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Original article

Synthesis and antitumor activity of new sulfonamide derivatives of thiadiazolo [3,2-*a*]pyrimidines

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ABSTRACT

New series of sulfonamide derivatives of [1,3,4]thiadiazolo[3,2-*a*]pyrimidine were synthesized and investigated as antitumor agents. Some of the newly prepared compounds were tested for their *in vitro* and *in vivo* antitumor activities. Preliminary biological studies revealed that compounds **4c**, **4f**, and **4j** exhibited the highest affinity to DNA, while compounds **4h**,**i**, **6a**–**c**, **8** and **12**–**14** exhibited moderate activity. Also, compounds **4j**, **4f** and **4c** showed the highest percentage increase in lifespan of mice inoculated with Ehrlich ascites cells over 5-flurouracil (positive control). The detailed synthesis, spectroscopic and biological data are reported.

1. Introduction

Sulfonamides constitute an important class of drugs. They possess various types of pharmacological activities such as antibacterial [1–3], anti-carbonic anhydrase [4,5], high-ceiling diuretic [6], hypoglycemic [7,8], antithyroid [7], anti-inflammatory [9], and antiglaucoma [6,7]. It is also known that aryl/heteroaryl sulfonamides may act as antitumor agents through perturbation of cell cycle in the G1 phase, distribution of microtubule assembly or angiogenesis inhibition [10–13]. Moreover, numerous sulfonamides were found to act as antitumor agents through carbonic anhydrase (CA) inhibition [13–16]. It has been shown that two carbonic anhydrase isozymes (CA IX and CA XII) are prominently associated with, and over expressed, in many tumors [17,18]. They are involved in crucial processes connected with cancer progression and response to therapy [19–21]. Aromatic or heteroaromatic sulfonamides have been shown to reverse the effect of tumor acidification, consequently they inhibit the growth of cancer cells and suppress tumor invasion mediated by the carbonic anhydrases [22–25]. These compounds contain the classical recognition fragment ($Ar-SO_2NH_2$) that coordinates the carbonic anhydrase active site (Zn^{2+}) and so inhibits the catalytic ability of this enzyme [14,16]. On the other hand, pyrimidine derivatives and heterocyclic annelated pyrimidines have attracted a great deal of interest owing to their medicinal activities [26–28]. These medicinal activities include anticancer [28], antiviral [29], antitumor [30], anti-inflammatory [31] and others. All these findings encouraged us to explore the synthesis of sulfonamides containing thiadiazolo[3,2-*a*] pyrimidine moieties and examine their activities as antitumor agents. Herein we report their synthesis, preliminary DNA-binding assay and antineoplastic evaluation against Ehrlich ascites carcinoma (EAC) in mice.

2. Results and discussion

2.1. Chemistry

A general approach to synthesize the designed compounds is outlined in Schemes 1–5. 2-Amino-1,3,4-thiadiazole-5-sulfonamide (**2**), the key intermediate necessary for this study, was synthesized from acetazolamide **1** *via* hydrochloric acid hydrolysis followed by aqueous sodium carbonate neutralization [32]. Compound **2** was reacted with the appropriate 1,3-di (substituted phenyl)-2-propen-1-one **3a**–**l** (chalcone analogues) [33–38] in refluxing propylene glycol to afford the corresponding 5,7-di (substituted phenyl)-5*H*-[1,3,4]thiadiazolo[3,2-*a*]pyrimidine-2-sulfonamides (**4a**–**l**) (Scheme 1).

The reaction of the intermediate **2** with the appropriate (4-substituted phenyldiazenyl)butyrate **5a**–**f** [39–41] in refluxing glacial acetic acid afforded the 6-(4-substituted phenyldiazenyl-

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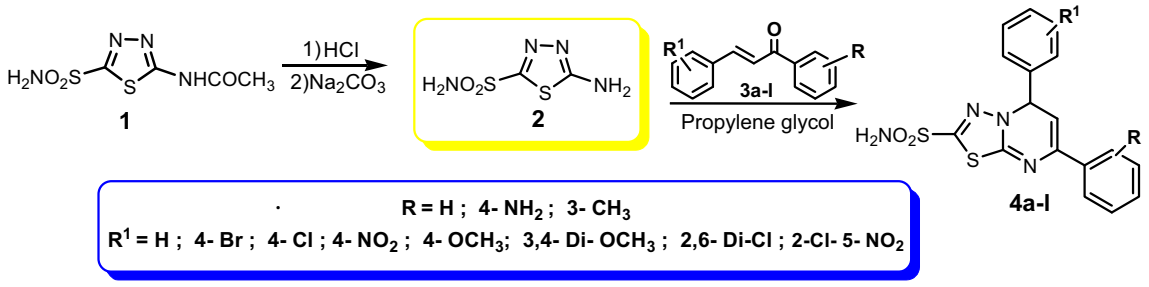


Scheme 1.

[1,3,4]thiadiazolo[3,2-*a*]pyrimidine-2-sulfonamides (**6a**–**f**)) (Scheme 2).

New derivatives of 5-oxo-7-substituted-5*H*-[1,3,4]thiadiazolo [3,2-*a*]pyrimidine-2-sulfonamide were prepared through the reaction of compound **2** with different dicarbonyl compounds as in Schemes 3 and 4. 6,7-Dihydro-5,7-dioxo-5*H*-[1,3,4]thiadiazolo[3,2-*a*]pyrimidine-2-sulfonamide (**7**) was obtained through the reaction of compound **2** with diethyl malonate in refluxing glacial acetic acid. Compound **7** upon heating with phosphorus oxychloride in the presence of *N,N*-dimethylaniline furnished the corresponding 7-chloro derivative **8**. Similarly, reaction of compound **2** with ethyl acetoacetate in refluxing glacial acetic acid furnished the 5-oxo derivative **9** in 60% yield (Scheme 3).

The key intermediate **2** was treated with dimethyl acetylene dicarboxylate in methanol to provide compound **10**. The structure of this compound was established on the basis of its elemental analysis and spectral data. For example, the ^{1}H-NMR spectrum showed a signal of the methyl ester at 3.95 ppm. Compound **10** was subsequently reacted with phenylhydrazine in refluxing absolute ethanol to give the corresponding 5-oxo-2-sulfamoyl-*N*′-phenyl-5*H*-[1,3,4]thiadiazolo[3,2-*a*]pyrimidine-7-carbohydrazide (**11**). On the other hand, hydrolysis of the ester **10** using 12% sodium hydroxide followed by acidification with hydrochloric acid produced compound **12** in 45% yield (Scheme 4).

In addition, reaction of compound **2** with acetylacetone using formic acid-phosphorous pentoxide as a condensation reagent, followed by addition of perchloric acid to provide the cyclic product **13** as perchlorate salt. Finally, treatment of compound **2** with ethyl cyanoacetate in methanolic solution of sodium methoxide produced 6,7-dihydro-5-imino-7-oxo-5*H*-[1,3,4]thiadiazolo[3,2-*a*] pyrimidine-2-sulfonamide mono sodium salt (**14**) which on acidification provided the corresponding derivative **15** (Scheme 5).

2.2. Biology

DNA as an affinity probe useful in evaluating the biologically active compounds: A variety of methods have been utilized for determining the interaction of small molecular weight compounds with DNA [42] such as DNA-binding assay and DNA/methyl green displacement assay.

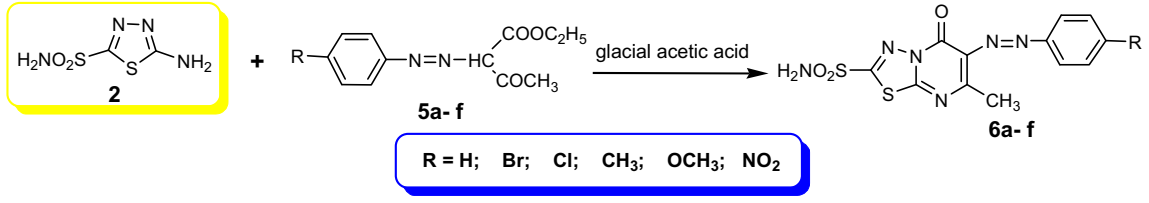
2.2.1. DNA-binding assay

In this method a fixed amount of the ligand is spotted on the RP-18 TLC plates followed by the addition of DNA on the same spot at the origin. The plates were then developed and the position of unbound DNA was determined by spraying the plates with anisaldehyde reagent [43]. The free DNA was detected as a blue spot (R_f, MeOH–H_2O, 8:2) on RP-18 TLC. It was demonstrated that, when DNA was mixed with compound known to interact with it, e.g. ethidium bromide, the complex was retained at the origin. Compounds with high binding affinity to DNA remained on the base line or migrated for a very short distance, while compounds with poor binding affinity did not cause DNA to be retained at the origin.

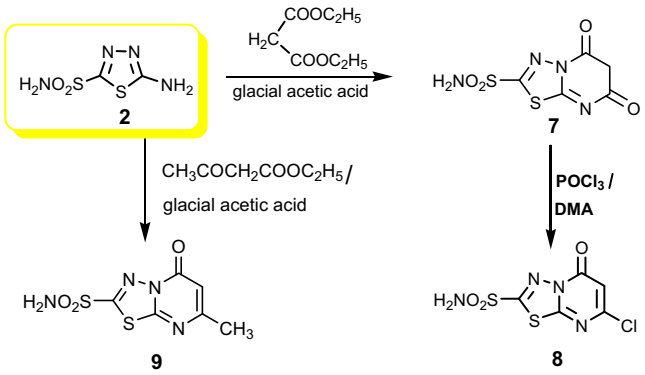
2.2.1.1. Results from DNA-binding assay. Compounds **4c**, **4f** and **4j** showed the highest binding affinity to DNA which was demonstrated by retaining the complex at the origin or by migrating for a very short distances. Compounds **4h**, **4i**, **6a**–**c**, **8**, **9**, **12**, and **14** showed moderate activities. While compounds **4d**, **4k**, **6d**, **6e** and **6f** showed weak activity, compound **6f** was the weakest in this study (Table 1). The results indicated that the diphenyl groups at positions –5 and –7 as in compounds **4c**, **4f**, and **4j** may contribute in the activity. Meanwhile, the presence of nitro group in the phenyl ring greatly decreases the binding affinity as in compounds **4d** and **4h**. In addition, the presence of chlorine atom in the phenyl ring either at 2- or 4-position increases the activity as in compounds **4c**, **4h** and **4j**. The presence of the dimethoxy groups in the phenyl ring as in compound **4f** produces the same activity as the chlorine atom.

2.2.2. Colorimetric assay for compounds that bind to DNA

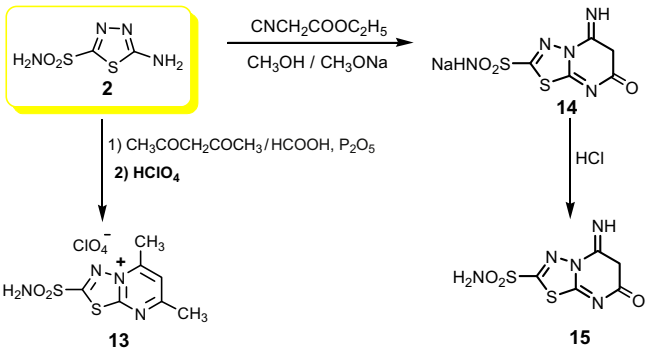
Methyl green reversibly binds polymerized DNA forming a stable complex at neutral pH, whereas free methyl green fades. The absorption maximum for the DNA/methyl green complex is 642.5–645 nm. This colorimetric assay [44] was used to measure the displacement of methyl green from DNA by compounds having ability to bind with DNA. The degree of displacement was determined spectrophotometrically by measuring the change in initial absorbance of DNA/methyl green solution in the presence of reference compound (ethidium bromide). The activity of compounds that showed high affinity for DNA have been determined and expressed



Scheme 2.



Scheme 3.



Scheme 5.

as IC_{50} (Concentrations required for a 50% decrease in the initial absorbance of the DNA/methyl green solution) as shown in (Table 2). These results indicate that compound **4c** possessed the highest binding affinity to DNA.

2.2.3. Antineoplastic activity against Ehrlich ascites carcinoma (EAC) in mice

The prolongation of lifespan of EAC bearing hosts, the reduction in viable tumor cell count and the recovery of normal hematological and biochemical profiles are three important measures that have been used in this *in vivo* testing for the evaluation of the antineoplastic activity for the compounds that showed the highest binding affinity to DNA. The results were comparable to that of the result obtained from the animals treated with the standard drug 5-flurouracil (20 mg/kg bw) [45,46].

2.2.3.1. Results. **Effect on survival time** [47]: The mean survival time (MST) of each group, consisting of 10 mice was noted. The antitumor efficacy of the test compounds **4c**, **4f** and **4j** was compared with 5-fluorouracil (as a positive control) (5-fluorouracil, 20 mg/kg/day, i.p. for 9 days) as shown in (Table 3). The MST of the treated groups was compared with that of the control group using the following calculations: % Increase in lifespan over control = (MST of treated group/MST of control group − 1) × 100, where MST = survival time (days of each mouse in a group)/Total no. of mice. Compound **4j** showed the highest % increase in lifespan of mice inoculated with EAC.

Effect on the hematological and biochemical parameters: Compound **4j** showed more or less normal hemoglobin (HB), RBCs, WBCs and ALT levels were improved as shown in (Table 4).

2.2.3.2. Determination of viable cell count of Ehrlich ascites cells after five days of treatment. Both compounds **4j** and **4f** which showed significant reduction of viable tumor cells but mice received compound **4j** showed the best results as shown in (Table 5).

2.2.3.3. Discussion. The results of the present study showed an antitumor activity of compounds **4j**, **4f**, and **4c** against EAC in Swiss albino mice. In the 5th day after inoculation of EAC in mice, increase in body weight and ascites was observed clearly, also the mice became slow and inactive. Mice received **4j** and 5-fluorouracil were more protected against ascites and showed slight increase in body weight unlike the control group. Mice received **4c** showed slight toxic symptoms such as dizziness, erection of tail, and became slow.

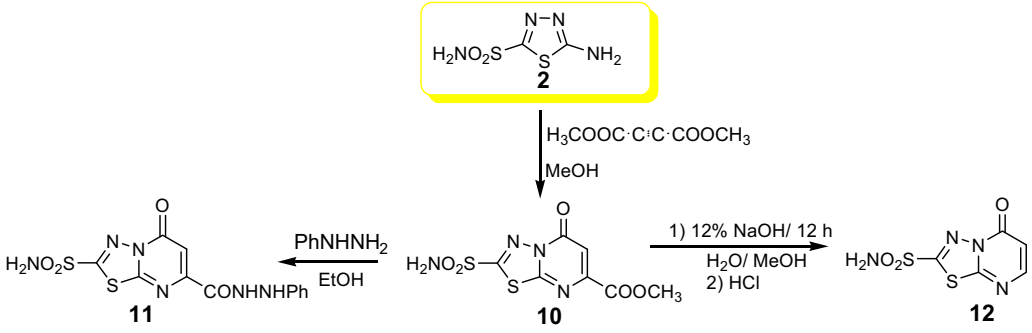
3. Experimental

3.1. Chemistry

All melting points (°C) were determined on fisher john melting point apparatus and are uncorrected. Infrared spectra were recorded in KBr disc using a Unicam SP 1000 infrared spectrophotometer and expressed in wave number (cm^{-1}). ^{1}H-NMR spectra were obtained on FT-NMR spectrometer at 200 MHz; the chemical shifts are expressed in δ (ppm) units using tetramethylsilan (TMS) as internal reference and DMSO-d_6 or $CDCl_3$ as solvent. Mass spectra were recorded on JEOL JMS-600H spectrometer using electron impact technique at 70 eV. Microanalyses (C, H, N) were in agreement with the proposed structures within ±0.4% of the theoretical values.

3.1.1. Chemical synthesis

*3.1.1.1. 2-Amino-1,3,4-thiadiazole-5-sulfonamide (**2**).* 2-Acetamido-1,3,4-thiadiazole-5-sulfonamide (**1**) (8.5 g, 0.0383 mol) was heated under reflux with concentrated hydrochloric acid (50 ml) for 7 h. The reaction mixture was concentrated and neutralized with



Scheme 4.

Table 1
DNA-binding affinity of the selected compounds.

Comp. No	DNA-binding affinity
4c	High
4d	Weak
4f	High
4h	Moderate
4j	High
4k	Weak
4l	Moderate
6a	Moderate
6b	Moderate
6c	Moderate
6d	Weak
6e	Weak
6f	Weak
8	Moderate
9	Moderate
11	Weak
12	Moderate
14	Moderate

aqueous sodium carbonate solution. The separated solid was collected by filtration, dried and recrystallized from ethanol to yield 5.38 g (78%) of compound **2**, m.p. 218–220 °C (reported m.p. for the hydrochloride salt = 199–200 °C [32]. IR; 3305–3038 (2-NH_2). MS *m*/*z* (%); 181 (0.10, M^+ +1); 180 (0.70, M^+); 117 (100.00).

*3.1.1.2. 5,7-Di (substituted phenyl)-5H-[1,3,4]thiadiazolo[3,2-a]pyrimidine-2-sulfonamides (**4a**–**l**).* A mixture of 2-amino-1,3,4-thiadiazole-5-sulfonamide (**2**) (0.45 g, 0.0025 mol) and the appropriate chalcone analogue **3a**–**l** [33–38] (0.0025 mol) in propylene glycol (10 ml) was heated for 3–5 h at 200–220 °C. The mixture was cooled and diluted with water (50 ml) with vigorous stirring. The separated solid was collected by filtration, washed with water, dried and recrystallized from ethanol to afford compounds **4a**,**h** or recrystallized from aqueous ethanol to produce compounds **4b**–**g**, **i**–**l**.

*3.1.1.2.1. 5,7-Diphenyl-5H-[1,3,4]thiadiazolo [3,2-a]pyrimidine-2-sulfonamide (**4a**).* Yield 0.20 g (55%); mp. 60–62 °C; MS *m*/*z* (%): 369 (0.94, M^+ −1), 368 (1.82, M^+ −2), 181 (100). Anal. for $C_{17}H_{14}N_4O_2S_2$ (370.45) C, H, N.

*3.1.1.2.2. 5-(4-Bromophenyl)-7-phenyl-5H-[1,3,4]thiadiazolo [3,2-a]pyrimidine-2-sulfonamide (**4b**).* Yield 0.27 g (60%); mp. 113–115 °C; MS *m*/*z* (%): 451 (17.30, M^+ +2), 450 (9.10, M^+ +1), 449 (34.20, M^+), 105 (100). Anal. for $C_{17}H_{13}BrN_4O_2S_2$ (449.34) C, H, N.

*3.1.1.2.3. 5-(4-Chlorophenyl)-7-phenyl-5H-[1,3,4]thiadiazolo [3,2-a]pyrimidine-2-sulfonamide (**4c**).* Yield 0.27 g (67%); mp. 88–89 °C; ^{1}H-NMR ($CDCl_3$): δ 3.89 (s, 1H, C_7-H), 6.60 (s, 1H, C_6-H); 7.21–8.07 (m, 11H, 9-Ar–H, NH_2, D_2O exchange). Anal. for $C_{17}H_{13}ClN_4O_2S_2$ (404.89) C, H, N.

*3.1.1.2.4. 5-(4-Nitrophenyl)-7-phenyl-5H-[1,3,4]thiadiazolo [3,2-a]pyrimidine-2-sulfonamide (**4d**).* Yield 0.29 g (70%); mp. 100–102 °C; ^{1}H-NMR (DMSO-d_6): δ 3.80 (s, 1H, C_5-H), 5.98 (s, 1H, C_6-H), 7.57–8.58 (m, 11H, 9-Ar–H, NH_2, D_2O-exchange). Anal. for $C_{17}H_{13}N_5O_4S_2$ (415.45) C, H, N.

*3.1.1.2.5. 5-(4-Methoxyphenyl)-7-phenyl-5H-[1,3,4]thiadiazolo [3,2-a]pyrimidine-2-sulfonamide (**4e**).* Yield 0.29 g (73%); mp. 75–77 °C; ^{1}H-NMR ($CDCl_3$): δ 3.70 (s, 1H, C_5-H), 3.95 (s, 3H, OCH_3), 6.47 (s, 1H, C_6-H), 7.26–8.17 (m, 9H, 9-Ar–H), 8.61 (s, 2H, NH_2, D_2O-exchange). Anal. for $C_{18}H_{16}N_4O_3S_2$ (400.47) C, H, N.

*3.1.1.2.6. 5-(3,4-Dimethoxyphenyl)-7-phenyl-5H-[1,3,4]thiadiazolo [3,2-a]pyrimidine-2-sulfonamide (**4f**).* Yield 0.30 g (70%); mp. 105–107 °C; ^{1}H-NMR ($CDCl_3$): δ 3.82 (s, 1H, C_5-H), 3.98 (s, 6H, 2–OCH_3), 6.60–7.83 (m, 11H, C_6-H, 8-Ar–H, NH_2, D_2O-exchange). Anal. for $C_{19}H_{18}N_4O_4S_2$ (430.50) C, H, N.

*3.1.1.2.7. 5-(2,6-Dichlorophenyl)-7-phenyl-5H-[1,3,4]thiadiazolo [3,2-a]pyrimidine-2-sulfonamide (**4g**).* Yield 0.33 g (75%); mp. 80–82 °C; IR: 3341 (NH_2) cm^{-1}. ^{1}H-NMR (DMSO-d_6): δ 4.03 (s, 1H, C_5–H), 5.94 (s, 1H, C_6-H), 7.09–8.07 (m, 10H, 8-Ar–H, NH_2, D_2O-exchange). Anal. for $C_{17}H_{12}Cl_2N_4O_2S_2$ (439.34) C, H, N.

*3.1.1.2.8. 5-(2-Chloro-5-nitrophenyl)-7-phenyl-5H-[1,3,4]thiadiazolo [3,2-a]pyrimidine-2-sulfonamide (**4h**).* Yield 0.36 g (80%); mp. 65–67 °C; ^{1}H-NMR ($CDCl_3$): δ 3.70 (s, 1H, C_7-H), 6.16 (s, 1H, C_6-H), 7.25–8.20 (m, 8H, 8-Ar–H), 8.62 (s, 2H, NH_2, D_2O-exchange). Anal. for $C_{17}H_{12}ClN_5O_4S_2$ (449.89) C, H, N.

*3.1.1.2.9. 4-(5-Phenyl-2-sulfamoyl-5H-[1,3,4]thiadiazolo [3,2-a]pyrimidin-7-yl)benzenamine (**4i**).* Yield 0.25 g (65%); mp. 65–67 °C; ^{1}H-NMR (DMSO-d_6): δ 3.62 (s, 1H, C_5-H), 4.64 (s, 2H, Ar–NH_2, D_2O-exchange), 6.50 (s, 1H, C_6-H), 7.18–7.90 (m, 11H, 9-Ar–H, SO_2NH_2, D_2O-exchange). Anal. for $C_{17}H_{15}N_5O_2S_2$ (385.46) C, H, N.

*3.1.1.2.10. 4-(5-(4-Chlorophenyl)-2-sulfamoyl-5H-[1,3,4]thiadiazolo [3,2-a]pyrimidin-7-yl)benzenamine (**4j**).* Yield 0.29 g (70%); mp. 117–120 °C; MS *m*/*z* (%): 420 (5.70, M^+), 419 (10.70, M^+ −1), 418 (14.00, M^+ −2), 101 (100). Anal. for $C_{17}H_{14}ClN_5O_2S_2$ (419.91) C, H, N.

*3.1.1.2.11. 4-(5-(4-Methoxyphenyl)-2-sulfamoyl-5H-[1,3,4]thiadiazolo [3,2-a]pyrimidin-7-yl)benzenamine (**4k**).* Yield 0.26 g (62%); mp. 115–117 °C; ^{1}H-NMR ($CDCl_3$): δ 3.78 (s, 1H, C_5-H), 3.96 (s, 3H, OCH_3), 4.20 (s, 2H, Ar–NH_2, D_2O-exchange), 5.91(s, 1H, C_6-H), 6.70–7.94 (m, 10H, 8-Ar–H, SO_2NH_2, D_2O-exchange). Anal. for $C_{18}H_{17}N_5O_3S_2$ (415.49) C, H, N.

*3.1.1.2.12. 5-Phenyl-7-m-tolyl-5H-[1,3,4]thiadiazolo [3,2-a]pyrimidine-2-sulfonamide (**4l**).* Yield 0.23 g (60%); mp. 86–88 °C; ^{1}H-NMR ($CDCl_3$): δ 2.42 (s, 3H, CH_3), 3.95 (s, 1H, C_5-H), 5.70 (s, 1H, C_6-H),

Table 2
Activity of compounds in the DNA/methyl green displacement assay.[a]

DNA-active compounds	DNA/methyl green IC_{50}, μg/ml
4c	40 ± 1
4f	62 ± 4
4h	80 ± 3
4j	64 ± 2
4l	72 ± 2
6a	77 ± 1
6b,**6c**	74 ± 2
8	79 ± 3
11, **4d**, **4k**, **6d**–**f**	81 ± 4
12	70 ± 1
9,**14**	76 ± 2
Ethidium bromide	1.4 ± 2

[a] Values represent the concentration (mean ± SD, *n* = 3–5 separate determinations) required for a 50 % decrease in the initial absorbance of the DNA/methyl green solution.

Table 3
Effect of test compounds **4c**, **4f**, and **4j** and 5-fluorouracil on the survival time of mice inoculated with EAC.

Group	Normal	Control (Ehrlich only)	**4j**	**4f**	**4c**	5-fluorouracil
% Increase in lifespan over control	71.43	0	71.43	57.14	42.86	42.86

Table 4
Effect of test compound **4j** and 5-fluorouracil on the hematological and biochemical parameters of mice inoculated with EAC.

Parameter	Normal	Control (Ehrlich only)	4j (100 μg/mouse)	5-fluorouracil (20 mg/kg)
Hb/g%	13.4	5.7	11.1	15.6
RBCs/10^6 mm^{-3}	5.1	3.15	5.96	5.87
Total WBCs/10^3 mm^3	4.35	47.4	9.45	14.7
ALT × 0.1/μ L^{-1}	3.7	14.4	6.7	6.9

Table 5
Viable cell count of EAC after 5 days of treatment with compounds **4c**, **4f**, **4j**, and 5-fluorouracil.

Group number	Group name	Count (cells)/100 μL
1	**4j**	17.6×10^6
2	**4f**	18.6×10^6
3	**4c**	99.2×10^6
4	5-fluorouracil	83.6×10^6
5	Ehrlich only	192.8×10^6

7.23–7.94 (m, 11H, 9-Ar–H, NH_2, D_2O-exchange). Anal. for $C_{18}H_{16}N_4O_2S_2$ (384.48) C, H, N.

*3.1.1.3. 7-Methyl-5-oxo-6-(4-substituted phenyldiazenyl)-5H-[1,3,4] thiadiazolo[3,2-a]pyrimidine-2-sulfonamides (**6a–f**).* A mixture of 2-amino-1,3,4-thiadiazole-5-sulfonamide (**2**) (0.45 g, 0.0025 mol) and the appropriate ethyl 3-oxo-2-(4-substituted phenyldiazenyl) butyrate **5a–f** [39–41] (0.0025 mol) in glacial acetic acid (10 ml) was heated under reflux for 12 h and refrigerated overnight. The separated solid was filtered, dried and recrystallized from aqueous ethanol to afford compounds **6a**, **c** or recrystallized from acetic acid/water to yield compounds **6b**, **d–f**.

*3.1.1.3.1. 7-Methyl-5-oxo-6-(phenyldiazenyl)-5H-[1,3,4]thiadiazolo [3,2-a]pyrimidine-2-sulfonamide (**6a**).* Yield 0.16 g (45%); mp. 255–257 °C; IR: 3207 (NH_2), 1656 (C=O) cm^{-1}. ^{1}H-NMR (DMSO-d_6): δ 2.18 (s, 3H, CH_3), 7.35–7.78 (m, 7H, 5-Ar–H, NH_2, D_2O-exchange). Anal. for $C_{12}H_{10}N_6O_3S_2$ (350.38) C, H, N.

*3.1.1.3.2. 7-Methyl-5-oxo-6-(4-bromophenyldiazenyl)-5H-[1,3,4] thiadiazolo [3,2-a]pyrimidine-2-sulfonamide (**6b**).* Yield 0.26 g (60%); mp. 247–249 °C; ^{1}H-NMR (DMSO-d_6): δ 2.22 (s, 3H, CH_3); 7.36–7.79 (m, 6H, 4-Ar–H, NH_2, D_2O-exchang.). Anal. for $C_{12}H_9BrN_6O_3S_2$ (429.27) C, H, N.

*3.1.1.3.3. 7-Methyl-5-oxo-6-(4-chlorophenyldiazenyl)-5H-[1,3,4] thiadiazolo [3,2-a]pyrimidine-2-sulfonamide (**6c**).* Yield 0.25 g (65%); mp. 258–260 °C; ^{1}H-NMR (DMSO-d_6): δ 2.22 (s, 3H, CH_3), 7.40–8.25 (m, 6H, 4-Ar–H, NH_2, D_2O-exchange). Anal. for $C_{12}H_9ClN_6O_3S_2$ (384.82) C, H, N.

*3.1.1.3.4. 7-Methyl-5-oxo-6-(4-methylphenyldiazenyl)-5H-[1,3,4] thiadiazolo [3,2-a]pyrimidine-2-sulfonamide (**6d**).* Yield 0.24 g (67%); mp. 250–252 °C; ^{1}H-NMR (DMSO-d_6): δ 2.08 (s, 3H, CH_3), 2.21(s, 3H, CH_3), 7.30–7.45 (m, 4H, Ar–H), 7.63 (s, 2H, NH_2, D_2O-exchange). Anal. for $C_{13}H_{12}N_6O_3S_2$ (364.40) C, H, N.

*3.1.1.3.5. 7-Methyl-5-oxo-6-(4-methoxyphenyldiazenyl)-5H-[1,3,4]thiadiazolo [3,2-a]pyrimidine-2-sulfonamide (**6e**).* Yield 0.27 g (72%); mp. 250–252 °C; ^{1}H-NMR (DMSO-d_6): δ 2.21 (s, 3H, CH_3), 3.92 (s, 3H, OCH_3), 7.06–7.21 (m, 4H, Ar–H), 7.59 (s, 2H, NH_2, D_2O-exchange). Anal. for $C_{13}H_{12}N_6O_4S_2$ (380.40) C, H, N.

*3.1.1.3.6. 7-Methyl-5-oxo-6-(4-nitrophenyldiazenyl)-5H-[1,3,4] thiadiazolo [3,2-a]pyrimidine-2-sulfonamide (**6f**).* Yield 0.28 g (70%); mp. 262–265 °C; MS *m/z* (%): 393 (2.66, $M^+ -2$), 368 (22.50), 180 (100). Anal. for $C_{12}H_9N_7O_5S_2$ (395.37) C, H, N.

*3.1.1.4. 6,7-Dihydro-5,7-dioxo-5H-[1,3,4]thiadiazolo [3,2-a]pyrimidine-2-sulfonamide (**7**).* Diethyl malonate (0.8 g, 0.005 mol) was added to a stirred solution of 2-amino-1,3,4-thiadiazole-5-sulfonamide (**2**) (0.9 g, 0.005 mol) in glacial acetic acid (10 ml). The mixture was heated under reflux for 12 h and refrigerated overnight. The separated solid was filtered, dried and recrystallized from acetic acid/water. Yield 0.81 g (65%); mp. 253–255 °C; IR: 3422 (NH_2), 1693 (C=O), 1654 (C=O) cm^{-1}. ^{1}H-NMR (DMSO-d_6): δ 3.06 (s, 2H, CH_2), 8.22 (s, 2H, NH_2, D_2O-exchange). MS *m/z* (%): 248 (1.60, M^+), 247 (3.30, $M^+ -1$), 117(100). Anal. for $C_5H_4N_4O_4S_2$ (248.24) C, H, N.

*3.1.1.5. 7-Chloro-5-oxo-5H-[1,3,4]thiadiazolo [3,2-a]pyrimidine-2-sulfonamide (**8**).* A mixture of 6,7-dihydro-5,7-dioxo-5*H*-[1,3,4] thiadiazolo[3,2-*a*]pyrimidine-2-sulfonamide (**7**) (1.24 g, 0.005 mol), phosphorus oxychloride (5 ml) and *N,N*-dimethylaniline (0.5 ml) was heated under reflux for 5 h. After cooling, the mixture was poured gradually on crushed ice and extracted with chloroform (3 × 10 ml). The combined organic extract was evaporated to dryness and residue obtained was recrystallized from ethanol/water. Yield 0.6 g (45%); mp. 253–255 °C; IR: 3303 (NH_2), 1680 (C=O) cm^{-1}. ^{1}H-NMR (DMSO-d_6): δ 6.73 (s, 1H, C_6-H), 7.79 (s, 2H, NH_2, D_2O-exchange). MS *m/z* (%): 268 (0.10, $M^+ +2$), 267 (0.70, $M^+ +1$), 135 (100). Anal. for $C_5H_3ClN_4O_3S_2$ (266.69) C, H, N.

*3.1.1.6. 7-Methyl-5-oxo-5H-[1,3,4]thiadiazolo[3,2-a]pyrimidine-2-sulfonamide (**9**).* Ethyl acetoacetate (0.65 g, 0.005 mol) was added dropwise to a stirred solution of 2-amino-1,3,4-thiadiazole-5-sulfonamide (**2**) (0.9 g, 0.005 mol) in glacial acetic acid (10 ml). The reaction mixture was heated under reflux for 12 h and refrigerated overnight. The separated solid was collected by filtration, dried and recrystallized from ethanol/water to give the desired compound **9**. Yield 0.74 g (60%); mp. 253–255 °C; IR: 3300 (NH_2), 1679 (C=O). ^{1}H-NMR (DMSO-d_6): δ 2.26 (s, 3H, CH_3), 7.43 (s, 1H, C_6-H), 8.36 (s, 2H, NH_2, D_2O-exchange). Anal. for $C_6H_6N_4O_3S_2$ (246.27) C, H, N.

*3.1.1.7. Methyl 5-Oxo-2-sulfamoyl-5H-[1,3,4]thiadiazolo[3,2-a]pyrimidine-7-carboxylate (**10**).* Dimethyl acetylene dicarboxylate (DMAD) (0.71 g, 0.005 mol) was added dropwise to a stirred solution of 2-amino-1,3,4-thiadiazole-5-sulfonamide (**2**) (0.9 g, 0.005 mol) in methanol (15 ml). The reaction mixture was heated under reflux for 12 h. After cooling, the separated solid was collected by filtration, dried and recrystallized from ethanol/water to afford the desired compound **10**. Yield 1.09 g (75%); mp. 188–190 °C; IR: 3265 (NH_2): 1750 ($COOCH_3$): 1628 (C=O). ^{1}H-NMR (DMSO-d_6): δ 3.95 (s, 3H, $COOCH_3$), 6.71 (s, 1H, C_6-H), 8.58 (s, 2H, NH_2, D_2O-exchange). Anal. for $C_7H_6N_4O_5S_2$ (290.28) C, H, N.

*3.1.1.8. 5-Oxo-2-sulfamoyl-N′-phenyl-5H-[1,3,4]thiadiazolo[3,2-a] pyrimidine-7-carbohydrazide (**11**).* Phenylhydrazine (0.39 g, 0.0036 mmol) was added to a stirred solution of methyl 5-oxo-2-sulfamoyl-5*H*-[1,3,4]thiadiazolo[3,2-*a*]pyrimidine-7-carboxylate (**10**) (0.87 g, 0.003 mol) in absolute ethanol (10 ml). The reaction mixture was heated under reflux for 8 h. After cooling, the separated solid was collected by filtration, dried and recrystallized from ethanol to afford the desired compound **11**. Yield 0.64 g (58%); mp. 262–264 °C; IR: 3409 (2–NH), 3303 (NH_2), 1698 (C=O), 1641 (C=O).^{1}H-NMR (DMSO-d_6): δ 6.82–7.48 (m, 6H, C_6-H, 5-Ar–H), 8.56 (s, 2H, NH_2, D_2O-exchange), 11.56 (s, 1H, CONHNH, D_2O-exchange), 11.95 (s, 1H, CONHNH, D_2O-exchange). Anal. for $C_{12}H_{10}N_6O_4S_2$ (366.38) C, H, N.

*3.1.1.9. 5-Oxo-5H-[1,3,4]thiadiazolo[3,2-a]pyrimidine-2-sulfonamide (**12**).* 5 ml (12%) Sodium hydroxide solution was gradually added to a solution of methyl 5-oxo-2-sulfamoyl-5*H*-[1,3,4]thiadiazolo[3,2-*a*]pyrimidine-7-carboxylate (**10**) (0.87 g, 0.003 mol) in methanol (15 ml). The reaction mixture was heated under reflux for 12 h. The solvent was evaporated; the obtained residue was triturated with water (5 ml) and acidified with hydrochloric acid. The separated solid was collected by filtration, washed with water, dried and recrystallized from methanol to yield 0.31 g (45%) of compound **12**; mp. >300 °C. IR: 3344 (NH_2), 1631 (C=O). MS *m/z* (%): 234 (4.30, $M^+ +2$), 233 (3.40, $M^+ +1$), 232 (20.10, M^+), 60 (100). Anal. for $C_5H_4N_4O_3S_2$ (232.24) C, H, N.

*3.1.1.10. 5,7-Dimethyl-[1,3,4]thiadiazolo[3,2-a]pyrimidin-4-ium-2-sulfonamide perchlorate (**13**).* Acetylacetone (0.33 g, 0.0033 mmol), 2-amino-1,3,4-thiadiazole-5-sulfonamide **2** (0.54 g, 0.003 mol) and were added to a solution of phosphorus pentoxide (0.43 g,

0.003 mol) in formic acid (10 ml). The reaction mixture was heated under reflux for 10 h and the solvent was evaporated. The obtained residue was triturated with water (5 ml), mixed with 70% perchloric acid in excess. The precipitated product was collected by filtration, dried and recrystallized from methanol to afford the desired compound **13**. Yield 0.55 g (53%); mp. >300 °C; IR: 3293 (NH_2). ^{1}H-NMR (DMSO-d_6): δ 2.75 (s, 3H, CH_3), 2.91(s, 3H, CH_3), 7.97 (s, 1H, C_6-H), 8.32 (s, 2H, NH_2, D_2O-exchange). MS *m*/*z* (%); 344 (47.10, M^+), 342 (100, $M^+ -2$), 330 (66.20), 303 (36.80), 193 (45.60). Anal. for $C_7H_9ClN_4O_6S_2$ (344.75) C, H, N.

*3.1.1.11. 6,7-Dihydro-5-imino-7-oxo-5H-[1,3,4]thiadiazolo[3,2-a] pyrimidine-2-sulfonamide mono sodium salt (**14**).* To a solution of sodium metal (0.23 g, 0.01 mol) in dry methanol (15 ml), 2-amino-1,3,4-thiadiazole-5-sulfonamide (**2**) (0.9 g, 0.005 mol) and ethyl cyanoacetate (0.56 g, 0.005 mol) were added and the resulting mixture was refluxed for 8 h. After cooling, the separated solid was collected by filtration, dried and recrystallized from methanol to afford the desired compound **14**. Yield 0.9 g (67%); mp. >300 °C; IR: 451 (2−NH), 1630 (C=O). MS *m*/*z* (%): 268 (7.40, $M^+ -1$), 267 (6.40, $M^+ -2$), 253 (6.40), 57 (100). Anal. for $C_5H_4N_5NaO_3S_2$ (269.24) C, H, N.

*3.1.1.12. 6,7-Dihydro-5-imino-7-oxo-5H-[1,3,4]thiadiazolo[3,2-a] pyrimidine-2-sulfonamide (**15**).* To a solution of sodium metal (0.23 g, 0.01 mol) in dry methanol (15 ml), 2-amino-[1,3,4-thiadiazole-5-sulfonamide (**2**) (0.9 g, 0.005 mol) and ethyl cyanoacetate (0.56 g, 0.005 mmol) were added and the resulting mixture was heated under reflux for 8 h. The solution was evaporated and the obtained residue was triturated with water (5 ml) and acidified with hydrochloric acid. The separated solid was collected by filtration, washed with water, dried and recrystallized from ethanol/water to give the desired compound **15**. Yield 0.74 g (60%); mp. 178–180 °C; IR: 3401 (NH_2), 3313 (NH), 1679 (C=O). ^{1}H-NMR ($CDCl_3$): δ 3.25 (s, 2H, CH_2), 7.52 (s, 2H, NH_2, D_2O-exchange), 7.76 (s, 1H, NH, D_2O-exchange). Anal. for $C_5H_5N_5O_3S_2$ (247.25) C, H, N.

3.2. Biology

3.2.1. DNA-binding assay

Analysis of the DNA-binding affinity of the tested compounds was performed using RP-18 TLC plates. TLC plates (RP-18 F_{254}; 0.25 mm; Merck) were predeveloped with MeOH–H_2O (8:2). Test compounds (5 mg/ml in MeOH) were then applied at the origin, followed by the addition of DNA (1 mg/ml in H_2O and MeOH mixture) at the same positions at the origin. The plates were then developed with the same solvent system and the position of the DNA was determined by spraying with anisaldehyde reagent [43]. The reagent yields a blue color reaction with DNA, and the intensity of the color was proportional to the quantity of DNA added to the plate. Ethidium bromide was used as a positive control.

3.2.2. Colorimetric assay for compounds that bind to DNA (DNA/Methyl green displacement assay)

DNA/methyl green (20 mg, Sigma, St. Louis, MO, USA) was suspended in 100 ml of 0.05 M Tris-HCl buffer, pH 7.5, containing 7.5 mM $MgSO_4$ and stirred at 37 °C with a magnetic stirrer for 24 h. Unless otherwise indicated, samples to be tested were dissolved in EtOH in eppendorf tubes. Solvent was removed under vacuum and 200 μl of DNA/methyl green solution was added to each tube. The absorption maxima for the DNA/methyl green complex is 642.5–645 nm. Samples were incubated in the dark at ambient temperature. After 24 h, the final absorbance of samples was determined. Readings were corrected for initial absorbance and normalized as a % of the untreated DNA/methyl green absorbance value. Concentrations required for a 50% decrease in the initial absorbance of the DNA/methyl green solution (IC_{50}'s) were determined for each compound as shown in (Table 2).

3.2.3. Antineoplastic activity against Ehrlich ascites carcinoma (EAC) in mice

3.2.3.1. Materials

3.2.3.1.1. Experimental animals. Adult Swiss male albino mice (20–25 g) were procured from pharmacology department, Mansoura University, Egypt and used throughout the study. They were housed in microlon boxes in a controlled environment (temperature 25 + 2 °C and 12 h dark/light cycle) with standard laboratory diet and sterilized water.

3.2.3.1.2. Tumor cells. Ehrlich ascites carcinoma (EAC) was obtained from National Cancer Institute, Cairo, Egypt. After harvesting and preparation of the cells, their total number and viability were determined by counting using trypan blue [47]. The desired concentration of tumor cells (2×10^6 cells per 0.2 ml) was obtained by dilution with saline (0.9% sodium chloride solution). Viability of tumor cells obtained and used in this experiment was always higher than 90.0%. Below this percentage the cells were discarded and the entire procedure was repeated.

3.2.3.2. Procedure. Male Swiss albino mice were divided into 6 groups ($n = 10$).

Group I – Normal control.
Group II – Disease control, EAC cell line only.
Group III – EAC cell line treated with **4c**.
Group IV – EAC cell line treated with **4f**.
Group V – EAC cell line treated with **4j**.
Group VI – EAC cell line treated with standard [5-fluorouracil (20 mg/kg)]

All the groups were inoculated with 2×10^6 cells/mouse intraperitoneally except normal group; this was taken as day zero. The treatment started 24 h after inoculation (100 μg/mouse). The control group was treated with the same volume of 0.9% sodium chloride solution. 5-Fluorouracil (20 mg/kg) was used as a standard drug. All the treatments were given for nine days.

3.2.3.3. Determination of viable cell count of Ehrlich ascites cells after five days of treatment. From each group take 100 μL sample of Ehrlich ascites cells (from three mice) and make 20 fold dilution in saline. The cells were stained by giemsa stain and measure the number of viable cells under microscope.

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