ISSN 1070-3632, Russian Journal of General Chemistry, 2020, Vol. 90, No. 5, pp. 787–793. © Pleiades Publishing, Ltd., 2020.
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New Bis-4*H*-1,2,4-triazoles and Their In Vitro Study as DNA Methylation Inhibitors

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Received December 22, 2019; revised December 22, 2019; accepted December 26, 2019

Abstract—Bis-1,2,4-triazoles with aryl and alkyl linkers were synthesized. In order to increase their hydrophilicity and potential biological activity, pharmacophore electron-deficient groups (carboxylic, carboxamide, nitrile) were introduced into the molecule. Functionalized compounds revealed a new type of biological activity such as inhibition of the level of methylation of tumor DNA.

Keywords: bis-1,2,4-triazole, bistiosemicarbazide, linker, cyclization, tumor DNA

DOI: 10.1134/S1070363220050047

A use of combination of various pharmacophore groups with a 1,2,4-triazole scaffold in design of macromolecular drugs resulted in obtaining new structures with diverse biological activities [1–3]. Recently, we have reported a new type of biological activity of 1,2,4-triazole derivatives bearing pharmacologically active alkylsulfanyl fragments and exhibiting an inhibitory effect on the level of tumor DNA methylation [4, 5]. DNA methylation inhibitors are effectively used to treat various types of cancer [6]. The introduction of electron-withdrawing groups into the 1,2,4-triazole ring (carboxyl, sulfanyl, carboxamide, hydroxyl), as well as halogen-substituted aromatic fragments, contributes to a significant increase in fungicidal [7, 8], antitumor [9, 10] activity due to a possible increase stability and bioavailability of these compounds [11]. The introduction into the structure of another heterocyclic system [12, 13], the second 1,2,4-triazole ring, as well as various aromatic [14] or aliphatic [15, 16] linkers leads to bistriazolyl derivatives and opens up new possibilities in rational drug design.

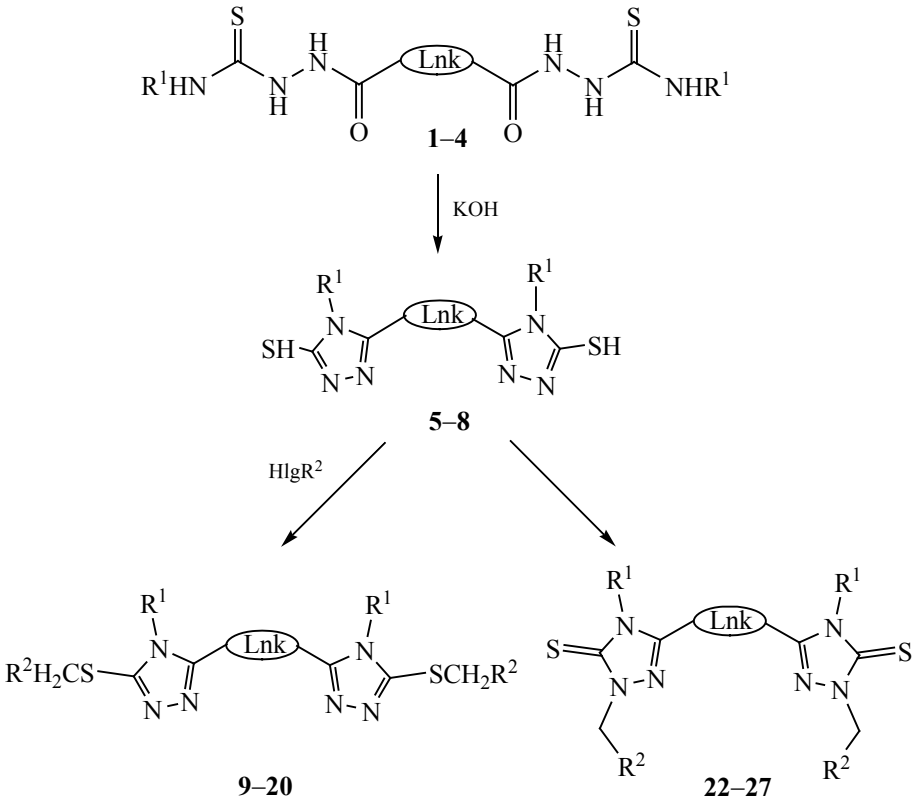
Herein, we reported the synthesis of new bis-1,2,4-triazolyl derivatives **5–27**, the structure of which involves functionalized 1,2,4-triazole fragments linked by aromatic or alkyl linkers (Scheme 1).

The introduction of various aryl substituents into the 1,2,4-triazole molecule leads to an increase in biological activity [11], which determined the choice of an aromatic linker for the synthesis of bis-1,2,4-triazoles **5** and **6**.

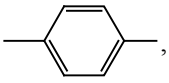
For the synthesis of bistriazoles with aromatic and aliphatic linkers **5–8**, we used bisthiosemicarbazides **1–4** obtained by the reaction of terephthalic and sebacic acid bishydrazides with phenyl or allyl isothiocyanates when boiled in ethanol. The intramolecular cyclization of thiosemicarbazides **1–4** in an alkaline medium led to the formation of bis-1,2,4-triazoles **5–8**. The obtained compounds are white crystalline substances that are poorly soluble in organic solvents and in water. In order to possibly increase the hydrophilicity and, therefore, bioavailability of the compounds, as well as to introduce new pharmacophore groups, compounds **5–8** were alkylated with some halides containing electron-deficient groups (2-chloroacetamide, 2-chloroacetic acid, ethyl chloroacetate) in an aqueous alcohol solution in the presence of KOH excess. Bis-1,2,4-triazole **7** was also alkylated with Cl- or F-substituted benzyl chlorides to afford compounds **17** and **18**. To compare the biological activity of mono- and disubstituted derivatives, we obtained asymmetric acetamide **21** by reacting bis-1,2,4-triazole **5** with an equimolar amount of 2- chloroacetamide (Scheme 2).

Under similar conditions, we were not able to obtain a monoalkyl derivative from bis-1,2,4-triazolyl-1,8-octane **7**. Using 2-chloroacetamide and 2-chloroacetic acid,

Scheme 1.



5–8, Lnk =  , R^1 = Ph (**5**), R^2 = All (**6**); Lnk = $(CH_2)_8$, R^1= Ph (**7**), R^2=All (**8**).

9–20, Lnk =  , R^1 = Ph, R^2=$CONH_2$ (**9**), COOH (**10**); R^1 = All, R^2=$CONH_2$ (**11**),

COOH (**12**),COOEt **(13)**; Lnk = $(CH_2)_8$, R^1 = Ph, R^2 = $CONH_2$ (**14**), COOH (**15**), COOEt (**16**), 2-ClC_6H_4 (**17**),

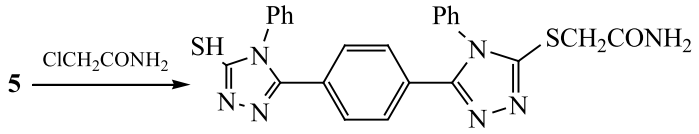
4-F-C_6H_4 (**18**); R^1 = All, R^2 = $CONH_2$ (**19**), COOH (**20**); **22–27**, Lnk =  , R^1 = Ph,

R^2 = CH_2CN (**22**), morpholin-4-yl (**23**) , R^1 =All, R^2= CH_2CN (**24**); Lnk = $(CH_2)_8$, R^1 = Ph, R^2 = CH_2CN (**25**), morpholin-4-yl (**26**); R^1 = All, R^2 = CH_2CN (**27**).

bis-alkyl derivatives **14** and **15** were prepared with low yields. Probably, the mobility of the alkyl chain has a significant effect on the reaction course, which has also been noted in the study of triazole derivatives with long chain alkyl groups [17].

Structure of bis-1,2,4-triazoles **5–27** was established by IR, ^1H, and ^{13}C NMR spectroscopy methods. In the IR spectra, there are characteristic absorption bands of the amide, carboxyl and ester groups, as well as C=C and C=N bonds in the expected ranges. In the ^1H NMR spectra of compounds **9–13** and **14–20**, the singlets of SCH_2 groups at 3.90–3.98 and 3.74–4.35 ppm, respectively, along with ^{13}C NMR spectroscopy data indicate the occurrence of alkylation of compounds **5–8** at the thiol group. In addition, the multiplet and triplet signals of the protons of the aliphatic linker $(CH_2)_8$ at 1.30–1.45, 1.66–1.77, and 2.63 ppm confirm the structure of compounds **14–20**. Chromatographically pure bis-1,2,4-triazoles **9–20** are stable white crystalline substances.

Scheme 2.



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In order to obtain derivatives with a more active lateral function, we performed cyanoethylation of bistriazoles **5–8** by heating with freshly distilled acrylonitrile in the presence of a basic triethylamine catalyst (Scheme 1). The formation of compounds **22**, **24**, **25**, **27** was confirmed by the appearance in the ^{1}H NMR spectra of triplet signals of the CH_2CN and NCH_2 groups in the ranges of 3.13–3.51 and 4.50 ppm (**22**, **24**), 2.99–3.03 and 4.14–4.37 ppm (**25**, **27**), respectively.

The reaction of bistriazoles **5** and **7** with morpholine in the presence of formalin (Scheme 1) proceeded at the N–H bond of the tautomeric thioxo form of the 1,2,4-triazole ring, as evidenced by signals of the NCH_2N group in the 5.13 and 5.02 ppm regions in the ^{1}H NMR spectra of compounds **28** and **26**, respectively. The obtained Mannich bases are stable white crystalline substances.

We studied in vitro the effect of bis-1,2,4-triazoles derivatives obtained on the level of tumor DNA methylation on the sarcoma 180 (C-180) model according to the method reported in [18]. Methylation disorder occurs in the early stages of the tumor. Unlike mutations that are irreversible, DNA methylation can be stabilized. This opens up new opportunities for the early diagnosis and treatment of cancer. In recent years, a number of approaches have appeared that allow DNA to be demethylated in cancer, thereby restoring gene expression [19].

A clear difference between the DNA samples from the tumor tissue after exposure to the test compounds was found only in relation to the amount of 5-methylcytosine (5-MC) (Table 1). Compound **12** and **21**, which inhibit the level of methylation of tumor DNA by 65.6 and 56.3%, respectively, have pronounced activity. Compounds **6** (43.8%), **24** (37.5%), **22** (34.4%), and **11** (31.3%) possess relatively weak demethylating activity. All of them, along with compounds **12** and **21**, are derivatives of bis-1,2,4-triazoles with an aromatic linker synthesized from terephthalic acid bishydrazide. The greatest activity was shown by 2,2'-{benzene-1,4-diylbis[(4-phenyl-4*H*-1,2,4-triazole-5,3-diyl)sulfanediyl]}diacetic acid **10**, which inhibits the level of tumor methylation DNA by 82.8%, which exceeds the activity of the control drug doxorubicin by 15.6%. Compound **10** was selected for further in vivo studies.

In the series of bis-1,2,4-triazoles with octamethylene linker (CH_2), only compound **15** exhibits moderate activity, which inhibits the level of DNA methylation by 42.2%. Most active compounds have phenyl group at position 4 of the triazole ring and a carboxyl group in the side alkylsulfanyl chain.

In conclusion, new substituted bistriazoles containing phenyl and octamethylene linkers were synthesized. It

Table 1. The effect of synthesized compounds on the level of sarcoma-180 DNA methylation

Compound	Content of bases in DNA, mol %		Methylation inhibition, %
	5-MC±ζ	G+C+5-MC	
DNA source (S-180)	0.64±0.02	42.66	–
Doxorubicin	0.21±0.01	43.40	67.2
5	0.82±0.01	42.22	–
6	0.36±0.02	42.52	43.8
7	1.63±0.01[a]	44.10	–
8	1.57±0.02	43.44	–
9	0.55±0.02	40.56	–
10	0.11±0.02	42.78	82.8
11	0.44±0.01	42.86	31.3
12	0.22±0.02	42.50	65.6
13	0.70±0.02	43.42	–
14	0.63±0.01[a]	42.16	–
15	0.37±0.01	44.18	42.2
16	1.01±0.02	42.72	–
17	0.79±0.02	43.26	–
18	0.61±0.01	45.00	–
19	0.86±0.02	42.92	–
20	0.55±0.01	43.40	–
21	0.28±0.01	43.18	56.3
22	0.42±0.01	42.68	34.4
23	0.73±0.01	42.46	–
24	0.40±0.01	43.48	37.5
25	1.25±0.02[a]	44.64	–
26	1.54±0.02	44.86	–
27	1.01±0.02	41.34	–

[a] $p < 0.05$.

was found that bistriazoles with a rigid phenyl linker significantly inhibit the level of DNA methylation in in vitro experiments on the sarcoma-180 model, while bistriazoles with a flexible octamethylene linker are less active.

EXPERIMENTAL

All solvents were purified by distillation before use. The crystalline starting compounds were recrystallized from suitable solvents.

IR spectra were recorded on a Nicolet Avatar 330 FT-IR spectrometer from vaseline oil. ^{1}H and ^{13}C NMR spectra were recorded on a Varian Mercury-300 instrument from DMSO-d_6 (300 or 75 MHz, respectively), and the internal standard was TMS. Melting points were determined on a Boëtius micro-heating table. The reaction progress and individuality of the obtained compounds were monitored by TLC on Silicagel 60 F254 plates in dioxane–benzene (2 : 1) or acetone–benzene (1 : 4) systems, developing with UV light. Elemental analysis was performed on an EA 3000 Eurovector automated analyzer.

The starting bisthiosemicarbazides **1–4** were obtained by boiling a mixture of 0.1 mol of sebacic acid hydrazide and 0.2 mol of allyl isothiocyanate in 120 mL of ethanol according to the procedure [20].

General procedure for the synthesis of bis-4*H*-1,2,4-triazole-3-thiols 5–8. A mixture of 10 mmol of thiosemicarbazide **1–4** and 1.68 g (3 mmol) of KOH in 30 mL of water was boiled for 7–8 h. After cooling, the reaction mixture was filtered, the filtrate was acidified with glacial acetic acid. The precipitate was filtered off, washed with water, ethanol and recrystallized from ethanol.

5,5'-(Benzene-1,4-diyl)bis(4-phenyl-4*H*-1,2,4-triazole-3-thiol) (5). Yield 91%, mp >260°C. ^{1}H NMR spectrum, δ, ppm: 7.31 s ($4H_{Ar}$), 7.37–7.41 m ($4H_{Ar}$), 7.55–7.60 m ($6H_{Ar}$), 14.07 br. s (2H, SH). Found, %: C 61.52; H 3.58; N 19.47. $C_{22}H_{16}N_6S_2$. Calculated, %: C 61.66; H 3.76; N 19.61.

5,5'-(Benzene-1,4-diyl)bis(4-allyl-4*H*-1,2,4,-triazole-3-thiol) (6). Yield 86%, mp >260°C. IR spectrum, ν, cm^{-1}: 3040 br (H–C=), 1709 s (C=C), 1520 s (N–C=S). ^{1}H NMR spectrum, δ, ppm: 4.70–4.80 m (4H, NCH_2), 4,87 br. d (2H, $=CH_2$, J = 17.0 Hz), 5.13 br. d (2H, $=CH_2$, J = 10.4 Hz), 5.77–5.91 m (2H, $=CH_2$), 7.77–7.89 m ($4H_{Ar}$), 14.02 br. s (2H, SH). Found, %: C 53.69; H 4.64; N 23.40. $C_{16}H_{16}N_6S_2$. Calculated, %: C 53.91; H 4.52, N 23.58.

5,5'-(Octane-1,8-diyl)bis(4-phenyl-4*H*-1,2,4-triazole-3-thiol) (7). Yield 38%, mp 204–205°C. IR spectrum, ν, cm^{-1}: 1649 s (C=C), 1570 s (N–C=S). ^{1}H NMR spectrum, δ, ppm: 0.98–1.16 m (8H, CH_2), 1.31–1.45 m (4H, CH_2), 2.38 t (4H, CH_2, J = 7.4 Hz), 7.36–7.42 m ($4H_{Ar}$), 7.50–7.59 m ($6H_{Ar}$), 13.63 br. s (2H, SH). Found, % C 62.17; H 6.26; N 18.31. $C_{24}H_{28}N_6S_2$. Calculated, %: C 62.04; H 6.07; N 18.09.

5,5'-(Octane-1,8-diyl)bis(4-allyl-4*H*-1,2,4-triazole-3-thiol) (8). Yield 90%, mp 173–175°C. IR spectrum, ν, cm^{-1}: 3060 w (H–C=), 1580 s (C=C), 1500 s (N–C=S). ^{1}H NMR spectrum, δ, ppm: 1.29–1.44 m (8H, CH_2), 1.64–1.7 m (4H, CH_2), 2.57 t (4H, CH_2, J = 7.5 Hz), 4.60 d. t (4H, CH_2, J = 5.3, 1.6 Hz), 5.10 d. t. d (2H, =CH, J = 17.1, 1.6, 1.2 Hz), 5.21 d. t. d (2H, =CH, J = 10.3, 1.6, 1.2 Hz), 5.87 d. d. t (2H, =CH, J = 17.1, 10.3, 5.3 Hz), 13.34 br. s (2H, SH). ^{13}C NMR spectrum, δ_C, ppm: 24.5 ($2CH_2$), 25.0 ($2CH_2$), 28.2 ($2CH_2$), 28.3 ($2CH_2$), 44.5 ($2NCH_2$), 116.9 ($=CH_2$), 131.3 (=CH), 151.2 (2C), 166.5 (CS). Found, %: C 55.26; H 7.32; N 21.22. $C_{18}H_{28}N_6S_2$. Calculated, %: C 55.07; H 7.19; N 21.41.

***S*-Alkylation of bis-4*H*-1,2,4-triazole-3-thiols 5–8** was performed by procedure reported in [21] using 2-fold molar amount of alkylating agent, the reaction mixture was heated for 8–10 h.

2,2'-{Benzene-1,4-diylbis[(4-phenyl-4*H*-1,2,4-triazole-3,5-diyl)sulfanediyl]}diacetamide (9). Yield 74%, mp >260°C. IR spectrum, ν, cm^{-1}: 3318 br (NH_2), 1689 s ($C{=}O_{amide}$). ^{1}H NMR spectrum, δ, ppm: 3.93 s (4H, SCH_2), 7.19 br. s (2H) and 7.63 br. s (2H, $CONH_2$), 7.29 s ($4H_{Ar}$), 7.35–7.40 m ($4H_{Ar}$), 7.50–7.57 m ($6H_{Ar}$). Found, %: C 57.68; H 4.20; N 20.44. $C_{26}H_{22}N_8O_2S_2$. Calculated, %: C 57.55; H 4.09; N 20.65.

2,2'-{Benzene-1,4-diylbis[(4-phenyl-4*H*-1,2,4-triazole-3,5-diyl)sulfanediyl]}diacetic acid (10). Yield 56%, mp >260°C. IR spectrum, cm^{-1}: 3450 br (O–H), 1731 s (C=O), 1377 s [δ(O–H)]. ^{1}H NMR spectrum, δ, ppm: 3.94 s (4H, CH_2), 7.28 s ($4H_{Ar}$), 7.49–7.56 m ($6H_{Ar}$), 12.56 br. s (1H, COOH). Found, %: C 57.53; H 3.56; N 15.51. $C_{26}H_{20}N_6O_4S_2$. Calculated, %: C57.34; H 3.70; N 15.43.

2,2'-{Benzene-1,4-diylbis[(4-allyl-4*H*-1,2,4-triazole-3,5-diyl)sulfanediyl]}diacetamide (11). Yield 53%, mp >260°C. IR spectrum, ν, cm^{-1}: 3363 br (NH_2). ^{1}H NMR spectrum, δ, ppm: 3.95 s (4H, SCH_2), 4.68–4.74 m (4H, NCH_2), 4.87 d. t. d (2H, $=CH_2$, J = 17.4 Hz), 5.25 br. d (2H, $=CH_2$, J = 11.6 Hz), 5.94–6.07 m (2H, =CH), 7.22 br. s and 7.67 br. s (4H, NH_2), 7.77–7.84 m ($4H_{Ar}$).

Found, %: C 51.22; H 4.59; N 23.71. $C_{20}H_{22}N_8O_2S_2$. Calculated, %: C 51.05; H 4.71; N 23.81.

2,2'-{Benzene-1,4-diylbis[(4-allyl-4*H*-1,2,4-triazole-3,5-diyl)sulfanediyl]}diacetic acid (12). Yield 53%, mp >260°C. IR spectrum, cm^{-1}: 3420 br (O–H), 3060 w (H–C=), 1717 s (C=O), 1376 s [δ(OH)]. Found, %: C 50.72; H 4.40; N 17.62. $C_{20}H_{20}N_6O_4S_2$. Calculated, %:C 50.83; H 4.27; N 17.79.

Diethyl 2,2'-{benzene-1,4-diylbis[(4-allyl-4*H*-1,2,4-triazole-3,5-diyl)sulfanediyl]}diacetate (13). Yield 57%, mp >260°C. IR spectrum, ν, cm^{-1}: 3084 w (H–C=), 1736 s (C= O). ^{1}H NMR spectrum, δ, ppm: 1.19 t (6H, CH_3, *J* = 7.1 Hz), 4.12 q (4H, OCH_2, *J* = 7.1 Hz), 4.13 s (4H, SCH_2), 4.68–4.74 m (4H, NCH_2), 4.87 br. d (2H, $=CH_2$, *J* = 17.3 Hz), 5.26 br. d (2H, $=CH_2$, *J* = 10.4 Hz), 5.93–6.06 m (2H, =CH), 7.28–7.82 m ($4H_{Ar}$). Found, %: C 54.32; H 5.24; N 15.99. $C_{24}H_{28}N_6O_4S_2$. Calculated, %: C 54.53; H 5.34; N 15.90.

2,2'-{Octane-1,8-diylbis[(4-phenyl-4*H*-1,2,4-triazole-3,5-diyl)sulfanediyl]}diacetamide (14). Yield 51%, mp 219–220°C. IR spectrum, ν, cm^{-1}: 3320 br (NH_2), 3091 br (H–C=), 1693 s ($C=O_{amide}$). ^{1}H NMR spectrum, δ, ppm: 1.01–1.17 m (8H, CH_2), 1.36–1.47 m (4H, CH_2), 2.48 t (4H, CH_2), 3.84 s (4H, SCH_2), 7.18 br. s and 7.62 br. s (4H, NH_2), 7.40–7.46 m ($4H_{Ar}$), 7.55–7.64 m ($6H_{Ar}$). Found, %: C 61.28; H 6.36; N 20.36. $C_{28}H_{34}N_8S_2$. Calculated, %: C 61.51; H 6.27; N 20.50.

2,2'-{Octane-1,8-diylbis[(4-phenyl-4*H*-1,2,4-triazole-3,5-diyl)sulfanediyl]}diacetic acid (15). Yield 45%, mp 216–218°C. IR spectrum, cm^{-1}: 3420 br (O–H), 1718 s (C=O), 1377 m [δ(OH)]. ^{1}H NMR spectrum, δ, ppm: 0.99–1.19 m (8H, CH_2), 1.35–1.47 m (4H, CH_2), 2.49 t (4H, CH_2, *J* = 7.2 Hz), 3.96 s (4H, CH_2), 7.39–7.46 m ($4H_{Ar}$), 7.55–7.62 m ($6H_{Ar}$), 12.94 br. s (2H, COOH). ^{13}C NMR spectrum, δ_C, ppm: 24.3 ($2CH_2$), 26.1 ($2CH_2$), 28.0 ($2CH_2$), 28.1 ($2CH_2$), 34.4 ($2SH_2$), 127.9 (4CH), 129.9 (4CH), 132.8 (2CH), 199.1 (2C), 155.5 (2C), 169.2 (2CO). Found, %: C 57.70; H 5.67; N 14.28. $C_{28}H_{32}N_6O_4S_2$. Calculated, %: C 57.91; H 5.55; N 14.47.

Diethyl 2,2'-{octane-1,8-diylbis[(4-phenyl-4*H*-1,2,4-triazole-3,5-diyl)sulfanediyl]}diacetate (16). Yield 38%, mp 100–102°C. IR spectrum, ν, cm^{-1}: 1734 s (C=O). ^{1}H NMR spectrum, δ, ppm: 1.00–1.18 m (8H, CH_2), 1.17 t (6H, CH_3, *J* = 7.0 Hz), 1.36–1.47 m (4H, CH_2), 2.50 t (4H, CH_2, *J* = 7.2 Hz), 3.98 s (4H, SCH_2), 4.08 q (4H, CH_2, *J* = 7.0 Hz), 7.40–7.45 m ($4H_{Ar}$), 7.56–7.64 m ($6H_{Ar}$). ^{13}C NMR spectrum, δ_C, ppm: 13.9 ($2CH_3$), 24.3 ($2CH_2$), 26.0 ($2CH_2$), 27.9 ($2CH_2$), 28.0 ($2CH_2$), 33.9 ($2SCH_2$), 61.1 ($2OCH_2$), 127.1 (4CH), 129.9 (6CH), 132.9 (2C), 155.6 (2C), 168.0 (2CO). Found, %: C 60.24; H 6.55; N 13.10. $C_{32}H_{40}N_6O_4S_2$. Calculated, %: C 60.35; H 6.33; N 13.20.

3,3'-(Octane-1,8-diyl)bis[4-phenyl-5-(2-chlorobenzylsulfanyl)-4*H*-1,2,4-triazole] (17). Yield 85%, mp 151–152°C. IR spectrum, ν, cm^{-1}: 3061 w (H–C=), 1445 s (C=N). ^{1}H NMR spectrum, δ, ppm: 0.99–1.14 m (8H, CH_2), 1.35–1.45 m (4H, CH_2), 2.48 t (4H, CH_2, *J* = 7.6 Hz), 4.39 s (4H, SCH_2), 7.21–7.34 m ($8H_{Ar}$), 7.37–7.44 m ($4H_{Ar}$), 7.49–7.55 m ($6H_{Ar}$). Found, %: C 64.18; H 5.16; N 11.89. $C_{38}H_{38}Cl_2N_6S_2$. Calculated, %: C 64.02; H 5.37; N 11.79.

3,3'-(Octane-1,8-diyl)bis[4-phenyl-5-(4-fluorobenzylsulfanyl)-4*H*-1,2,4-triazole (18). Yield 29%, mp 159–162°C. IR spectrum, ν, cm^{-1}: 3080 w (H–C=), 1600 s (C=C), 1461 s (C=N). ^{1}H NMR spectrum, δ, ppm: 0.98–1.15 m (8H, CH_2), 1.35–1.46 m (4H, CH_2), 2.48 t (4H, CH_2, *J* = 7.6 Hz), 4.28 s (4H, SCH_2), 7.05–7.13 m ($4H_{Ar}$), 7.23–7.36 m ($8H_{Ar}$), 7.50–7.56 m ($6H_{Ar}$). Found, %: C 66.72; H 5.92; N 12.20. $C_{38}H_{38}F_2N_6S_2$. Calculated, %: C 66.93; H 5.76; N 12.33.

2,2'-{Octane-1,8-diylbis[(4-allyl-4*H*-1,2,4-triazole-3,5-diyl)sulfanediyl]}diacetamide (19). Yield 98%, mp 167–169°C. IR spectrum, ν, cm^{-1}: 3346 br (NH_2), 3051 m (H–C=), 1600 s (C=O). ^{1}H NMR spectrum, δ, ppm: 1.31–1.43 m (8H, CH_2), 1.66–1.77 m (4H, CH_2), 2.63 t (4H, CH_2, *J* = 7.5 Hz), 3.74 s (4H, SCH_2), 4.56 d. t (4H, CH_2, *J* = 4.9, 1.7 Hz), 4.93 d. t. d (2H, =CH, *J* = 17.1, 1.7, 1.0 Hz), 5.21 d. t. d (2H, =CH, *J* = 10.4, 1.7, 10.2 Hz), 5.90 d. d. t (2H, =CH, *J* = 17.1, 10.4, 4.9 Hz), 6.92 br. s (2H, CONH), 7.51 br (2H, CONH). ^{13}C NMR spectrum, δ_C, ppm: 24.1 ($2CH_2$), 26.0 ($2CH_2$), 28.2 ($2CH_2$), 28.3 ($2CH_2$), 36.5 ($2SCH_2$), 45.0 ($2NH_2$), 116.6 ($=CH_2$), 131.7 (=CH),148.5 (2C),154.8 (2C), 168.3 (2CO). Found, %: C 52.38; H 6.58; N 22.21. $C_{22}H_{34}N_8O_2S_2$. Calculated, %: C 52.15; H 6.76; N 22.12.

2,2'-{Octane-1,8-diylbis[(4-allyl-4*H*-1,2,4-triazole-3,5-diyl)sulfanediyl]}diacetic acid (20). Yield 40%, mp 122–123°C. IR spectrum, cm^{-1}: 3420 br (O–H), 1721 s (C=O), 1381 s [δ(OH)]. ^{1}H NMR spectrum, δ, ppm: 1.30–1.45 m (8H, CH_2), 1.66–1.77 m (4H, CH_2), 2.63 t (4H, CH_2, *J* = 7.5 Hz), 3.85 s (4H, SCH_2), 4.57 d. t (4H, CH_2, *J* = 4.8, 1.6 Hz), 4.93 d. t. d (2H, $=CH_2$, *J* = 17.1, 1.6, 1.0 Hz), 5.22 d. t. d (2H, $=CH_2$, *J* = 10.5, 1.6, 1.0 Hz), 5.90 d. d. t (2H, =CH, *J* = 17.1, 10.5, 4.8 Hz), 11.58 br. s (2H, COOH). ^{13}C NMR spectrum, δ_C, ppm: 24.1 ($2CH_2$), 26.1 ($2CH_2$), 28.2 ($2CH_2$), 28.3 ($2CH_2$), 35.2

($2CH_2$), 116.7 (2=CH_2), 131.6 (2=CH), 147.9 (2C), 154.9 (2C), 168.7 (2CO). Found, %: C 51.71; H 6.45; N 16.27. $C_{22}H_{32}N_6O_4S_2$. Calculated, %: C 51.95; H 6.34; N 16.52.

2-({5-[4-(5-Sulfanyl-4-phenyl-4*H*-1,2,4-triazol-3-yl)phenyl]-4-phenyl-4*H*-1,2,4-triazol-3-yl}sulfanyl)-acetamide (21). Yield 72%, mp >260°C. IR spectrum, ν, cm^{-1}: 3322 br (NH_2), 3072 br (H–CH=), 1689 s ($C=O_{amide}$). ^1H NMR spectrum, δ, ppm: 3.94 s (2H, CH_2), 7.14–7.69 m ($16H_{Ar}$, NH_2), 14.00 br. s (1H, SH). Found, %: C 59.62; H 3.83; N 20.41. $C_{24}H_{19}N_7OS_2$. Calculated, %: C 59.36; H 3.94; N 20.19.

***N*-Cyanoethylation of bis-4*H*-1,2,4-triazole-3-thiols 5–8.** A mixture of 1 mmol of compound **5–8**, 3.24 g (60 mmol) of freshly distilled acrylonitrile, 8 mL of water, and 6.0 g (60 mmol) of triethylamine was boiled for 10–12 h. The solution was evaporated, and the crystalline residue was recrystallized from ethanol.

***N*-Aminomethylation of bis-4*H*-1,2,4-triazole-3-thiols 5 and 7** was performed by procedure [5] using 2-fold amount of morpholine and paraform.

3,3'-[(Benzene-1,4-diyl)bis(5-thioxo-4-phenyl-4,5-dihydro-1*H*-1,2,4-triazole-3,1-diyl)]dipropanenitrile (22). Yield 57%, mp >260°C. IR spectrum, ν, cm^{-1}: 2249 s (C≡N). ^1H NMR spectrum, δ, ppm: 3.15 t (4H, CH_2CN, J = 6.5 Hz), 4.51 t (4H, NCH_2, J = 6.5 Hz), 7.28 s ($4H_{Ar}$), 7.30–7.37 m ($4H_{Ar}$), 7.46–7.54 m ($6H_{Ar}$). ^{13}C NMR spectrum, δ_C, ppm: 16.0 (**C**H_2CN), 44.4 (NCH_2), 118.2 (CN), 126.9, 128.4 (8CH), 129.4 (4CH), 129.7 (2CH), 134.4, 148.5, 168.1. Found, %: C 62.76; H 4.38; N 20.78. $C_{28}H_{22}N_8S_2$. Calculated, %: C 62.90; H 4.15; N 20.46.

5,5'-(Benzene-1,4-diyl)bis{2-[(morpholin-4-yl)methyl]-4-phenyl-2,4-dihydro-1*H*-1,2,4-triazole-3-thione} (23). Yield 71%, mp >260°C. IR spectrum, ν, cm^{-1}: 3071 cp (H–C=). ^1H NMR spectrum, δ, ppm: 2.72–2.83 m [8H, $N(CH_2)_2$], 3.54–3.63 m [8H, $O(CH_2)_2$], 5.14 s (4H, NCH_2N), 7.28 s ($4H_{Ar}$), 7.32–7.38 m ($4H_{Ar}$), 7.43–7.51 m ($6H_{Ar}$). Found, %: C 61.44; H 5.33; N 17.67. $C_{32}H_{34}N_8O_2S_2$. Calculated, %: C 61.32; H 5.46; N 17.88.

3,3'-[(Benzene-1,4-diyl)bis(4-allyl-5-thioxo-4,5-dihydro-1*H*-1,2,4-triazole-3,1-diyl]dipropanenitrile (24). Yield 98%, mp >260°C. IR spectrum, ν, cm^{-1}: 2252 s (C≡N). ^1H NMR spectrum, δ, ppm: 3.11–3.18 m (4H, CH_2, NCH_2), 4.47–4.54 m (4H, CH_2CN), 4.79–4.85 m (4H, NCH_2), 4.90 br. d (2H, =CH_2, J = 17.4 Hz), 5.16 br. d (2H, =CH_2, J = 10.6 Hz), 5.79–5.93 m (2H, =CH), 7.87–7.94 m ($4H_{Ar}$). ^{13}C NMR spectrum, δ_C, ppm: 16.0 ($2CH_2$), 44.3 ($2NHCH_2$), 47.1 ($2NCH_2$), 117.4 (2CH), 118.0 (2=CH_2), 127.6 (2C), 129.0 (4CH), 131.2 (2=CH), 149.5 (2C), 167.3 (2CN). Found, %: C 57.35; H 4.67; N 24.35. $C_{22}H_{22}N_8S_2$. Calculated, %: C 57.12; H 4.39; N 24.22.

3,3'-[(Octane-1,8-diyl)bis(5-thioxo-4-phenyl-4,5-dihydro-1*H*-1,2,4-triazole-3,1-diyl)]dipropanenitrile (25). Yield 66%, mp 109–110°C. IR spectrum, ν, cm^{-1}: 2250 m (C≡N). ^1H NMR spectrum, δ, ppm: 1.00–1.18 m (8H, CH_2), 1.34–1.45 m (4H, CH_2), 2.45 t (4H, CH_2, J = 7.5 Hz), 3.09 t (4H, CH_2CN, J = 6.4 Hz), 4.41 t (4H, NCH_2, J = 6.4 Hz), 7.40–7.45 m ($4H_{Ar}$), 7.51–7.61 m ($6H_{Ar}$). ^{13}C NMR spectrum, δ_C, ppm: 16.0 ($2CH_2$), 24.8 ($2CH_2$), 24.9 ($2CH_2$), 27.7 ($2CH_2$), 27.9 ($2CH_2$), 43.9 ($2NCH_2$), 118.0 (4CH), 129.5 (4CH), 129.6 (2CH), 133.9 (2C), 151.3 (2C), 167.1 (2C=S). Found, %: C 64.36; H 4.38; N 19.81. $C_{30}H_{25}N_8S_2$. Calculated, %: C 64.15; H 4.49; N 19.95.

5,5'-(Octane-1,8-diyl)bis{2-[(morpholin-4-yl)-methyl]-4-phenyl-2,4-dihydro-1*H* -1,2,4-triazole-3-thione} (26). Yield 67%, mp 209–210°C. ^1H NMR spectrum, δ, ppm: 1.01–1.19 m (8H, CH_2), 1.33–1.46 m (4H, CH_2), 2.44 t (4H, CH_2, J = 7.4 Hz), 2.68–2.75 m [8H, $N(CH_2)_2$], 3.53–3.59 m [8H, $O(CH_2)_2$], 7.39–7.45 m ($4H_{Ar}$), 7.52–7.60 m ($6H_{Ar}$). Found, %: C 61.43; H 6.78; N 16.83. $C_{34}H_{46}N_8O_2S_2$. Calculated, %: C 61.60; H 6.99; N 16.90.

3,3'-[(Octane-1,8-diyl)bis(4-allyl-5-thioxo-4,5-dihydro-1*H*-1,2,4-triazole-3,1-diyl)]dipropanenitrile (27). Yield 99%, mp 67–69°C. IR spectrum, ν, cm^{-1}: 2251 m (C≡N). ^1H NMR spectrum, δ, ppm: 1.30–1.45 m (8H, CH_2), 1.66–1.77 m (4H, CH_2), 2.63 t (4H, CH_2, J = 7.5 Hz), 2.99 t (4H, CH_2CN, J = 6.6 Hz), 4.37 t (4H, NCH_2, J = 6.6 Hz), 4.65 d. t (4H, CH_2, J = 5.3, 1.6 Hz), 5.11 d. t. d (2H, = CH_2, J = 17.2, 1.6, 1.0 Hz), 5.23 d. t. d (2H, =CH_2, J = 10.4, 1.6, 1.0 Hz), 5.88 d. d. t (2H, =CH, J = 17.2, 10,4, 5.3 Hz). ^{13}C NMR spectrum, δ_C, ppm: 15.7 ($2CH_2$), 24.3 ($2CH_2$), 24.9 (CH_2), 28.0 ($2CH_2$), 28.3 ($2CH_2$), 43.8 ($2NCH_2$), 45.7 ($2NCH_2$), 116.4 (2CN), 117.3 (2=CH_2), 130.7 (2=CH), 151.1 (2C), 166.2 (2C=S). Found, %: C 57.66; H 6.95; N 22.61. $C_{24}H_{34}N_8S_2$. Calculated, %: C 57.80; H 6.87; N 22.47.

CONFLICT OF INTEREST

No conflict of interest was declared by the authors.

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