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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Synthesis, antioxidant, and antitumor evaluation of certain new *N*-substituted-2-amino-1,3,4-thiadiazoles

Wafaa S. Hamama · Moustafa A. Gouda · Marwa H. Badr · Hanafi H. Zoorob

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Abstract Reaction of 2-amino-1,3,4-thiadiazole (**1**) with chloroacetyl chloride afforded the chloroacetamide **2** which used as starting compound for the synthesis of 2-thiocyanatoacetamide **3** and *N*-(1,3,4-thiadiazol-2-yl) acetamides **5**–**9** via reaction of **1** with various reagents. Treatment of **9** with 4-(piperidin-1-yl)benzaldehyde or DMF-DMA afforded the arylidenes **10** and **11**, respectively. Cyclization of the later compound with hydrazine hydrate gave the pyrazole derivative **12**. Furthermore, coupling of **9** with 4,6-dimethyl-1*H*-pyrazolo[3,4-*b*]pyridin-3-diazonium chloride afforded hydrazone derivative **13**, which cyclized in acetic acid to afford 1,2,4-triazine derivative **14**. Moreover, 1,3,4-thiadiazoles **15**, **19**, **22**, and **23** were achieved via reaction of **1** with different nucleophiles. Finally, 1-phenyl-1*H*-pyrazol-5(4*H*)-one when subjected to react either with **15** or with diazonium salt of **1** afforded pyrazole derivative **16** or *bis*-1,3,4-thiadiazole derivative **18**, respectively. Some of these compounds were screened for their cytotoxicity and antioxidant activities which showed promising results.

Keywords Thiadiazoles · Synthesis · Antitumor and antioxidant activities

W. S. Hamama (✉) · M. A. Gouda · M. H. Badr · H. H. Zoorob
Chemistry Department, Faculty of Science, Mansoura University, El-Gomhoria Street, Mansoura 35516, Egypt
e-mail: wshamama@yahoo.com

Present Address:
M. A. Gouda
Department of Chemistry, Faculty of Science and Arts, Ulla, Taibah University, Medina, Kingdom of Saudi Arabia

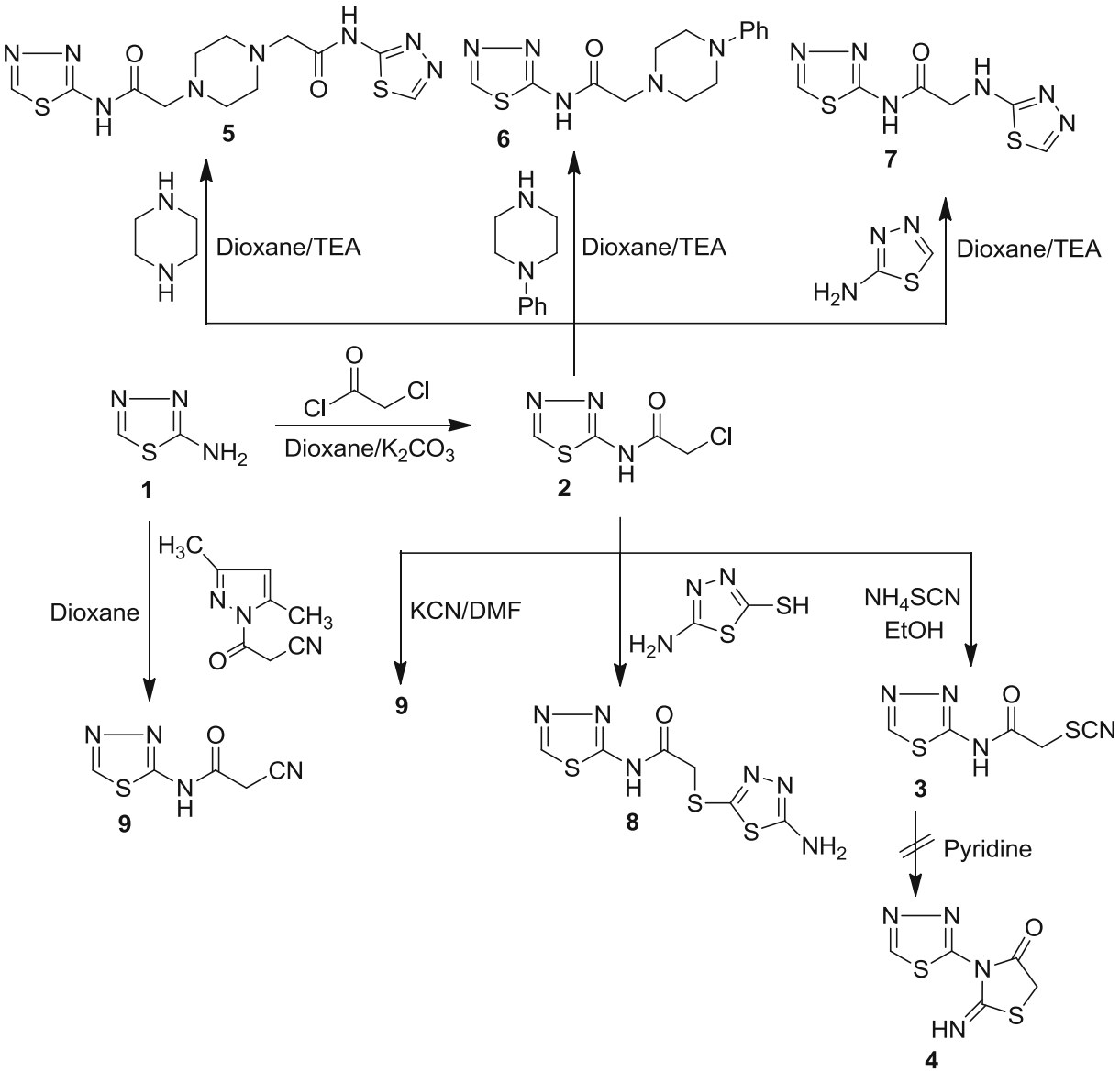
Introduction

1,3,4-Thiadiazoles are a valuable group of bioactive compounds showing antituberculosis, anticancer, antibacterial, antifungal, and anticonvulsant activities (Clerici *et al*., 2001; Prasad *et al*., 1986; Madapa *et al*., 2009; Sharma and Bahel, 1982; Jatav *et al*., 2008; Gagnon *et al*., 2009). Moreover, *N*-substituted 2-amino-1,3,4-thiadiazoles bearing acetyl and halo nucleus show broad range of biological activities (Molyneux, 2004; Re *et al*., 1999). Furthermore, thiadiazole substituted by amide groups possesses antifungal activities (Liu *et al*., 2009), antioxidant properties, and radioprotective effects (Cressier *et al*., 2009). 2-Amino-1,3,4-thiadiazole acts as a building block for the synthesis of 1,3,4-thiadiazole derivatives and various intermediates (Matysiak and Niewiadomy, 2006; Matysiak and Opolski, 2006; Terzioglu and Gürsoy, 2003; Bhati and Kumar, 2008), possessing biological activities. Therefore, some new amide derivatives were synthesized, starting from 2-amino-1,3,4-thiadiazole (Lauer and Zenchoff, 1976), in order to investigate their antioxidant and antitumor activities.

Results and discussion

Chemistry

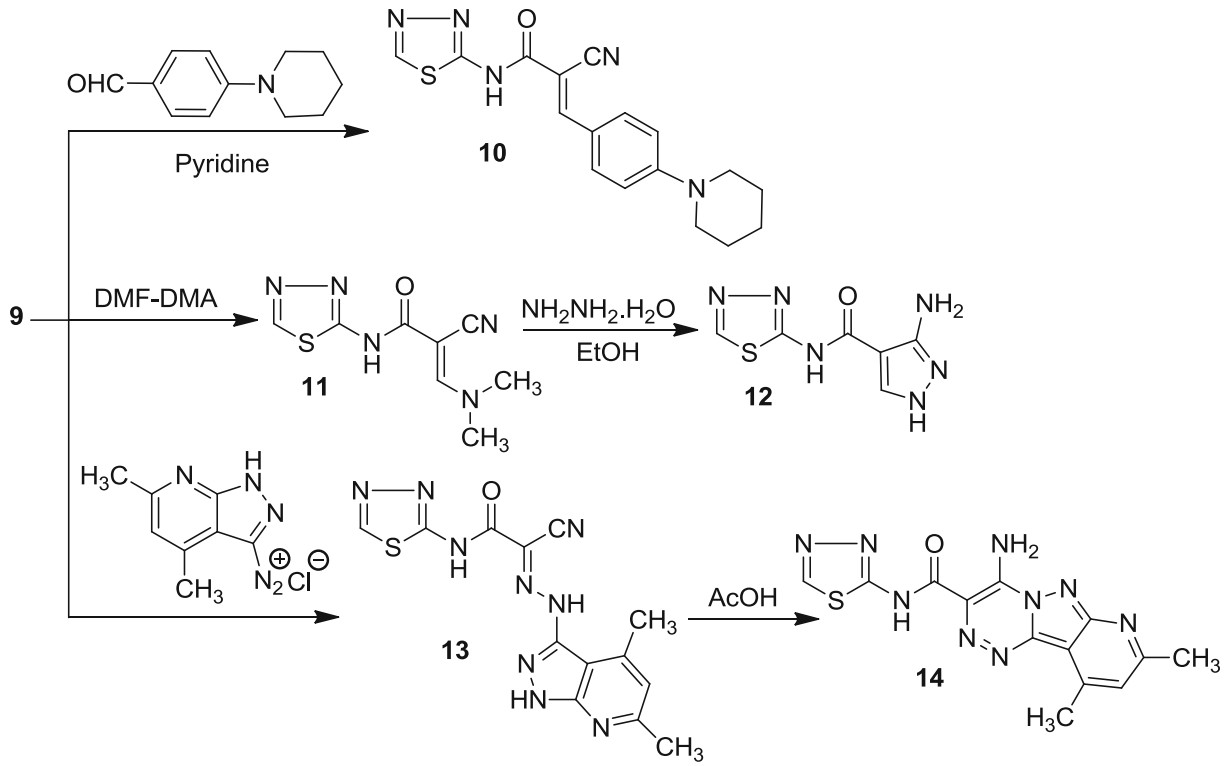
The target compounds were synthesized as outlined in Schemes 1, 2, 3, 4. Reaction of 2-amino-1,3,4-thiadiazole (**1**) with chloroacetyl chloride afforded chloroacetamide derivative **2** which gave the corresponding thiocyanate **3** upon treatment with ammonium thiocyanate upon refluxing in ethanol. Attempts for the preparation of thiazoline **4** through cyclization of **3** under the influence of pyridine were failed. Treatment of **2** with piperazine, *N*-phenyl



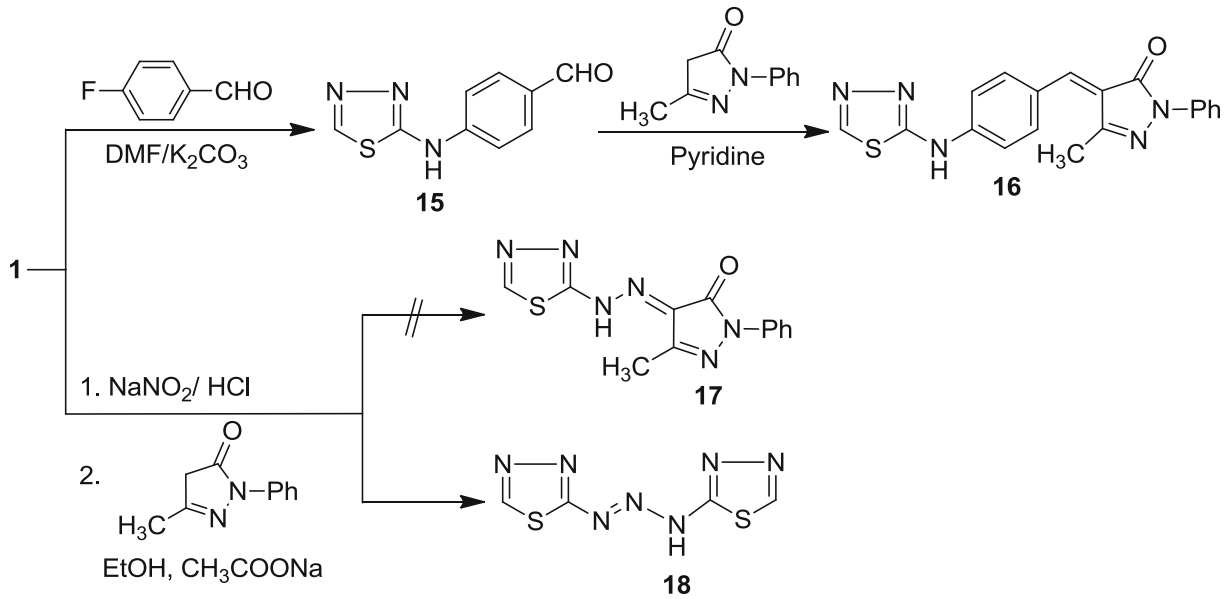
Scheme 1 Synthesis of 2-chloro-*N*-(1,3,4-thiadiazol-2-yl)acetamide (**2**) and its reactions with different nucleophiles

piperazine, 2-amino-1,3,4-thiadiazole, and 2-amino-1,3,4-thiadiazol-5-thiol afforded the corresponding thiadiazole derivatives **5**–**8**, respectively. Moreover, heating of **2** with potassium cyanate in dimethylformamide (DMF) afforded cyanoacetamide **9**. In another route, compound **9** was obtained in good yield via cyanoethylation of **1** with 3,5-dimethyl-1-cyanoacetyl pyrazole (Ried and Schleimer, 1958), in dioxane (Scheme 1).

Condensation of compound **9** with 4-(piperidin-1-yl)benzaldehyde in pyridine afforded the corresponding arylidene derivative **10**. Pyrazole nucleus has pronounced pharmacological applications as antianxiety and antipyretic (Wustrow *et al*., 1998), analgesic, and anti-inflammatory drugs (Eid *et al*., 1978; Menozzi *et al*., 1997; Penning *et al*., 1997) and certain alkyl pyrazoles were reported to exhibit significant bacteriostatic, bactericidal, and fungicidal activities (Potts, 1986). These biological data prompted us to synthesize some new pyrazole derivatives incorporating 2-amino-1,3,4-thiadiazole moiety, thus the aminopyrazole **12** was obtained via treatment of **9** with dimethylformamide dimethyl acetal (DMF-DMA) in dry dioxane followed by cyclization of the formed 2-cyano-3-dimethylamino-*N*-(1,3,4-thiadiazol-yl)-acrylamide (**11**) with hydrazine hydrate. In continuation of our interest in the synthesis of nitrogen heterocyclic systems (Hamama *et al*., 2012a, c), we have found that diazotized heterocyclic amine is an excellent building block for the synthesis of the target compounds. Thus, coupling of compound **9** with 4,6-dimethyl-1*H*-pyrazolo[3,4-*b*]pyridin-3-diazonium chloride (El-Dean *et al*., 1991), in pyridine at 0–5 °C afforded the



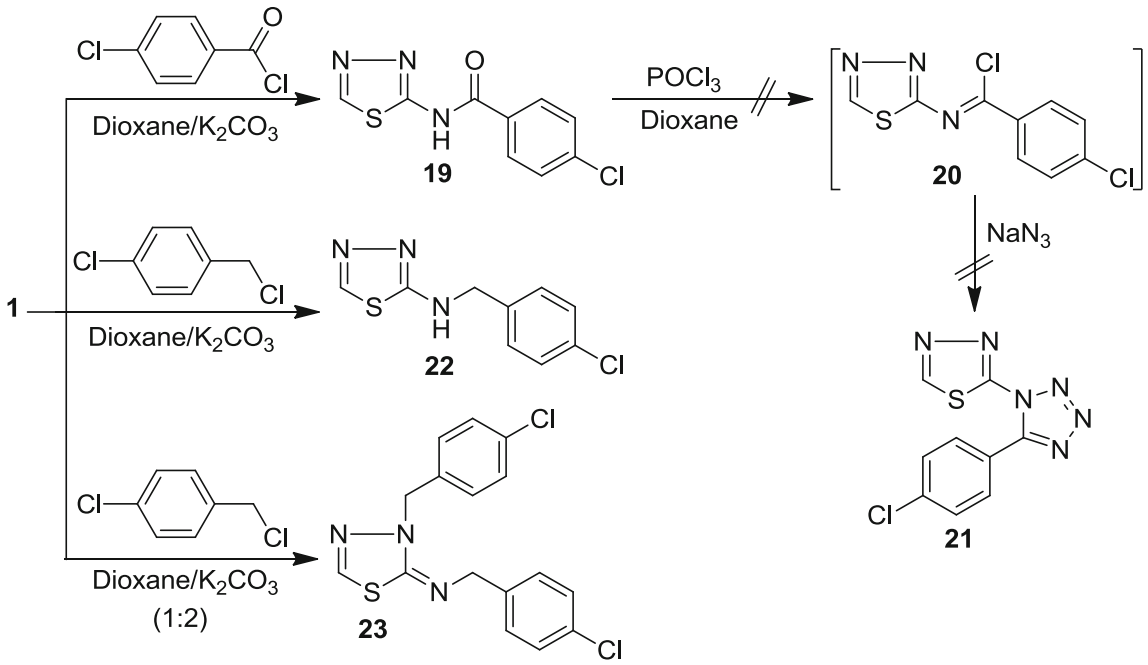
Scheme 2 Reactions of 2-cyano-*N*-(1,3,4-thiadiazol-2-yl)acetamide (**9**) with aldehyde, DMF-DMA and diazonium salt



Scheme 3 Synthesis of pyrazol-5(4*H*)-one **16** and bis(1,3,4-thiadiazole) **18**

corresponding hydrazono compound **13**. When compound **13** is refluxed in acetic acid, it can be cyclized to pyrazolo[5,1-*c*][1,2,4]triazine derivative **14** (Scheme 2).

Furthermore, we also investigated the reactivity of **1** toward nucleophilic substitution reaction with the aim of preparing 4-(1,3,4-thiadiazol-2-ylamino)benzaldehyde. Thus, treatment of **1** with 4-flourobenzaldehyde in DMF/K_2CO_3 afforded 4-(1,3,4-thiadiazol-2-ylamino)benzaldehyde **15**, which condensed with 1-phenyl-1*H*-pyrazol-5(4*H*)-one to afford the corresponding arylidene derivative **16**. Attempts for the investigation of the reactivity of diazonium salt of **1** toward 1-phenyl-1*H*-pyrazol-5(4*H*)-one



Scheme 4 Reactions of 2-amino-1,3,4-thiadiazole (**1**) with different halogenated compounds

(Vogel *et al*., 1994), in pyridine to give (4*Z*)-4-(2-(1,3,4-thiadiazol-2-yl)hydrazono)-1-phenyl-1*H*-pyrazol-5-(4*H*-pyrazol-5(4*H*)-one (**17**) were failed, but we obtained (*E*)-(1,3,4-thiadiazol-2-ylimino)-2-(1,3,4-thiadiazol-2-yl)hydrazine (**18**) (Scheme 3).

Moreover, compound **1** reacted with 4-chlorobenzoyl-chloride in dioxane/K_2CO_3 to give the corresponding *N*-aroyl derivative **19**. Subsequently, reaction of compound **19** with phosphorus oxychloride in dioxane followed by in situ addition of sodium azide did not furnish the corresponding tetrazole derivative **21**. Finally, treatment of compound **1** with one equivalent amount of 4-chlorobenzyl chloride afforded the *N*-(4-chlorobenzyl) derivative **22** (bioisostere of **19**). Moreover, compound **23** was achieved when two equivalent of 4-chlorobenzyl chloride were used (Scheme 4).

Biological activity

ABTS antioxidant assay

The antioxidant activity of the synthesized compounds was evaluated by Lissi *et al.*, 1999. Some of the *N*-substituted-2-amino-1,3,4-thiadiazoles exhibited an antioxidant effect as shown in Table 1. Compared with the control (L-ascorbic acid), the antioxidant potency of compounds **9** was found to be high, while compounds **13** and **16** showed a moderate antioxidant activity and the rest tested compounds showed a weak activity. On the other hand, compounds **9**, **10**, **13**, **16**, **22**, and **23** exhibited a high antioxidant activity compared to the starting material **1**. From the structure activity relationship (SAR) we found that, the presence of 4-chlorobenzyl, cyanoacetyl or pyrazole moiety in the 2-amino-1,3,4-thiadiazoles enhanced the antioxidant activity of the 2-amino-1,3,4-thiadiazoles.

Table 1 *ABTS* antioxidant activity assay *N*-substituted-2-amino-1,3,4-thiadiazoles derivatives

Compound no.	ABTS	
	Absorbance of samples	% Inhibition
Control of ABTS	0.498	0.0
Ascorbic acid	0.057	88.55
1	0.356	28.51
2	0.363	27.10
3	0.370	25.70
6	0.370	25.70
8	0.395	20.68
9	0.145	70.88
10	0.355	28.71
11	0.380	23.69
12	0.404	18.87
13	0.240	51.80
15	0.366	26.50
16	0.261	41.56
18	0.376	24.49
19	0.366	26.50
22	28.91	0.354
23	0.309	37.95

Table 2 Assay for bleomycin-dependent DNA damage (DNA)

Compound no.	Bleomycin-dependent DNA damage Absorbance of samples
Ascorbic acid	0.098
1	0.090
2	0.102
3	0.099
6	0.096
8	0.101
9	0.080
10	0.114
11	0.127
12	0.102
13	0.109
15	0.100
16	0.105
18	0.103
19	0.100
22	0.108
23	0.121

Bleomycin-dependent DNA damage

Damage to DNA in the presence of a Bleomycin-Fe complex has been adopted as a sensitive and specific method to examine potential pro-oxidant agents (Gutteridge *et al*., 1981). If the samples to be tested are able to reduce the bleomycin-Fe^{3+} to Bleomycin-Fe^{2+}, DNA degradation in this system will be stimulated, resulting in a positive test for pro-oxidant activity. DNA degradation is accompanied by the formation of a product similar to Malondialdehyde (MDA). L-ascorbic acid, a reducing agent, can reduce Fe^{3+} to Fe^{2+}. Table 2 showed that compounds **1**, **6**, and **9** showed a high pro-antioxidant action compared to the L-ascorbic acid, whereas compounds **23**, **10**, and **11** have weak effects. On the other hand the rest of compounds showed good pro-oxidant action in this system.

Antitumor

Effect of drugs on the viability of Ehrlich ascites cells (EAC) in vitro (Hamama et al., 2012b): To examine whether these substances have a direct cytotoxic effect on Ehrlich ascites cells (EAC) viability, the percentage of viable cells was estimated by the trypan blue (Sheeja *et al*., 1997), exclusion test. Seventeen *N*-substituted-2-amino-1,3,4-thiadiazoles were tested for cytotoxicity against well-known established model EAC in vitro. Results for the ED_{100}, ED_{50}, and ED_{25} values of the active compounds are summarized in Table 3. The data showed clearly that compounds **1** and **9** showed high activity, whereas compounds **8**, **17**, **18**, **19**, and **21** showed moderate activity on the other hand the rest of compounds have weak activities. From the structure activity relationship's (SAR's) we found that *N*-substitution of 2-amino-1,2,3-thiadiazol decrease the antitumor activity of 2-amino-1,3,4-thiadiazole.

Table 3 In vitro cytotoxicity of *N*-substituted-2-amino-1,3,4-thiadiazoles derivatives (Ehrlich ascites cells dead %)

Compound no.	% Dead		
	ED_{100} (μg/cm^3)	ED_{50} (μg/cm^3)	ED_{25} (μg/cm^3)
5-FU	97.0	63.0	40.0
1	92.9	56.0	37.0
2	28.0	16.0	7.8
5	37.0	18.0	8.7
6	46.1	24.0	13.0
7	39.1	18.9	9.4
8	42.5	27.0	14.4
11	26.0	14.1	7.8
13	19.0	10.2	5.0
14	80.7	48.2	25.0
15	38.3	17.9	8.7
16	31.0	17.0	8.8
17	42.1	21.0	10.0
18	61.5	33.0	19.3
19	52.0	24.0	11.0
20	6.9	13.0	6.9
21	41.0	23.0	12.0
23	29.0	16.0	7.4

Where, ED_{100}, ED_{50}, and ED_{25} are the effective doses at 25, 50, and 100 μl, respectively, of the compounds used. The dead % refers to the % of the dead tumor cells and 5-fluorouracil (5-FU) is 5-fluorouracil as a well-known cytotoxic agent

Conclusion

The prepared new ring systems seem to be interesting for biological studies. Furthermore, the present investigation offers rapid and effective new procedures for the synthesis of a new class of *N*-substituted-2-amino-1,3,4-thiadiazoles. The new compounds were investigated for antioxidant activity. Compounds **1** and **9** exhibited a high antioxidant activity when compared to the ascorbic acid; these compounds manifested the best protective effect against DNA damage induced by Bleomycin. Furthermore, *N*-substituted-2-amino-1,3,4-thiadiazoles were tested for cytotoxicity against well-known established model EAC in vitro. The data showed clearly that *N*-substitution of 2-amino-1,2,3-thiadiazol decrease the antitumor activity of 2-amino-1,3,4-thiadiazole.

Experimental section

General

All melting points are in degree centigrade (uncorrected) and were determined on Gallenkamp electric melting point apparatus. Elemental analyses were carried out at Micro analytical Center, Faculty of Science, Cairo University. IR spectra were recorded (KBr), (υ cm^{-1}) on a Mattson 5000 FTIR Spectrophotometer at Micro analytical Center Faculty of Science, Mansoura University. The ^{1}H NMR spectra of **3**, **6**, **7**, **8**, **10**, **12**, **13**, **16**, and **18** were measured on a Varian Spectrophotometer at 300 MHz, using TMS as an internal reference and DMSO-d_6 or $CDCl_3$ as solvent at Chemistry Department, Faculty of Science, Cairo University. The ^{1}H NMR and ^{13}C NMR spectra of **2**, **9**, **11**, **19**, **22**, and **23** were acquainted on a JEOL ECX-400 spectrometer Chemistry department, School of Engineering and Science University of Jacobs, Bremen, Germany, operating at 400 MHz for the ^{1}H NMR and 100 MHz for the ^{13}C NMR at room temperature in $CDCl_3$ or DMSO-d_6, using a 5 mm probe. High Resolution Mass Spectra (HRMS) were recorded using both a Bruker HCT ultra and a high resolution (Bruker Daltonics micrOTOF) instruments from methanol or dichloromethane solutions using the positive Electrospray Ionization Mode (ESI). Mass spectra were recorded on (Kratos, 70 eV) MS equipment and/or a Varian MAT 311 A Spectrometer, at Microanalytical Center, Faculty of Science, Cairo University. Reaction mixtures were monitored by thin layer chromatography (TLC) using EM science silica gel coated plates with visualization by irradiation with ultraviolet lamp. Biological activities were screened for the tested compounds at Pharmacognosy Department, Faculty of Pharmacy, Mansoura University, Mansoura, Egypt.

Chemistry

*2-Chloro-N-(1,3,4-thiadiazol-2-yl)acetamide (**2**)*

A mixture of **1** (0.51 g, 5 mmol), chloroacetyl chloride (0.4 mL, 5 mmol) in dioxane (20 mL) was refluxed for 5 h. The reaction mixture was left to cool, poured into ice cold water and the precipitated solid was filtered off, dried, and crystallized from ethanol to give **2**. Yield 80 % (white crystals); m.p. 200 °C; $R_f = 0.38$ [pet. ether 60–80)/ethyl acetate (2:3)]; IR (KBr) ν_{max} (cm^{-1}) 3171 (NH), 2900 (CH, str.), 1709 (C=O); ^{1}H NMR (DMSO-d_6) δ (ppm) 4.4 (s, 2H, CH_2), 9.1 (s, 1H, CH, thiadiazole); ^{13}C NMR (DMSO-d_6) δ (ppm) 165.9 (C=O), 159.1 (C_2, thiadiazole), 149.6 (C_5, thiadiazole), 42.8 (CH_2). MS (ESI, −98.7 v) (+) ESI showed two quasi-molecular ion peaks at 199.7 (M^++Na) and 376.8($2M^+$+Na) pointing 177.5 as the molecular mass of **2**; HRMS(micrOTOF):*m*/*z* for $C_4H_{4Cl}N_3OS$, Calcd., 177.61 Found, 175.9788(M^+-H).

*N-(1,3,4-Thiadiazol-2-yl)-2-thiocyanatoacetamide (**3**)*

A mixture of **2** (0.89 g, 5 mmol), ammonium thiocyanate (0.38 g, 5 mmol) in ethanol (20 mL) was refluxed for 2 h. The reaction mixture was left to cool and the formed precipitate was filtered off, dried, and recrystallized from DMF to furnish **3**. Yield 80 % (yellow crystals); m.p. 270 °C (DMF); $R_f = 0.25$ [pet. ether (60–80)/ethyl acetate (1:4)]; IR (KBr) ν_{max} (cm^{-1}) 2918 (CH, str.), 2260 (CN), 1715 (C=O); ^{1}H NMR (DMSO-d_6) δ (ppm) 4.09 (d, 2H, CH_2), 9.29 (s, 1H, CH, thiadiazole), 12.7 (s, 1H, NH). Anal. Calcd for $C_5H_4N_4OS_2$ (200.24): C, 29.99; H, 2.01; N, 27.98 %. Found: C, 30.03; H, 2.08; N, 28.07 %.

*Reaction of chloroacetamide derivative **2** with different 1°ry and 2°ry amines*

General procedure: A mixture of **2** (0.89 g, 5 mmol), appropriate amines namely; piperazine (0.43 g, 5 mmol), *N*-phenyl piperazine (0.81 g, 5 mmol), 2-amino-1,3,4-thiadiazole (0.51 g, 5 mmol) or 2-amino-1,2,3-thiadiazole-5-thiol (0.67 g, 5 mmol) and TEA (0.7 mL, 5 mmol) in dioxane was refluxed for 2 h. The reaction mixture was left to cool and the formed precipitate was filtered off, dried, and recrystallized from DMF to furnish the corresponding thiadiazoles **5**–**8**, respectively.

*2-(4-(2-(5-Amino-1,3,4-thiadiazol-2-ylthio)acetyl) piperazin-1-yl)-N-(1,3,4-thiadiazol-2-yl)acetamide (**5**)*

Yield 60 % (pale green crystals); m.p. 210 °C (ethanol); $R_f = 0.6$[pet. ether (80–100)/ethyl acetate (1:1)]; IR (KBr) ν_{max} (cm^{-1}) 3442 (br, 2NH), 2829, 2874 (CH, aliphatic), 1713 (br, 2CO); MS: *m*/*z* (%) = 369 (M^++1, 1.4), 283 (0.8), 245 (0.8), 202 (2.9), 176 (2.3), 139 (100), 112 (25.4). Anal. Calcd for $C_{12}H_{16}N_8O_2S_2$ (368.44): C, 39.12; H, 4.38; N, 30.41 %. Found: C, 39.17; H, 4.43; N, 30.46 %.

*2-(4-Phenyl piperazine-1-yl)-N-(1,3,4-thiadiazol-2-yl)acetamide (**6**)*

Yield 70 % (white crystals); m.p. 191 °C (ethanol); $R_f = 0.34$ [pet. ether (60–80)/ethyl acetate (3:7)]; IR (KBr) ν_{max} (cm^{-1}) 3451 (NH), 2938 (CH, str.), 1696, 1598 (C=C); ^{1}H NMR ($CDCl_3$) δ (ppm) 2.82–2.95 (m, 2H, piperazine), 3.22–3.41 (m, 2H, piperazine), 3.69 (s, 2H, CH_2), 6.85–7.66 (m, 5H, Ar–H), 9.19 (s, 1H, CH, thiadiazole), 11.80 (s, 1H, NH); MS: *m*/*z* (%) = 303 (M^+, 2.62), 273 (3.3), 220 (6.7), 195 (100), 178 (12.6), 152 (11.3), 133 (6.4), 122 (7.1), 85 (76.2), 77 (35.2), 63 (51.3). Anal. Calcd

for $C_{14}H_{17}N_5OS$ (303.38): C, 55.42; H, 5.65; N, 23.08 %. Found: C, 55.47; H, 5.71; N, 23.13 %.

*2-(1,3,4-Thiadiazol-2-ylamino)-N-(1,3,4-thiadiazol-2-yl) acetamide (**7**)*

Yield 50 % (yellow crystals); m.p. 226 °C (ethanol); $R_f = 0.24$ [pet. ether (60–80)/ethyl acetate (3:7)]; IR (KBr) ν_{max} (cm^{-1}) 3183 (NH), 2980 (CH, str.), 1702 (C=O), 1616 (C=N); ^{1}H NMR (DMSO-d_6) δ (ppm) 4.45 (s, 2H, CH_2), 8.94 (s, 1H, CH, thiadiazole), 9.22 (s, 1H, CH, thiadiazole), 10.42 (s, 1H, NH), 13.16 (s, 1H, NHCO). Anal. Calcd for $C_6H_6N_6OS_2$ (242.28): C, 29.74; H, 2.50; N, 34.69 %. Found: C, 29.71; H, 2.46; N, 34.64 %.

*2-(5-Amino-1,3,4-thiadiazol-2-ylthio)-N-(1,3,4-thiadiazol-2-yl) acetamide (**8**)*

Yield 80 % (white crystals); m.p above 300 °C (DMF); $R_f = 0.17$[pet. Ether (60–80)/ethyl acetate (3:7)]; IR (KBr) ν_{max} (cm^{-1}) 3315 (NH_2), 3132 (NH), 1678 (C=O), 1592 (C=C); ^{1}H NMR (DMSO-d_6) δ (ppm) 4.13 (s, 2H, CH_2), 7.30 (s, 1H, NH_2), 9.18 (s, 1H, CH, thiadiazole), 12.81 (s, 1H, NH). Anal. Calcd for $C_6H_6N_6OS_3$ (274.35): C, 26.27; H, 2.20; N, 30.63 %. Found: C, 26.34; H, 2.28; N, 30.67 %.

*Synthesis of 2-cyano-N-(1,3,4-thiadiazol-2-yl) acetamide (**9**)*

Method A: A mixture of **2** (0.89 g, 5 mmol) and potassium cyanide (0.39 g, 6 mmol) in DMF (15 mL) was heated at 60 °C for 8 h. The reaction mixture was left to cool then poured into ice cold water and the formed precipitate was filtered off, dried, and crystallized from ethanol to give **9**.

Method B: A mixture of **1** (1.42 g, 14 mmol) and 3-(3, 4-dimethyl-1*H*-pyrazol-1-yl)-3-oxopropanenitrile (2.28 g, 14 mmol) in dioxane (20 mL) was refluxed for 5 h. The solvent was evaporated under *vacuum* and the residue was crystallized form ethanol to give **9**. Yield 90 % (white crystals); m.p. 241 °C (ethanol); $R_f = 0.11$ [pet. ether (60–80)/ethyl acetate (2:3)]; IR (KBr) ν_{max} (cm^{-1}) 3423 (br, NH), 2859 (CH, str.), 2181 (CN), 1610 (br, CO); ^{1}H NMR (DMSO-d_6) δ (ppm) 4.05 (s, 2H, CH_2), 9.17 (s, 1H, C-5H, thiadiazole), 12.11 (s, 1H, NHCO); ^{13}C NMR (DMSO-d_6) δ (ppm) 162.9 (C=O), 158.9 (C_2), 149.6 (C_5), 115.5(CN), 49.1 (CH_2); MS (ESI, −98.7v) (+)ESI showed one quasi-molecular ion peak at 190.8 (M^++Na), the (−)-ESI mass spectrum showed one quasi-molecular ion peak at 166.7 ([M-2H]) pointing 168 as the molecular mass of **9**; HRMS(micrOTOF):*m/z* for $C_5H_4N_4OS$, Calcd., 168.18. Found, 167.0040 (M^+-H).

*(E)-2-Cyano-3-(4-(piperidin-1-yl)phenyl-N-(1,3,4-thiadiazol-2-yl)acrylamide (**10**)*

A mixture of **9** (0.84 g, 5 mmol), 4-(piperidin-1-yl)benzaldehyde (0.95 g, 5 mmol) in pyridine (7 mL) was refluxed for 6 h then the reaction mixture was left to cool and poured into ice cold water. The formed precipitate filtered off, dried, and crystallized from ethanol to give **10**. Yield 70 % (reddish brown crystals); m.p. 268 °C (ethanol); $R_f = 0.4$[pet. ether (60–80)/ethyl acetate (1:1.5)]; IR (KBr) ν_{max} (cm^{-1}) 3394 (NH), 2937 (CH, str.), 2201 (CN), 1678 (C=O), 1608 (C=N); ^{1}H NMR ($CDCl_3$) δ (ppm) 1.60–1.78 (m, 6H, $3CH_2$), 3.44–3.52 (m, 4H, CH_2N), 7.96 (d, 2H, Ar–H, $J = 9$ Hz), 6.85 (d, 2H, Ar–H, $J = 9$ Hz), 8.27 (s, 1H, methine), 8.87 (s, 1H, C-5H, thiadiazole), 12.15 (s, 1H, NHCO). Anal. Calcd for $C_{17}H_{17}N_5OS$ (339.41): C, 60.16; H, 5.05; N, 20.63 %. Found: C, 60.27; H, 5.14; N, 20.72 %.

*2-Cyano-3-(dimethylamino)-N-(1,3,4-thiadiazol-2-yl) acrylamide (**11**)*

A mixture of **9** (0.84 g, 5 mmol) and DMF-DMA (0.66 mL, 5 mmol) in DMF (5 mL) was heated at 60 °C. The reaction mixture was left to cool and the precipitated solid was collected by filtration and recrystallized from ethanol to give **11**. Yield 60 % (reddish brown crystals); m.p. 224 °C (ethanol); $R_f = 0.25$ [pet. ether (60–80)/ethyl acetate (2:5)]; IR (KBr) ν_{max} (cm^{-1}) 3217 (NH), 2935 (CH, str.), 2199 (CN), 1674(C=O), 1611 (C=N); ^{1}H NMR (DMSO-d_6) δ (ppm) 3.20 (s, 3H, CH_3), 3.27 (s, 3H, CH_3), 8.02 (s, 1H, CH, methine), 9.11 (s, 1H, C-5H, thiadiazole), 9.83 (s, 1H, NH); ^{13}C NMR (DMSO-d_6) δ (ppm) 160(C=O), 157.9 (C_2, thiadiazole), 157.7 (C_3-acrylamide), 148.7 (C_5, thiadiazole), 71.4 (C_2, acrylamide), 118.7 (CN), 47.7 (2 CH_3); HRMS(micrOTOF):*m/z* for $C_8H_9N_5OS$, Calcd., 223.25. Found, 246.039(M^++Na), 469.0920 ($2M^+$+Na).

*3-Amino-N-(1,3,4-thiadiazol-2-yl)-1H-pyrazole-4-carboxamide (**12**)*

A mixture of **11** (1.12 g, 5 mmol), hydrazine hydrate (0.24 mL, 5 mmol) in ethanol (10 mL) was heated under reflux for 2 h. The formed precipitate was filtered off, washed with ethanol, and recrystallization from DMF to give **12**. Yield 70 % (white crystals); m.p. 286 °C (DMF); $R_f = 0.3$[pet. ether (60–80)/ethyl acetate (2:5)]; IR (KBr) ν_{max} (cm^{-1}) 3472, 3362, 3204 (NH_2, NH), 1670 (C=O), 1622 (C=N); ^{1}H NMR (DMSO-d_6) δ (ppm) 6.2 (s, 2H, NH_2), 8.09 (s, 1H, C_5-H, pyrazole), 9.09 (s, 1H, C_5-H, thiadiazole), 11.88 (s, 1H, NH, pyrazole), 11.21 (s, 1H,

NHCO). Anal. Calcd for $C_6H_6N_6OS$ (210.22): C, 34.28; H, 2.88; N, 39.98 %. Found: C, 34.35; H, 2.94; N, 39.94 %.

*2-(2-(4-Methyl-1H-pyrazolo[3,4-b]pyridine-3-yl)diazenyl)-2-cyano-N-(1,3,4-thia-diazol-2-yl)acetamide (**13**)*

To a well-stirred cooled solution of 4,6-dimethyl-1*H*-pyrazolo[3,4-*b*]pyridin-3-amine (0.81 g, 5 mmol) in conc. HCl (3 mL), a solution of $NaNO_2$ (0.35 g, 5.1 mmol in 5 mL H_2O) was added dropwise. The above cooled diazonium solution was added slowly to a well-stirred solution of **9** (0.84 g, 5 mmol) in pyridine (10 mL). The reaction mixture was stirred for 2 h. The crude product was filtered off, dried well, and recrystallized from the EtOH-benzene to afford **13**. Yield 80 % (reddish brown crystals); m.p. above 300 °C (DMF); $R_f = 0.4$ [ethyl acetate/methanol 20:3]; IR (KBr) ν_{max} (cm^{-1}) 3386, 3268, (2NH), 2925 (CH, str.), 2194 (CN), 1681 (C=O); ^{1}H NMR (DMSO-d_6) δ (ppm) 2.84 (s, 3H, CH_3), 2.95 (s, 3H, CH_3), 6.96 (s, 1H, C_5-H, pyridine), 9.11 (s, 1H, C_5-H, thiadiazole), 11.92 (s, 1H, NH, pyrazole), 12.91 (s, 1H, NHCO), 14.12 (s, 1H, NH–N=C). Anal. Calcd for $C_{13}H_{11}N_9OS$ (341.35): C, 45.74; H, 3.25; N, 36.93 %. Found: C, 45.85; H, 3.32; N, 37.02 %.

*8-Amino-2,4-dimethyl-1,5,6,8a,9-pentaazafluorene-7-carboxylic acid (1,3,4-thiadiazol-2-yl)-amide (**14**)*

A suspension of **13** (1.71 g, 5 mmol) in acetic acid (10 mL) was refluxed for 7 h. The reaction mixture was poured into ice cold water. The formed precipitate was filtered off, dried and recrystallized from DMF to give **14**. Yield 50 % (yellow crystals); m.p. above 300 °C (DMF); $R_f = 0.4$ [ethyl acetate containing drops of methanol]; IR (KBr) ν_{max} (cm^{-1}) 3442, 3372, 3262 (NH_2, NH), 1677 (C=O), 1600 (C=C). Anal. Calcd for $C_{13}H_{11}N_9OS$ (341.35): C, 45.74; H, 3.25; N, 36.93 %. Found: C, 45.83; H, 3.31; N, 36.97 %.

*4-(1,3,4-Thiadiazol-2-ylamino)benzaldehyde (**15**)*

A mixture of **1** (0.51 g, 5 mmol), *p*-fluorobenzaldehyde (0.62 g, 5 mmol) and potassium carbonate (0.69 g, 5 mmol) in DMF (10 mL) was refluxed on water bath for 10 h, poured into ice-water and the resultant solid was filtered and recrystallized from ethanol to give **15**. Yield 90 % (yellow crystals); m.p. 73 °C (ethanol); $R_f = 0.48$ [pet.ether(80–100)/ethyl acetate (4:1)]; IR (KBr) ν_{max} (cm^{-1}) 3417 (NH), 2908 (CH, str.), 1662 (CHO), 1598 (C=C); MS: *m/z* (%) = 203 (M^+-2H, 0.98), 176 (4.9), 148 (8.4), 121 (5.2), 106 (3.5), 95 (3.7), 78 (44.0), 63 (81.6), 45 (100). Anal. Calcd for $C_9H_7N_3OS$ (205.24): C, 52.67; H, 3.44; N, 20.47 %. Found: C, 52.61; H, 3.47; N, 20.43 %.

*(4E)-4-(4-(1,3,4-Thiadiazol-2-ylamino)benzylidene)-3-methyl-1-phenyl-1H-pyrazol-5(4H)-one (**16**)*

A mixture of **15** (1.03 g, 5 mmol), 3-methyl-1-phenyl-1*H*-pyrazol-5(4*H*)-one (0.87 g, 5 mmol) and piperidine (0.5 mL, 5 mmol) in ethanol (20 mL) was refluxed for 2 h. The reaction mixture was left to cool and the formed precipitate was filtered off, dried, and recrystallized from DMF to give **16**. Yield 65 % (red crystals); m.p. 210 °C (DMF); $R_f = 0.48$[pet. ether (60–80)/ethyl acetate (4:3)]; IR (KBr) ν_{max} (cm^{-1}) 3421 (NH), 2918 (CH, str.), 1668 (C=O), 1572 (C=C); ^{1}H NMR ($CDCl_3$) δ (ppm) 2.32 (s, 3H, CH_3), 6.72-8.03 (m, 6H, Ar–H, NH), 8.55 (s, 1H, methine), 8.58 (s, C_5-H, thiadiazole). MS: *m/z* (%) = 361 (M^+, 0.23), 360 (M^+-1, 0.23), 277 (1.3), 261 (1.3), 218 (1.4), 185 (8.2), 172 (100), 156 (12.9), 128 (17.8), 91 (7.1), 78 (32.8), 63 (42.4), 43 (26.8). Anal. Calcd for $C_{19}H_{15}N_5OS$ (361.42): C, 63.14; H, 4.18; N, 19.38 %. Found: C, 63.22; H, 4.19; N, 19.46 %.

*(E)-1-(1,2,3-Thiadiazol-2-ylimino)-2-(1,2,3-thiadiazol-2-yl)hydrazine (**18**)*

Method A: To a well-stirred cooled solution of **1** (0.51 g, 5 mmol) in conc. HCl (3 mL), a solution of $NaNO_2$ (0.35 g, 5.1 mmol in 5 mL H_2O) was added dropwise. The above cooled diazonium solution was added slowly to a well-stirred solution of 1-phenyl-1*H*-pyrazol-5(4*H*)-one (0.87 g, 5 mmol) in ethanol (20 mL) and sodium acetate (1.64 g, 20 mmol). The reaction mixture was stirred for 2 h. The crude product was filtered off, dried well, and recrystallized from the EtOH to give **18**.

Method B: Hydrochloric acid (0.9 mL) in water (3.4 mL) was added to **1** (0.61 g, 6 mmol) in ice cold water then add sodium nitrite (0.2 g in 0.5 mL water) drop wise for 10 min. The reaction was stirred for 30 min., then raise pH by adding sodium acetate (0.9 g) in water (1.8 mL), stirring the mixture for 30 min. The formed precipitate was filtered off, washed with water, and recrystallized from ethanol to afford **18**. Yield 85 % (yellow crystals); m.p. 228 °C (ethanol); $R_f = 0.6$[ethyl acetate containing drops of methanol]; IR (KBr) ν_{max} (cm^{-1}) 3454 (NH), 1570 (–N=N–); ^{1}H NMR (DMSO-d_6) δ (ppm) 9.26 (br., s, 2H, C_5-H, thiadiazole), 14.13 (br, s, 1H, NH); MS (EI, 76 eV) *m/z* (%) = 213 (M^+, 4.27), 113 (100), 114 (5.32), 84 (19.25), 45 (84.9). Anal. Calcd for $C_4H_3N_7S_2$ (213.24): C, 22.53; H, 1.42; N, 45.98 %. Found: C, 22.61; H, 1.47; N, 46.04 %.

*Reaction of 2-amino-1,3,4-thiadiazole (**1**) with 4-chlorobenzoyl chloride and 4-chlorobenzyl chloride*

General procedure: A mixture of **1** (0.51 g, 5 mmol), appropriate aromatic chloro derivatives namely; 4-chlorobenzoyl

chloride (0.88 g, 5 mmol), 4-chlorobenzyl chloride (0.81 g, 5 mmol) or 4-chlorobenzyl chloride (1.61 g, 10 mmol) in dioxane (20 mL) was refluxed for 2 h, The reaction mixture was left to cool and the formed precipitate was filtered off, dried, and recrystallized from ethanol to furnish **19**, **22**, and **23**, respectively.

*4-Chloro-N-(1,3,4-thiadiazol-2-yl)benzamide (**19**)*

Yield 65 % (white crystals); m.p. 270 °C (ethanol); $R_f = 0.6$ [pet. ether 60–80)/ethyl acetate (3:7)]; IR (KBr) ν_{max} (cm^{-1}) 3437 (NH), 1672 (C=O); ^{13}C NMR (DMSO-d_6) δ (ppm) 165.1 (C=O), 160.3 (C_2, thiadiazole), 149.5 (C_5, thiadiazole), 138.3, 131.2, 130.8 (2C), 129 (2C); MS (ESI, −98.7 v) (+)-ESI mass spectrum showed one quasi-molecular ion peak at 261.8 (M^++Na), (−)-ESI mass spectrum showed one quasi-molecular ion peak at 237.7 (M^+-H) pointing 239 as the molecular mass of **23**; HRMS(micrOTOF): *m/z* for $C_9H_6N_3OSCl$, Calcd., 239.5. Found, 237.9866 (M^+-H).

*N-(4-Chlorobenzyl)-1,3,4-thiadiazol-2-amine (**22**)*

Yield 30 % (yellow crystals); m.p 143 °C (ethanol); $R_f = 0.34$ [pet. ether (80–100)/ethyl acetate (4:2.5)]; IR (KBr) ν_{max} (cm^{-1}) 3417 (NH), 2946(CH, str.), 1596 (C=C), ^1H NMR (DMSO-d_6) δ (ppm) 5.5 (s, 2H, CH_2), 7.3–7.4 (m, 4H, Ar–H), 8.8 (s, 1H, CH, thiadiazole), 10.6 (s, 1H, NH); ^{13}C NMR (DMSO-d_6) δ (ppm) 167.4 (C_2, thiadiazole), 146 (C_5, thiadiazole), 133.7, 133.4, 130.7, 129.3 (2C), 39.4 (CH_2); HRMS(micrOTOF):*m/z* for $C_9H_8ClN_3S$, Calcd., 225.7. Found, 226.0218(M + H).

*(5E)-N-(3-(4-Chlorobenzyl)-1,3,4-thiadiazol-2(3H)-ylidene)(4-chlorophenyl) methan-amin (**23**)*

Yield 40 % (yellow crystals); m.p. 227 °C (ethanol); $R_f = 0.73$ [pet. ether (60–80)/ethyl acetate (2:3)]; IR (KBr) ν_{max} (cm^{-1}) 2901 (CH, str.), 1631 (C=N), ^1H NMR (DMSO-d_6) δ (ppm): 4.2 (s, 2H, CH_2–N=C), 5.3 (s, 2H, CH_2–N–N), 7.2–7.4 (m, 8H, Ar–H), 8.6 (s, 1H, CH thiadiazole); ^{13}C NMR (DMSO-d_6) δ (ppm) 159.5 (C_2, thiadiazole), 146.2 (C_5, thiadiazole), 131.1 (2C‘), 130.4 (2C), 129.1 (2C), 128.7 (2C), 39.8 (CH_2), 39.6 (CH_2). Anal. Calcd for $C_{16}H_{13}Cl_2N_3S$ (350.27): C, 54.86; H, 3.74; N, 12.00 %. Found: C, 54.96; H, 3.82; N, 12.08 %.

Materials and methods

Antioxidant activity screening assay; ABTS method (Lissi et al., 1999)

Antioxidant activity determinations were evaluated from the bleaching of ABTS derived radical cations. The radical cation derived from ABTS [2,2′-azino-*bis* (3-ethyl benzothiazoline-6-sulfonic acid)] was prepared by reaction of ABTS (60 µl) with MnO_2 (3 mL, 25 mg/mL) in (5 mL) aqueous buffer solution (pH 7). After shaking the solution for a few minutes, it was centrifuged and filtered. The Absorbance (A control) of the resulting green–blue solution (ABTS radical solution) was recorded at λ_{max} 734 nm. The absorbance (A test) was measured upon the addition of (20 µl of 1 mg/mL) solution of the tested sample in spectroscopic grade MeOH/buffer (1:1 v/v) to the ABTS solution. The inhibition ratio (%) was calculated using the following formula: % Inhibition = [A (control) − A (test)/A (control)] × 100

Ascorbic acid (20 µl, 2 mM) solution was used as a standard antioxidant (positive control). Blank sample was run using solvent without ABTS (Table 2).

Bleomycin-dependent DNA damage

The assay was done according to Aeschbach *et al.*, 1994 and Chan and Tang, 1996, with minor modifications. The reaction mixture (0.5 mL) contained DNA (0.5 mg/mL), Bleomycin sulfate (0.05 mg/mL), $MgCl_2$ (5 mM), $FeCl_3$ (50 mM) and the samples were dissolved in DMSO to be tested at concentration (20 µl of 1 mg/mL). L-Ascorbic acid was used as a positive control. The mixture was incubated at 37 °C for 1 h. The reaction was terminated by the addition of 0.05 mL EDTA (0.1 M). The color was developed by adding thiobarbituric acid (TBA) (0.5 mL) (1 %, w/v) and HCl (0.5 mL) (25 %, v/v) followed by heating at 80 °C for 10 min. After centrifugation, the extent of DNA damage was measured by the increase in absorbance at 532 nm.

Antitumor activity

Different concentrations of the tested compounds were prepared (ED_{100}, ED_{50}, and ED_{25} 1 g mL^{-1} DMSO). The amount of DMSO was adjusted to give a final concentration of 0.1 %. Ascites fluid obtained was aseptically aspirated from the peritoneal cavity of the donor animal (National Cancer Institute, Cairo, Egypt), which contains Ehrlich cell. The cells were grown partially floating and attach in AQ_4 a suspension culture (RPMI 1660 medium, Sigma Chemical, St. Louis), supplemented with 10 % fetal bovine serum (GIBCO, UK). They were maintained at 37 °C in humidified atmosphere with 5 % CO_2 for 2 h. The viability of the cell used in control experiments (DMSO only without drug) exceeded 95 % as determined by microscopic examination using a hemocytometer and trypan blue stain (stain only the dead cells).

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