coplanar with triazole and phenyl rings. A small distortion from planarity is observed in **11**; the dihedral angle between propenoamide and phenyl best planes amounts to 5.9(5)°, which may be caused by intermolecular hydrogen bonding. A *cis*

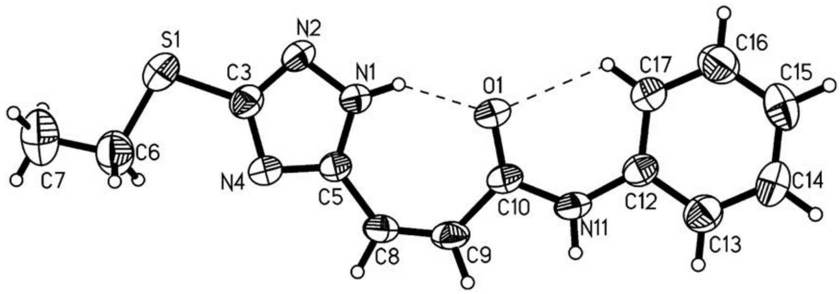

Fig. 1. Molecular structure of **8** with the atom-labelling scheme. The displacement ellipsoids are drawn at the 50% probability level. Dashed lines indicate hydrogen bonds.

arrangement of O1 with respect to the protonated N1 atom allows formation of strong intramolecular hydrogen bond N1–H1···O1 [with N1···O1 distance of 2.619(5), **8**, 2.610(4), **11** and 2.567(3) Å, **12**]. This interaction, together with weak C17–H17···O1 hydrogen bond [with C17···O1 distances of 2.902(5), **8**, 2.849(3), **11**, 2.867(4) Å, **12**], may control the conformation of the 3-(1,2,4-triazole)arylamide part of molecules in the solid state.

3.2. Biological evaluation

Some synthesized compounds were studied for their antitumour activity (Table 2). Compounds **9–11** were evaluated for their cytotoxic effect against two cancer cell lines: human lung cancer cell line A549 and human breast carcinoma cell line T47D. These compounds were examined against human lung tumour cells *in vitro*. They were unfortunately toxic at the higher doses (100 and 50 $\mu g\,mL^{-1}$) for normal fibroblast cultures. The growth of inhibition activity in breast carcinoma cell line was observed for compound **9** in concentration of 0.32 mM and 0.16 mM after 72 h of incubation.

4. Conclusion

In this paper we reported an efficient and convenient method of obtaining biologically active *N*-substituted amides of 3-(3-ethylthio-1,2,4-triazol-5-yl)propenoic acid by the condensation reaction of primary amines with the isothiosemicarbazide. The reactions were carried out under moderately drastic conditions and the solid products were isolated in medium and good yields. Interestingly, during cyclization reaction nucleophilic ring opened and decomposed, which has not been reported so far.

Compounds **9–11** were found effective *in vitro* against lung cell line. The most effective were two examined doses 100 and 50 $\mu g\,mL^{-1}$ (IC50). The cytotoxicity of compounds **9–11** against breast carcinoma cell line was weak. Moreover, the lowest cytotoxicity of compound **9** in concentrations of 0.16 mM and 0.03 mM against the normal skin fibroblast cell line was noticed in this study. Unfortunately, compound **10** (in concentrations of 0.28 mM and 0.14 mM), and compound **11** (in concentrations of 0.35 mM and 0.17 mM) have distinctly marked cytotoxicity.

5. Experimental protocol

5.1. Chemistry

Melting points were determined using Fischer–Johns block and presented without any corrections. Elemental analyses were performed on a Perkin–Elmer 2400 CHN Analyser and were in range of ±0.4% for each element analyzed. IR spectra were recorded in KBr on a Perkin–Elmer 1725X FTIR spectrometer. NMR spectra (^{1}H and ^{13}C) were recorded on a Bruker Avance 300 MHz spectrometer in solution noted and with TMS as an internal standard. Chemicals were purchased from Sigma–Aldrich or Lancaster Synthesis and used without further purification. *S*-Ethyl isothiosemicarbazide hydrobromide was obtained by the method described in Ref. [25].

5.1.1. *Procedure for synthesis of* exo*-S-ethyl-7-oxabicyclo-[2.2.1]-hept-5-ene-2,3-dicarbonyl isothiosemicarbazide (**1**)*

2 g (0.01 mol) of *S*-ethyl isothiosemicarbazide hydrobromide and 1.66 g (0.01 mol) of *exo*-7-oxabicyclo-[2.2.1]-hept-5-ene-2,3-dicarboxylic anhydride in 5 mL of glacial acetic acid were left for 72 h at room temperature. The reaction mixture was neutralized with 25% aq. ammonia solution and left for crystallization for 2 h. The crystalline precipitate was filtered off and crystallized from ethanol–water. Yield 89%, m.p. 191–193 °C. Spectroscopic data for $C_{11}H_{13}N_3O_3S$: ^{1}H NMR (δ, ppm, DMSO-d$_6$, TMS): 1.28 (t, 3H, $J = 7.2$ Hz, –SCH$_2$<u>CH$_3$</u>), 2.78 (s, 2H, CH–CH), 2.92 (q, 2H, $J = 7.2$ Hz, –S<u>CH$_2$</u>CH$_3$), 5.09 (s, 2H, CH–O–CH), 6.54 (s, 2H, CH=CH), 7.01 (s, 2H, NH$_2$); ^{13}C NMR (DMSO-d$_6$): 14.77 (–SCH$_2$<u>CH$_3$</u>), 23.98 (–S<u>CH$_2$</u>CH$_3$), 45.67 (<u>CH</u>–<u>CH</u>), 80.08 (<u>CH</u>–O–<u>CH</u>), 136.23 (<u>CH</u>=<u>CH</u>), 163.86 (<u>C</u>–SCH$_2$CH$_3$), 173.30 (2C=O).

5.1.2. *General procedure for synthesis of N-substituted amides of 3-(3-ethylthio-1,2,4-triazol-5-yl)propenoic acid (**2–12**)*

1.34 g (0.005 mol) of **1** and 0.005 mol of *n*-propyl-, *n*-butyl-, iso-pentyl-, cyclohexyl-, benzyl-, 2-phenylethyl-, phenyl-, 2-chlorophenyl-, 4-bromophenyl-, 3-methylphenyl-, 4-methoxyphenyl-amine in 3 mL of glacial acetic acid were refluxed for 2 h. After cooling and standing for

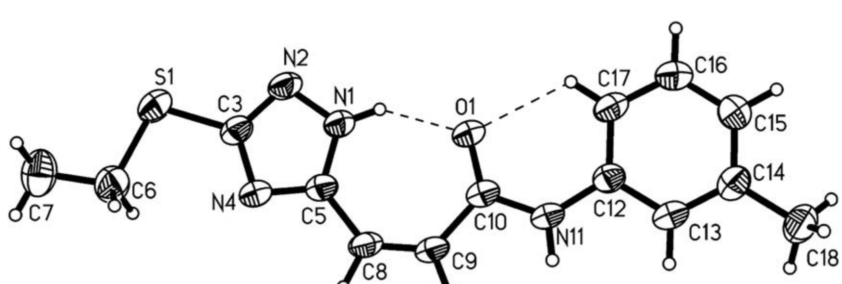

Fig. 2. Molecular structure of **11**.

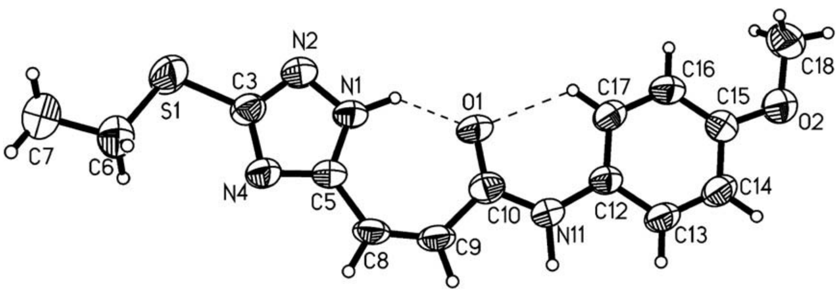

Fig. 3. Molecular structure of **12**.

a few hours at room temperature the precipitate was filtered off and crystallized from ethanol–water.

*5.1.2.1. N-Propyl amide of 3-(3-ethylthio-1,2,4-triazol-5-yl)propenoic acid (**2**).* Yield 65%, m.p. 73–75 °C. Spectroscopic data for $C_{10}H_{16}N_4OS$: IR (KBr) (ν, cm^{-1}): 3239 (NH), 2931 (CH), 1659 (C=O), 1558 (C=N), 1444 (CH), 1255 (C–N); ^1H NMR (δ, ppm, DMSO-d_6, TMS): 0.89 (t, 3H, $J = 7.4$ Hz, $-CH_2CH_2\underline{CH_3}$), 1.30 (t, 3H, $J = 7.3$ Hz, $-SCH_2\underline{CH_3}$), 1.52 (hex., 2H, $J = 7.2$ Hz, $-CH_2\underline{CH_2}CH_3$), 3.08 (q, 2H, $J = 7.1$ Hz, $-S\underline{CH_2}CH_3$), 3.17 (q, 2H, $J = 6.8$ Hz, $-\underline{CH_2}CH_2CH_3$), 6.33 (d, 1H, $J = 13.2$ Hz, $-CH{=}\underline{CH}CONH$), 6.81 (d, 1H, $J = 12.7$ Hz, $-\underline{CH}{=}CHCONH$), 9.02 (bs, 1H, NH_{amide}), 14.98 (bs, 1H, $NH_{triazole}$); ^{13}C NMR (DMSO-d_6): 11.40 ($-CH_2CH_2\underline{CH_3}$), 15.02 ($-SCH_2\underline{CH_3}$), 21.89 ($-CH_2\underline{CH_2}CH_3$), 25.47 ($-S\underline{CH_2}CH_3$), 40.84 ($-\underline{CH_2}CH_2CH_3$), 123.32 ($-CH{=}\underline{CH}CONH$), 126.99 ($-\underline{CH}{=}CHCONH$), 152.19 ($C_{triazole}$), 159.90 ($\underline{C}-SCH_2CH_3$), 165.27 ($C_{amide}$).

*5.1.2.2. N-Butyl amide of 3-(3-ethylthio-1,2,4-triazol-5-yl)propenoic acid (**3**).* Yield 70%, m.p. 65–67 °C. Spectroscopic data for $C_{11}H_{18}N_4OS$: IR (KBr) (ν, cm^{-1}): 3234 (NH), 2960 (CH), 1659 (C=O), 1555 (C=N), 1445 (CH), 1257 (C–N); ^1H NMR (δ, ppm, DMSO-d_6, TMS): 0.89 (t, 3H, $J = 7.2$ Hz, $-CH_2CH_2CH_2\underline{CH_3}$), 1.28–1.38 (m, 5H, $-SCH_2\underline{CH_3} + -CH_2CH_2\underline{CH_2}CH_3$), 1.48 (hex., 2H, $-CH_2\underline{CH_2}CH_2CH_3$), 3.09 (q, 2H, $J = 7.2$ Hz, $-S\underline{CH_2}CH_3$), 3.26 (q, 2H, $J = 6.5$ Hz, $-\underline{CH_2}CH_2CH_2CH_3$), 6.30 (d, 1H, $J = 11.9$ Hz, $-CH{=}\underline{CH}CONH$), 6.78 (d, 1H, $J = 12.4$ Hz, $-\underline{CH}{=}CHCONH$), 9.04 (bs, 1H, NH_{amide}), 14.94 (bs, 1H, $NH_{triazole}$); ^{13}C NMR (DMSO-d_6): 13.51 ($-CH_2CH_2CH_2\underline{CH_3}$), 15.00 ($-SCH_2\underline{CH_3}$), 19.64 ($-CH_2CH_2\underline{CH_2}CH_3$), 25.51 ($-S\underline{CH_2}CH_3$), 30.67 ($-CH_2\underline{CH_2}CH_2CH_3$), 38.76 ($-\underline{CH_2}CH_2CH_2CH_3$), 123.27 ($-CH{=}\underline{CH}CONH$), 127.11 ($-\underline{CH}{=}CHCONH$), 152.63 ($C_{triazole}$), 160.00 ($\underline{C}-SCH_2CH_3$), 165.22 ($C_{amide}$).

*5.1.2.3. N-Isopentyl amide of 3-(3-ethylthio-1,2,4-triazol-5-yl)propenoic acid (**4**).* Yield 72%, m.p. 90–92 °C. Spectroscopic data for $C_{12}H_{20}N_4OS$: IR (KBr) (ν, cm^{-1}): 3235 (NH), 2956 (CH), 1657 (C=O), 1558 (C=N), 1441 (CH), 1259 (C–N); ^1H NMR (δ, ppm, DMSO-d_6, TMS): 0.89 (s, 3H, $CH_{3isopentyl}$), 0.91 (s, 3H, $CH_{3isopentyl}$), 1.28 (t, 3H, $J = 7.3$ Hz, $-SCH_2\underline{CH_3}$), 1.38 (q, 2H, $J = 7.2$ Hz, $-CH_2\underline{CH_2}CH(CH_3)_2$), 1.57–1.66 (m, 1H, $-CH_2CH_2\underline{CH}(CH_3)_2$), 3.09 (q, 2H, $J = 7.3$ Hz, $-S\underline{CH_2}CH_3$), 3.27 (q, 2H, $J = 6.7$ Hz, $-\underline{CH_2}CH_2CH(CH_3)_2$), 6.29 (d, 1H, $J = 12.5$ Hz, $-CH{=}\underline{CH}CONH$), 6.78 (d, 1H, $J = 12.8$ Hz, $-\underline{CH}{=}CHCONH$), 9.04 (bs, 1H, NH_{amide}), 14.94 (bs, 1H, $NH_{triazole}$); ^{13}C NMR (DMSO-d_6): 15.01 ($-SCH_2\underline{CH_3}$), 22.21 ($-CH_2CH_2CH(\underline{CH_3})_2$), 25.19 ($-CH_2CH_2\underline{CH}(CH_3)_2$), 25.50 ($-S\underline{CH_2}CH_3$), 37.29 ($-\underline{CH_2}CH_2CH(CH_3)_2$), 37.49 ($-CH_2\underline{CH_2}CH(CH_3)_2$), 123.23 ($-CH{=}\underline{CH}CONH$), 127.13 ($-\underline{CH}{=}CHCONH$), 155.10 ($C_{triazole}$), 169.90 ($\underline{C}-SCH_2CH_3$), 165.14 ($C_{amide}$).

Table 1
Selected torsion angles (°).

	8	**11**	**12**
N1–C5–C8–C9	4.2(7)	2.2(4)	4.8(6)
C5–C8–C9–C10	−0.5(8)	0.4(4)	0.3(6)
C8–C9–C10–O1	0.8(7)	−0.2(4)	0.5(6)
C8–C9–C10–N11	178.6(4)	179.0(2)	−179.5(3)
C9–C10–N11–C12	−179.1(4)	179.2(2)	179.6(3)
C10–N11–C12–C17	0.8(7)	6.9(4)	−2.8(5)

*5.1.2.4. N-Cyclohexyl amide of 3-(3-ethylthio-1,2,4-triazol-5-yl)-propenoic acid (**5**).* Yield 78%, m.p. 107–109 °C. Spectroscopic data for $C_{13}H_{20}N_4OS$: ^1H NMR (δ, ppm, DMSO-d_6, TMS): 1.14–1.27 (m, 5H, cyclohexyl), 1.30 (t, 3H, $J = 7.3$ Hz, $-SCH_2\underline{CH_3}$), 1.56–1.84 (m, 5H, cyclohexyl), 3.09 (q, 2H, $J = 7.3$ Hz, $-S\underline{CH_2}CH_3$), 3.65–3.74 (m, 1H, $CH_{cyclohexyl}$), 6.29 (d, 1H, $J = 11.8$ Hz, $-CH{=}\underline{CH}CONH$), 6.78 (d, 1H, $J = 12.6$ Hz, $-\underline{CH}{=}CHCONH$), 8.95 (bs, 1H, NH_{amide}), 14.93 (bs, 1H, $NH_{triazole}$).

*5.1.2.5. N-Benzyl amide of 3-(3-ethylthio-1,2,4-triazol-5-yl)propenoic acid (**6**).* Yield 74%, m.p. 95–97 °C. Spectroscopic data for $C_{14}H_{16}N_4OS$: ^1H NMR (δ, ppm, DMSO-d_6, TMS): 1.27 (t, 3H, $J = 7.3$ Hz, $-SCH_2\underline{CH_3}$), 3.04 (q, 2H, $J = 7.3$ Hz, $-S\underline{CH_2}CH_3$), 4.44 (d, 2H, $J = 5.9$ Hz, $\underline{CH_2}$–Ph), 6.34 (d, 1H, $J = 12.9$ Hz, $-CH{=}\underline{CH}CONH$), 6.82 (d, 1H, $J = 12.9$ Hz, $-\underline{CH}{=}CHCONH$), 7.25–7.38 (m, 5H, Ph), 9.65 (bs, 1H, NH_{amide}), 14.68 (bs, 1H, $NH_{triazole}$); ^{13}C NMR (DMSO-d_6): 15.01 ($-SCH_2\underline{CH_3}$), 25.56 ($-S\underline{CH_2}CH_3$), 42.71 ($\underline{CH_2}$–Ph), 123.66 ($-CH{=}\underline{CH}CONH$), 126.90 ($-\underline{CH}{=}CHCONH$), 127.06 (*p*-PhC), 127.52 (*m*-Ph2C), 128.41 (*o*-Ph2C), 138.34 (*i*-PhC), 152.98 ($C_{triazole}$), 159.8 ($\underline{C}-SCH_2CH_3$), 165.27 (C_{amide}).

*5.1.2.6. N-(Ethylphenyl) amide of 3-(3-ethylthio-1,2,4-triazol-5-yl)-propenoic acid (**7**).* Yield 71%, m.p. 93–95 °C. Spectroscopic data for $C_{15}H_{18}N_4OS$: IR (KBr) (ν, cm^{-1}): 3437 (NH), 2972 (CH), 1655 (C=O), 1566 (C=N), 1439 (CH), 1263 (C–N); ^1H NMR (δ, ppm, DMSO-d_6, TMS): 1.28 (t, 3H, $J = 7.1$ Hz, $-SCH_2\underline{CH_3}$), 2.79–2.84 (m, 2H, $CH_2\underline{CH_2}Ph$), 3.07 (q, 2H, $J = 7.1$ Hz, $-S\underline{CH_2}CH_3$), 3.33–3.46 (m, 2H, $\underline{CH_2}CH_2$–Ph), 6.28 (d, 1H, $J = 11.8$ Hz, $-CH{=}\underline{CH}CONH$), 6.76 (d, 1H, $J = 13.1$ Hz, $-\underline{CH}{=}CHCONH$), 7.23–7.29 (m, 5H, Ph), 9.17 (bs, 1H, NH_{amide}), 14.87 (bs, 1H, $NH_{triazole}$); ^{13}C NMR (DMSO-d_6): 15.04 ($-SCH_2\underline{CH_3}$), 25.51 ($-S\underline{CH_2}CH_3$), 34.59 ($CH_2\underline{CH_2}$–Ph), 40.67 ($\underline{CH_2}CH_2$–Ph), 123.45 ($-CH{=}\underline{CH}CONH$), 126.17 (*p*-PhC), 127.11 ($-\underline{CH}{=}CHCONH$), 128.32 (*m*-Ph2C), 128.59 (*o*-Ph2C), 139.11 (*i*-PhC), 155.11 ($C_{triazole}$), 159.90 ($\underline{C}-SCH_2CH_3$), 165.32 (C_{amide}).

*5.1.2.7. N-Phenyl amide of 3-(3-ethylthio-1,2,4-triazol-5-yl)propenoic acid (**8**).* Yield 79%, m.p. 205–207 °C. Spectroscopic data for $C_{13}H_{14}N_4OS$: IR (KBr) (ν, cm^{-1}): 3439 (NH), 2964 (CH), 1664 (C=O), 1555 (C=N), 1444 (CH), 1257 (C–N); ^1H NMR (δ, ppm, DMSO-d_6, TMS): 1.24 (t, 3H, $J = 7.2$ Hz, $-SCH_2\underline{CH_3}$), 3.05 (q, 2H, $J = 7.3$ Hz, $-S\underline{CH_2}CH_3$), 6.44 (d, 1H, $J = 13.0$ Hz, $-CH{=}\underline{CH}CONH$), 6.85 (d, 1H, $J = 12.4$ Hz, $-\underline{CH}{=}CHCONH$), 7.09–7.16 (m, 1H, *p*-Ph), 7.29–7.39 (m, 2H, *m*-Ph), 7.68–7.70 (m, 2H, *o*-Ph), 10.94 (bs, 1H, NH_{amide}), 14.40 (bs, 1H, $NH_{triazole}$); ^{13}C NMR (DMSO-d_6): 15.02 ($-SCH_2\underline{CH_3}$), 25.56 ($-S\underline{CH_2}CH_3$), 119.89 (*o*-Ph2C), 123.20 (–CH=$\underline{CH}$CONH), 124.25 (*p*-PhC), 127.81 (–$\underline{CH}$=CHCONH), 128.79 (*m*-Ph2C), 138.35 (*i*-PhC), 151.80 ($C_{triazole}$), 159.90 ($\underline{C}-SCH_2CH_3$), 163.80 (C_{amide}).

*5.1.2.8. N-(2-Chlorophenyl) amide of 3-(3-ethylthio-1,2,4-triazol-5-yl)propenoic acid (**9**).* Yield 81%, m.p. 112–114 °C. Spectroscopic data for $C_{13}H_{13}N_4OSCl$: IR (KBr) (ν, cm^{-1}): 3202 (NH), 2973 (CH), 1671 (C=O), 1544 (C=N), 1444 (CH), 1256 (C–N); ^1H NMR (δ, ppm, DMSO-d_6, TMS): 1.24 (t, 3H, $J = 7.2$ Hz, $-SCH_2\underline{CH_3}$), 3.08 (q, 2H, $J = 7.3$ Hz, $-S\underline{CH_2}CH_3$), 6.50 (bs, 1H, –CH=$\underline{CH}$CONH), 6.90 (d, 1H, $J = 12.9$ Hz, –$\underline{CH}$=CHCONH), 7.24 (t, 1H, $J = 7.1$ Hz, *p*-Ph), 7.36 (t, 1H, $J = 7.3$ Hz, *m*-Ph), 7.53 (d, 1H, $J = 7.2$ Hz, *m*-Ph), 7.83 (d, 1H, $J = 7.5$ Hz, *o*-Ph), 11.08 (bs, 1H, NH_{amide}), 14.44 (bs, 1H, $NH_{triazole}$); ^{13}C NMR (DMSO-d_6): 14.93 ($-SCH_2\underline{CH_3}$), 25.66 ($-S\underline{CH_2}CH_3$), 123.93 (–CH=$\underline{CH}$CONH), 126.53 (*o*-PhC), 126.69 (*o*-PhC–Cl), 126.82 (*p*-PhC), 127.36 (*m*-PhC), 127.62 (–$\underline{CH}$=CHCONH), 129.52 (*m*-PhC), 134.43 (*i*-PhC), 154.82 ($C_{triazole}$), 159.90 ($\underline{C}-SCH_2CH_3$), 163.89 (C_{amide}).

*5.1.2.9. N-(4-Bromophenyl) amide of 3-(3-ethylthio-1,2,4-triazol-5-yl)propenoic acid (**10**).* Yield 78%, m.p. 230–232 °C. Spectroscopic data for $C_{13}H_{13}N_4OSBr$: IR (KBr) (ν, cm^{-1}): 3432 (NH), 2929 (CH), 1664 (C=O), 1547 (C=N), 1490 (CH), 1259 (C–N); ^1H NMR (δ, ppm, DMSO-d_6, TMS): 1.22 (t, 3H, $J = 7.3$ Hz, $-SCH_2\underline{CH_3}$), 3.02 (q, 2H, $J = 7.3$ Hz, $-S\underline{CH_2}CH_3$), 6.41 (d, 1H, $J = 12.7$ Hz, –CH=$\underline{CH}$CONH), 6.83 (d, 1H, $J = 12.7$ Hz, –$\underline{CH}$=CHCONH), 7.51–7.56 (m, 2H, *o*-, *m*-, *p*-Ph), 7.64–7.68 (m, 2H, *o*-, *m*-, *p*-Ph), 10.98 (bs, 1H, NH_{amide}), 14.28 (bs, 1H, $NH_{triazole}$); ^{13}C NMR (DMSO-d_6): 15.00 ($-SCH_2\underline{CH_3}$), 25.69 ($-S\underline{CH_2}CH_3$), 115.65 (*p*-PhC), 121.64 (*o*-Ph2C), 123.60 (–CH=$\underline{CH}$CONH), 127.79 (–$\underline{CH}$=CHCONH), 131.58 (*m*-Ph2C), 137.97 (*i*-PhC), 154.90 ($C_{triazole}$), 159.90 ($\underline{C}-SCH_2CH_3$), 164.00 (C_{amide}).

*5.1.2.10. N-(3-Methylphenyl) amide of 3-(3-ethylthio-1,2,4-triazol-5-yl)propenoic acid (**11**).* Yield 67%, m.p. 184–186 °C. Spectroscopic data for $C_{14}H_{16}N_4OS$: IR (KBr) (ν, cm^{-1}): 3435 (NH), 3037 (CH), 1674 (C=O), 1565 (C=N), 1449 (CH), 1253 (C–N); ^1H NMR (δ, ppm, DMSO-d_6, TMS): 1.24 (t, 3H, $J = 7.2$ Hz, $-SCH_2\underline{CH_3}$), 2.31 (s, 3H, $\underline{CH_3}$–Ph), 3.06 (q, 2H, $J = 7.3$ Hz, $-S\underline{CH_2}CH_3$), 6.43 (d, 1H, $J = 12.5$ Hz, –CH=$\underline{CH}$CONH), 6.84 (d, 1H, $J = 12.5$ Hz, –$\underline{CH}$=CHCONH), 6.93 (d, 1H, $J = 7.5$ Hz, *p*-Ph), 7.21 (t, 1H, $J = 7.8$ Hz, *m*-Ph), 7.46 (d, 1H, $J = 8.5$ Hz, *o*-Ph), 7.54 (s, 1H, *o*-Ph), 10.92 (bs, 1H, NH_{amide}), 14.43 (bs, 1H, $NH_{triazole}$); ^{13}C NMR (DMSO-d_6): 15.01 ($-SCH_2\underline{CH_3}$), 21.14 ($\underline{CH_3}$–Ph), 25.66 ($-S\underline{CH_2}CH_3$), 117.03 (*o*-PhC), 120.35 (*o*-PhC), 123.60 (–CH=$\underline{CH}$CONH), 124.80 (*p*-PhC), 128.05 (–$\underline{CH}$=CHCONH), 128.61 (*m*-PhC), 138.01 (*m*-$\underline{Ph}$–CH_3), 138.40 (*i*-PhC), 152.50 ($C_{triazole}$), 159.90 ($\underline{C}-SCH_2CH_3$), 163.70 (C_{amide}).

*5.1.2.11. N-(4-Methoxyphenyl) amide of 3-(3-ethylthio-1,2,4-triazol-5-yl)propenoic acid (**12**).* Yield 71%, m.p. 218–220 °C. Spectroscopic data for $C_{14}H_{16}N_4O_2S$: IR (KBr) (ν, cm^{-1}): 3431 (NH), 2963 (CH), 1663 (C=O), 1558 (C=N), 1442 (CH), 1245 (C–N); ^1H NMR (δ, ppm, DMSO-d_6, TMS): 1.25 (t, 3H, $J = 7.2$ Hz, $-SCH_2\underline{CH_3}$), 3.07 (q, 2H, $J = 7.3$ Hz, $-S\underline{CH_2}CH_3$), 3.75 (s, 3H, $\underline{CH_3}$O–Ph), 6.42 (d, 1H, $J = 12.7$ Hz, –CH=$\underline{CH}$CONH), 6.85 (d, 1H, $J = 12.7$ Hz, –$\underline{CH}$=CHCONH), 6.93 (d, 2H, $J = 9.0$ Hz, *m*-Ph), 7.60 (d, 2H, $J = 9.0$ Hz, *o*-Ph), 10.84 (bs, 1H, NH_{amide}), 14.50 (bs, 1H, $NH_{triazole}$); ^{13}C NMR (DMSO-d_6): 15.03 ($-SCH_2\underline{CH_3}$), 25.63 ($-S\underline{CH_2}CH_3$), 55.17 ($\underline{CH_3}$OPh), 113.94 (*m*-Ph2C), 121.44 (*o*-Ph2C), 123.59 (–CH=$\underline{CH}$CONH), 127.89 (–$\underline{CH}$=CHCONH), 131.45 (*p*-PhC), 133.50 (*i*-PhC), 155.92 ($C_{triazole}$), 159.90 ($\underline{C}-SCH_2CH_3$), 163.33 (C_{amide}).

5.2. Tumour cells proliferation assay

The newly synthesized compounds **9**–**11** were evaluated for their anticancer activity in human tumour cell lines derived from lung and breast carcinoma cells. These studies were carried out on A549 (ECACC 86012804 human lung epithelial) and T47D (ECACC 85102201 human breast epithelial). The primary cell line of normal human skin fibroblasts was used in this experiment. The studies were carried out for the purpose to choose the compounds having promising antiproliferative and anticancer properties. The influence of new synthesized amides on normal human skin fibroblast cells was also determined.

The cell lines were incubated at 10^4 cells per mL density on microtiter plates. Tested compounds were then added at three examined concentrations: 10, 50 and 100 µg mL^{-1}, and cultures were incubated at standard conditions (37 °C, 5% CO_2 and 90% humidity) for 24, 48 and 72 h.

Determinations were made with 5-bromo-2′-deoxy-uridine (BrdU) labelling and detection kit (Roche) on Elisa reader (BIO-TEC Instruments, USA). Cell viability of normal and carcinoma cells was evaluated spectrophotometrically. Results of every spectrophotometrical measurement were noticed as percent of growth inhibition or growth stimulation.

All experiments were repeated in triplicates.

5.3. X-ray structure analyses

*Crystal data for **8***: $C_{13}H_{14}N_4OS$, orthorhombic, space group Pna2$_1$, $a = 10.693(2)$, $b = 24.154(5)$, $c = 5.105(2)$ Å, $V = 1318.5(6)$ Å^3, $Z = 4$, $d_x = 1.382$ g cm^{-3}, $T = 295(2)$ K. 3039 data were collected up to $\theta = 45°$ for a yellow crystal with dimensions $0.54 \times 0.40 \times 0.09$ mm. Final R indices for 1475 reflections with $I > 2\sigma(I)$ and 173 refined parameters are: $R_1 = 0.0590$, $wR_2 = 0.1078$ ($R_1 = 0.1633$, $wR_2 = 0.1431$ for all 3039 data).

*Crystal data for **11***: $C_{14}H_{16}N_4OS$, monoclinic, space group P2$_1$/c, $a = 5.067(1)$, $b = 25.433(4)$, $c = 10.879(4)$ Å, $\beta = 91.38(2)°$, $V = 1401.6(6)$ Å^3, $Z = 4$, $d_x = 1.367$ g cm^{-3}, $T = 295(2)$ K. 6465 data were collected up to $\theta = 45°$ for a yellow crystal with dimensions $0.59 \times 0.32 \times 0.17$ mm. Final R indices for 1854 reflections with $I > 2\sigma(I)$ and 174 refined parameters are: $R_1 = 0.0476$, $wR_2 = 0.1059$ ($R_1 = 0.1099$, $wR_2 = 0.1280$ for all 3242 data).

*Crystal data for **12***: $C_{14}H_{16}N_4O_2S$, monoclinic, space group P2$_1$/c, $a = 5.523(2)$, $b = 23.314(7)$, $c = 11.667(7)$ Å, $\beta = 102.82(5)°$,

Table 2
Inhibition (GI) of normal and tumour cells growth *in vitro* of the tasted compounds.

Compound	Time of incubation (h)	GI (%) **10**			**11**			**9**		
Doses		I	II	III	I	II	III	I	II	III
Cell line										
Normal cell line										
HSF	24	75	75	0	5	50	0	25	0	0
	48	75	75	0	50	50	0	50	0	0
	72	50	75	0	50	50	0	75	0	5
Cancer cell line										
A549	24	50	50	25	50	50	20	50	50	25
	48	50	50	25	50	50	5	50	50	25
	72	75	50	0	50	25	0	75	50	25
T47D	24	5	5	0	0	5	0	0	0	0
	48	20	10	0	0	5	5	10	10	0
	72	25	25	0	25	25	5	90	50	5

GI – growth inhibition factor; HSF – human skin fibroblasts; A549 – human lung cancer cell line; T47D – human breast cancer cell line; examined doses of compounds: I – concentration of 100 µg mL^{-1}, which corresponds to concentration of 0.32 mM for compound **9**, 0.28 mM for compound **10**, and 0.35 mM for compound **11**; II – concentration of 50 µg mL^{-1}, which corresponds to concentration of 0.16 mM for compound **9**, 0.14 mM for compound **10**, and 0.17 mM for compound **11**; III – concentration of 10 µg mL^{-1}, which corresponds to concentration of 0.03 mM for compound **9**, 0.03 mM for compound **10**, and 0.03 mM for compound **11**.

$V = 1465(1)$ Å^3, $Z = 4$, $d_x = 1.380$ g cm^{-3}, $T = 295(2)$ K. 6730 data were collected up to $\theta = 45°$ for a yellow crystal with dimensions $0.50 \times 0.42 \times 0.08$ mm. Final R indices for 1181 reflections with $I > 2\sigma(I)$ and 192 refined parameters are: $R_1 = 0.0607$, $wR_2 = 0.0966$ ($R_1 = 0.2250$, $wR_2 = 0.1350$ for all 3375 data).

Single-crystal diffraction data were measured at room temperature in the $\omega/2\theta$ mode on an Oxford Diffraction Xcalibur diffractometer using graphite-monochromated Mo K_α radiation ($\lambda = 0.71073$). The stability of intensities was monitored by measurement of 3 standards every 100 reflections. Crystal structures were solved by direct methods using SHELXS97 [26] and refined by the full-matrix least-squares on F^2 using the SHELXL97 [27]. All non-hydrogen atoms were refined with anisotropic displacement parameters. The H-atoms were positioned geometrically and allowed to ride on their parent atoms, with $U_{iso}(H) = 1.2$ U_{eq}(C, N).

The CCDC 704272 (compound **8**), CCDC 704273 (compound **11**) and CCDC 704274 (compound **12**) contain the supplementary crystallographic data for this paper. These data can be obtained free of charge at www.ccdc.ac.uk/conts/retrieving.html or from the Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, UK; fax: +44-1223-336-033; e-mail: deposit@ccdc.cam.ac.uk.

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