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ORIGINAL PAPER

Novel pyrrolidinone and pyrazolo[1,5-*a*][1,3,5]triazine derivatives bearing a biologically active sulfamoyl moiety as a new class of antitumor agents

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Abstract Sulfonamides possess many types of biological activities and have recently been reported to show substantial antitumor activity in vitro and/or in vivo. There are a variety of mechanisms for the anticancer activity. The present work reports the synthesis of some novel pyrrole, oxopyrrole, and related pyrroloacetamide derivatives, hydrazones, aminopyrazolinone, thiocarbamoyl, and pyrazolo[1,5-*a*][1,3,5]triazine derivatives bearing a substituted sulfonamide moiety. All newly synthesized compounds were evaluated for their in vitro anticancer activity against the breast cancer cell line MCF7. Most of the screened compounds showed interesting cytotoxic activities compared with doxorubicin as a reference drug.

Keywords Pyrrolidinone · Aminopyrazolinone · Pyrazolo[1,5-*a*][1,3,5]triazine · Antitumor activity

Introduction

Cancer is becoming the biggest health hazard for the world. The development of resistance against existing anticancer drugs highlights the importance of finding new anticancer molecules. However, it is rather difficult to search for a molecule that can selectively inhibit the proliferation of abnormal cells with only minimal or no effect on normal cells. Therefore, the search for anticancer agents continues in various laboratories worldwide.

Different authors have reported several sulfonamides possessing many types of biological activities and representatives of this class of pharmacological agents are widely used in the clinic as antibacterial [1], hypoglycemic [2], diuretic, anticarbonic anhydrase [3–5], and antithyroid [6] drugs. Recently, a host of structurally novel sulfonamide derivatives were reported to show substantial antitumor activity in vitro and/or in vivo [7–11]. The pyrrole nucleus and pyrrolo[2,3-*d*]pyrimidine play a vital role in many biological activities [12], and aminopyrazoles possess a wide variety of biological activities [13–16]. Also, 3-aminopyrazole was used as a starting material for the synthesis of some novel purine analogues such as pyrazolo[1,5-*a*]pyrimidine. Substituted pyrazolo[1,5-*a*]-pyrimidines were prepared as anticancer, antipyretic, hypotensive, and anxiolytic agents [17]. In addition, pyrazolotriazines are applied as herbicides [18]. Having the above facts in mind and in continuation of my effort to synthesize heterocyclic compounds containing a sulfonamide moiety [7, 8, 19], I was interested in the synthesis of novel 3-aminopyrazole and pyrazolo[1,5-*a*][1,3,5]triazine derivatives containing a sulfonamide moiety to investigate their anticancer activity.

Results and discussion

Chemistry

A series of novel pyrrole, oxopyrrole, and related pyrroloacetamide derivatives, hydrazones, aminopyrazolinone, thiocarbamoyl, and pyrazolo[1,5-*a*][1,3,5]triazine derivatives bearing a substituted sulfonamide moiety were synthesized (Schemes 1, 2, 3, 4) and biologically evaluated for their in vitro antitumor activity. The starting material N^4-chloroacetylsulfaphenazole (**2**) was synthesized from 4-amino-*N*-(1-phenyl-1*H*-pyrazol-5-yl)benzenesulfonamide

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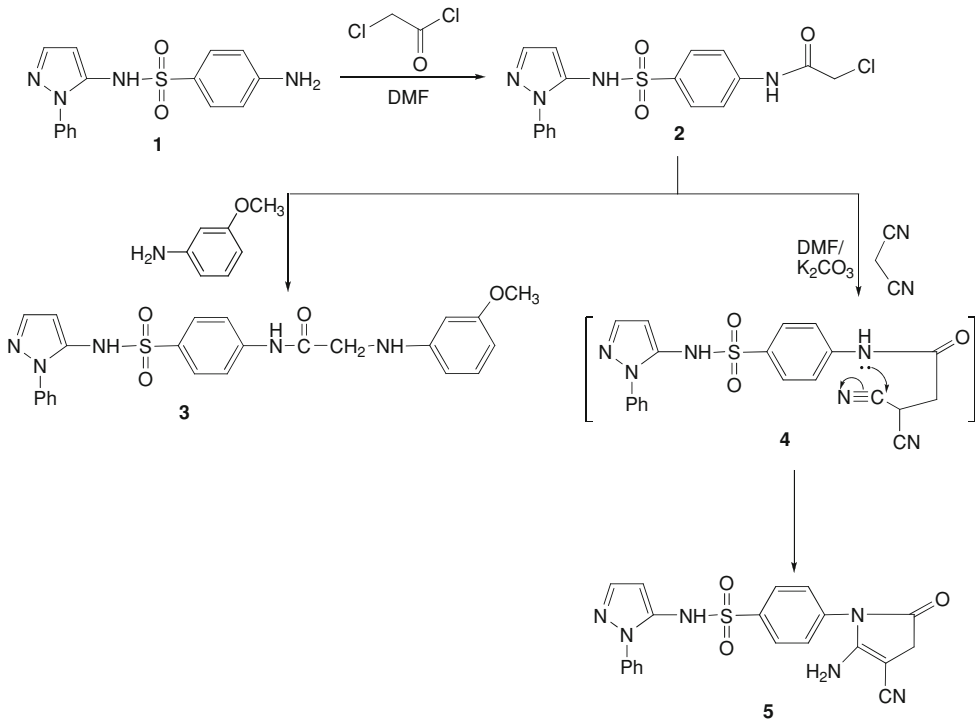
Scheme 1

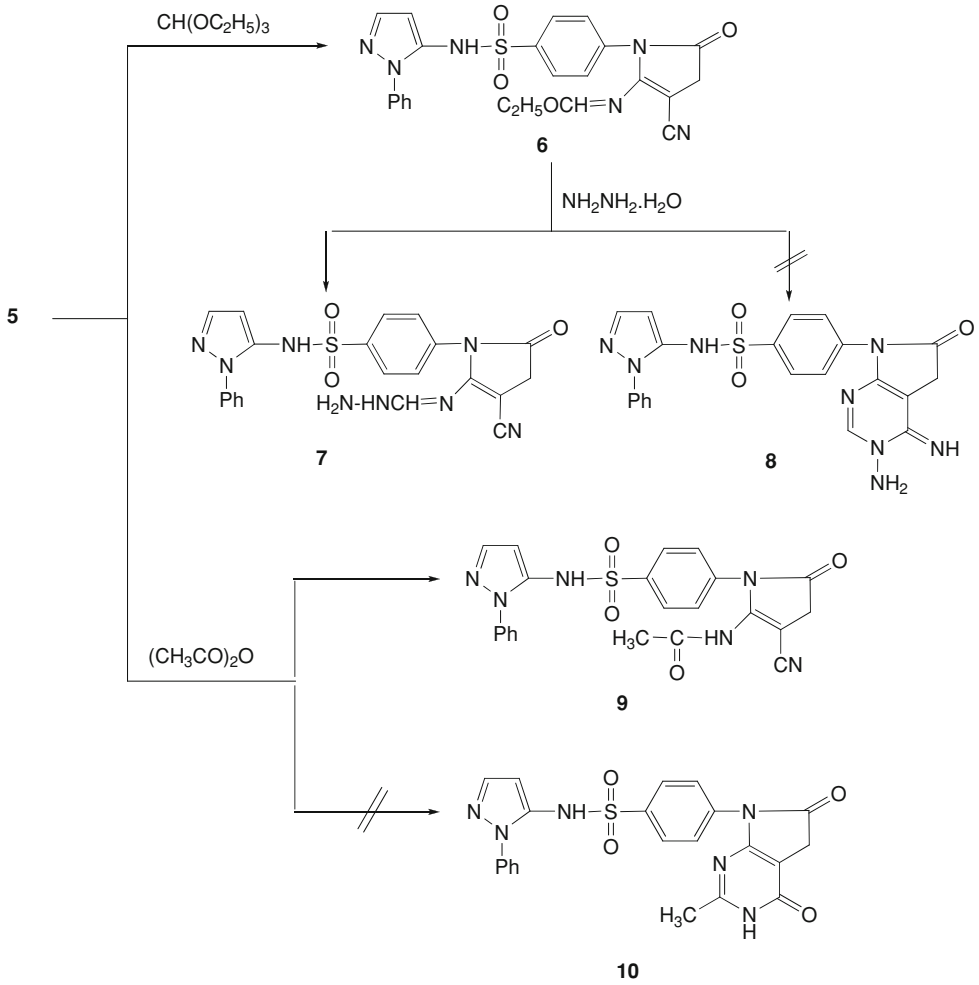


(sulfaphenazole, **1**) by acetylation with chloroacetyl chloride [19]. The reactivity of N^4-chloroacetylsulfaphenazole (**2**) towards some nitrogen nucleophiles and active methylene compounds was investigated. Thus, the nucleophilic substitution reaction of **2** with *m*-anisidine in the presence of anhydrous potassium carbonate in absolute ethanol under reflux afforded the acetamide derivative **3**. The structure of this compound was deduced from elemental analysis and spectral data. The ^{1}H NMR spectrum of compound **3** revealed singlet signals at $\delta = 3.6$, 5.9, and 6.3 ppm corresponding to methoxy and pyrazole protons and three singlet signals at $\delta = 8.0$, 8.20, and 10.9 ppm due to three NH protons. These three NH groups have three bands in the IR spectrum at 3,350, 3,223, and 3,193 cm^{-1}; an intense band at 1,735 cm^{-1} was due to the carbonyl group. The mass spectrum of **3** revealed a molecular ion peak at $m/z = 477$ [M + 2] and a base peak at $m/z = 281$.

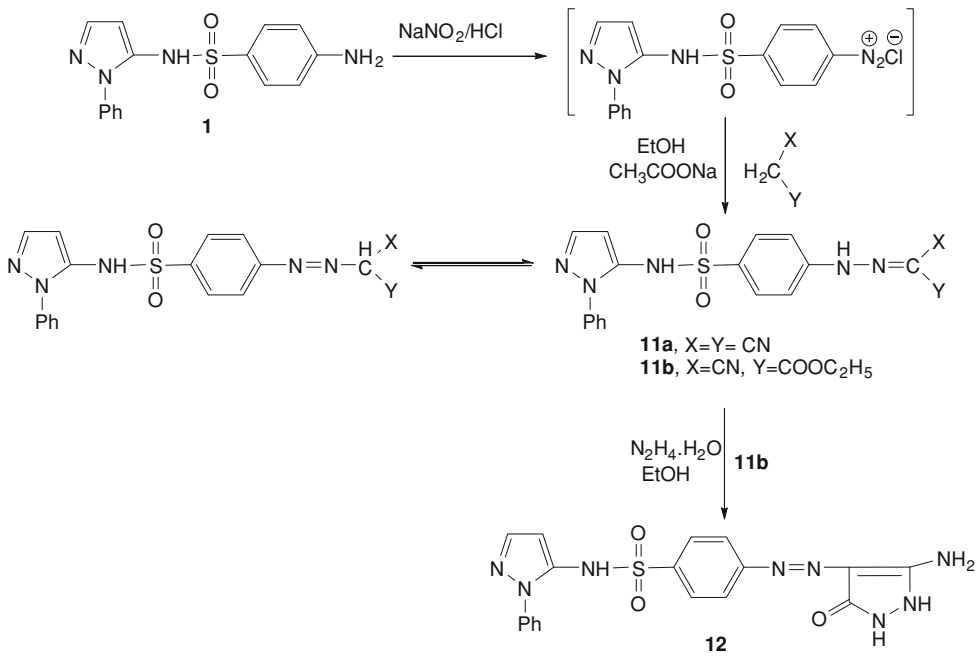
4-(5-Amino-4-cyano-2-oxo-2,3-dihydropyrrol-1-yl)-*N*-(1-phenyl-1*H*-pyrazol-5-yl)benzenesulfonamide (**5**) was prepared according to the previously reported procedure [19] (Scheme 1). The *o*-aminonitrile function of compound **5** was exploited to synthesize some new oxopyrrole and related pyrroloacetamide derivatives containing a sulfonamide moiety. Thus, the alkylation reaction of pyrrolidinone derivative **5** with excess triethyl orthoformate led to the formation of 4-[4-cyano-5-(ethoxymethyleneamino)-2,3-dihydro-2-oxo-1*H*-pyrrol-1-yl]-*N*-(1-phenyl-1*H*-pyrazol-5-yl)benzenesulfonamide (**6**), whose hydrazinolysis with hydrazine hydrate gave 4-[4-cyano-5-(hydrazinylmethyleneamino)-2,3-dihydro-2-oxo-1*H*-pyrrol-1-yl]-*N*-(1-phenyl-1*H*-pyrazol-5-yl)benzenesulfonamide (**7**, Scheme 2).

Attempts to cyclize the hydrazide derivative **7** by refluxing in ethanol containing piperidine and/or pyridine to afford pyrrolo[2,3-*d*]pyrimidine derivative **8** were unsuccessful on the basis of analytical and spectral data. The ^{1}H NMR spectrum of compound **7** (DMSO-d_6) exhibited the following signals: 4.3 (s, 2H, CH_2), 5.8, 6.6 (2 s, 2H, pyrazole-H), 7.0–8.3 (m, 10H, Ar–H + NH), 9.4 (s, H, CH=N), 10.8 (s, 1H, SO_2NH), 11.3 (s, 2H, NH_2). The mass spectrum of compound **7** had a molecular ion peak at $m/z = 464$ [M + 2] and a base peak at $m/z = 77$. Its IR spectrum was characterized by the presence of NH_2 absorption bands. Compound **5** reacted with acetic anhydride to afford the product of direct acetylation (**9**) rather than the expected compound **10**, which was ruled out due to the presence of a C≡N band at $\bar{\nu} = 2{,}200$ cm^{-1} in the IR spectrum of the product in addition to the ^{1}H NMR spectrum showing a signal due to the acetyl group which appeared at $\delta = 2.45$ ppm (Scheme 2). Diazotization of sulfaphenazole (**1**) with sodium nitrite and hydrochloric acid at 5–10 °C followed by coupling with the corresponding nucleophiles such as active methylene compounds (namely, malononitrile and ethyl cyanoacetate) [19] furnished the novel hydrazones **11a** and **11b**, respectively (Scheme 3). The reactions of hydrazones **11a**, **11b** with hydrazines (namely, hydrazine hydrate and thiosemicarbazide) were studied with the aim of forming pyrazole derivatives with potential biological activities [7, 19]. Thus, hydrazinolysis of hyrazo derivative **11b** with

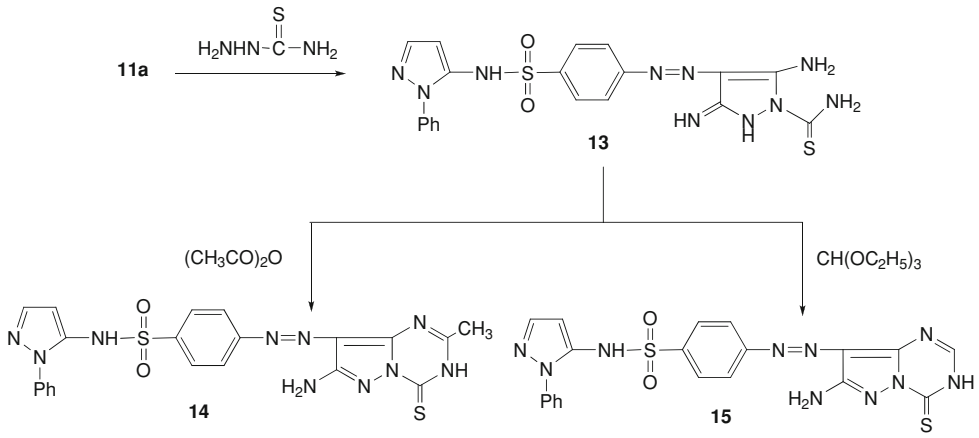
Scheme 2



Scheme 3



Scheme 4



hydrazine hydrate in ethanol under reflux followed by cyclocondensation afforded 3-amino-5-pyrazolinone derivative **12** (Scheme 3). The structure of the latter product was confirmed on the basis of analytical and spectral data. Its IR spectrum showed the following absorption bands: 3,310, 3,305 (NH_2/NH), 1,720 (C=O), and 1,360, 1,187 cm^{-1} (SO_2).

On the other hand, the iminopyrazole derivative **13** was successfully obtained by fusing the hyrazone derivative **11a** with thiosemicarbazide in dioxane containing a catalytic amount of triethylamine (Scheme 4). The ^{1}H NMR spectrum of compound **13** (DMSO-d_6) exhibited the following signals: 5.5, 6.0 (2 s, 2H, pyrazole-H), 6.54, 6.67 (2 s, 4H, $2NH_2$), 7.0–7.7 (m, 10H, Ar–H + NH), 10.5 (s, 1H, SO_2NH) ppm. Its ^{13}C NMR spectrum revealed 15 carbon types with the most important signals at $\delta = 177.00$, characteristic for C=S, and 60.32, due to C-4 of the newly formed pyrazole ring. The IR spectrum of **13** showed the following absorption bands: 3,450, 3,354, 3,209 (NH_2), 3,031 (CH-arom.), 1,330, 1,167 (SO_2), 1,088 (C=S) cm^{-1}. Its mass spectrum displayed a molecular ion peak at $m/z = 482$ [M^+] corresponding to the molecular formula $C_{19}H_{18}N_{10}O_2S_2$ with a base peak at $m/z = 164$. The iminopyrazole derivative **13** proved to be a versatile starting material for the synthesis of some novel pyrazolo[1,5-*a*][1,3,5]triazines. Thus, treatment of thiocarbamoyl derivative **13** with acetic anhydride afforded the pyrazolotriazine **14**. The structure of compound **14** was confirmed on the basis of analytical and spectral data. Its mass spectrum showed a molecular ion peak at $m/z = 506$ [M^+] and a base peak at $m/z = 138$. Finally, cyclocondensation of compound **13** with triethyl orthoformate as carbon donor moiety under reflux yielded another novel pyrazolo[1,5-*a*]-[1,3,5]triazine derivative **15**. The structure of compound **15** was proved by the presence of a signal at $\delta = 8.21$ ppm characteristic for the triazine proton in the ^{1}H NMR spectrum. The IR spectrum showed the disappearance of the characteristic bands for one amino functional group and its mass spectrum showed a molecular ion peak at $m/z = 492$ [M^+] and a base peak at $m/z = 159$.

In vitro antitumor screening test

Doxorubicin, the reference drug used in this study, is one of the most effective antitumor agents used to produce regression in acute leukemias, Hodgkin's disease, and other lymphomas. The cytotoxic effects of the newly synthesized compounds were evaluated in the MCF7 breast cancer cell line and doxorubicin (Adriablastina®) was used as a reference drug in three different concentrations. The primary anticancer assay was performed in accordance with the protocol of the Drug Evaluation Branch, the National Institute of Cancer, Cairo, Egypt [20]. The relationship

Table 1 In vitro cytotoxic activity of some selected synthesized compounds

Compd.	Non-viable cells (%)				IC_{50} (μg cm^{-3})
	Concentration (μg cm^{-3})				
	100	50	25	10	
2	0	0	0	0	>100[a]
3	0	0	0	0	>100[a]
5	24	20	52	78	2.14
6	0	0	0	0	>100[a]
7	21	22	51	81	2.48
9	0	0	0	0	>100[a]
11a	27	31	59	81	3.02
11b	0	0	0	0	>100[a]
12	21	19	43	67	1.88
13	29	38	55	72	2.95
14	0	0	0	0	>100[a]
15	0	0	0	0	>100[a]
Doxorubicin (reference)	40	51	54	58	51.0

[a] $IC_{50} > 100$ μg cm^{-3} is considered to be inactive

between surviving fraction and drug concentration was plotted to obtain the survival curve of the MCF7 breast cancer cell line. The response parameter calculated was the IC_{50} value which corresponds to the compound concentration causing 50% mortality in net cells as depicted in Table 1. The results showed that among the compounds tested in this study, **5**, **7**, **11a**, **12**, and **13**, all of them containing a sulfonamide moiety, exhibit the highest in vitro antitumor activity against MCF7 and are more effective than the positive control (doxorubicin) with IC_{50} values of 2.14, 2.48, 3.02, 1.88, and 2.95 μg cm^{-3}, whereas the other compounds are inactive.

Conclusions

This study reports the synthesis of pyrrolidinone **5**, cyanohydrazinylmethyleneaminone **7**, hydrazone **11a**, 3-amino-5-pyrazolinone **12**, and thiocarbamoyl **13**, which all contain a biologically active sulfonamide moiety. These compounds exhibit the highest in vitro antitumor activity against MCF7 and are more effective than the positive control doxorubicin. On the other hand compounds **2**, **3**, **6**, **9**, **11b**, **14**, and **15** showed no activity.

Experimental

Melting points were determined on a Stuart melting point apparatus. IR spectra were recorded on a Shimadzu-440 IR spectrophotometer using the KBr technique (Shimadzu, Japan). NMR spectra were measured on a Bruker Avance 300 (300 MHz) in DMSO-d_6 as a solvent, using tetramethylsilane (TMS) as an internal standard. The mass spectra were recorded on Shimadzu GCMS-QP-1000EX mass spectrometers at 70 eV. Elemental analyses were carried out by the Microanalytical Research Centre, Cairo University. Analytical results for C, H, N, and S were within ±0.4% of the calculated values. All reagents were of commercial quality and used without purification. Compounds **2**, **5**, **11a**, and **11b** were prepared according to the procedure reported in the literature [19].

2-(3-Methoxyphenylamino)-N-[4-[N-[(1-phenyl-1H-pyrazol-5-yl)amino]sulfonyl]phenyl]acetamide
(**3**, $C_{24}H_{23}N_5O_4S$)
A suspension of 391 mg **2** (1 mmol) and 113 mg *m*-anisidine (1 mmol) in 60 cm^3 dioxane containing three drops of triethylamine was heated under reflux for 2 h. The reaction mixture was poured into crushed ice/water and acidified with 2 N HCl. The solid product was collected and recrystallized from dioxane to give pale yellow crystals of **3** (81%). M.p.: >350 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ = 3.6 (s, OCH_3), 4.3 (s, CH_2), 5.9, 6.3 (2 s, pyrazole-H), 7.4–8.2 (m, Ar–H + 2NH), 10.9 (s, $NHSO_2$) ppm; ^{13}C NMR (300 MHz, DMSO-d_6): δ = 50.12 (CH_2, aliphatic), 55.05 (CH_3, aliphatic), 91.57 (pyrazole-4-CH), 105.62, 110.85, 121.78, 125.05, 127.55, 127.85, 129.11, 130.59, 135.70, 138.09, 139.23, 140.26, 140.69, 141.19, 142.54 (15C, aromatic carbons), 146.78 (pyrazole-3-CH), 169.32 (C=O, amide carbon) ppm; IR (KBr): $\bar{\nu}$ = 3,350, 3,223, 3,193 (3NH), 3,050 (CH-arom.), 2,968 (CH-aliph.), 1,735 (C=O), 1652 (C=N), 1,388, 1,150 (SO_2) cm^{-1}; MS (70 eV): m/z = 477 ($[M + 2]^+$, 31), 281 (100). Calcd. C 60.36, H 4.85, N 14.67, S 6.71. Found: C 60.23, H 4.55, N 14.87, S 6.31.

4-[4-Cyano-5-(ethoxymethyleneamino)-2,3-dihydro-2-oxo-1H-pyrrol-1-yl]-N-(1-phenyl-1H-pyrazol-5-yl)benzenesulfonamide (**6**, $C_{23}H_{20}N_6O_4S$)
Compound **5** (840 mg, 2 mmol) in excess triethyl orthoformate (60 cm^3) was heated under reflux for 5 h. The reaction mixture was cooled and triturated with cold ethanol, and the product was separated, collected by filtration, washed with petroleum ether (40–60 °C), and recrystallized from ethanol to give **6** (70%) as a brown powder. M.p.: 242 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ = 1.02 (t, 3H, CH_2CH_3, J = 6.6 Hz), 3.4 (q, 2H, CH_2CH_3, J = 6.6 Hz), 4.1 (s, 2H, CH_2), 5.8, 6.4 (2 s, 2H, pyrazole-H), 7.4-8.1 (m, 9H, Ar–H), 9.6 (s, H, CH=N), 10.2 (s, 1H, SO_2NH) ppm; ^{13}C NMR (300 MHz, DMSO-d_6): δ = 10.9 (CH_3, aliphatic), 19.88 (CH_2, aliphatic), 29.66 (pyrrole-3-CH_2), 75.79 (pyrrole-4-C–CN), 90.98 (pyrazole-4-CH), 117.09 (C≡N), 120.30, 121.90, 127.50, 127.50, 129.40, 136.49, 138.20, 139.79, 140.53 (9C, aromatic carbons), 146.52 (pyrazole-3-CH), 162.98 (N=$CHCH_2CH_3$), 163.78 (pyrrole-5-C–N=$CHCH_2CH_3$), 168.99 (C=O) ppm; IR (KBr): $\bar{\nu}$ = 3,243 (NH), 3,065 (CH-arom.), 2,996 (CH-aliph.), 2,218 (C≡N), 1,683 (C=O), 1,636 (C=N), 1,376, 1,161 (SO_2) cm^{-1}. Calcd. C 57.97, H 4.23, N 17.64, S 6.73. Found: C 58.17, H 3.93, N 17.74, S 7.03.

4-[4-Cyano-5-(hydrazinylmethyleneamino)-2,3-dihydro-2-oxo-1H-pyrrol-1-yl]-N-(1-phenyl-1H-pyrazol-5-yl)benzenesulfonamide (**7**, $C_{21}H_{18}N_8O_3S$)
To a solution of 238 mg **6** (0.5 mmol) in 50 cm^3 dioxane was added 5 cm^3 hydrazine hydrate (100 mmol) and the reaction mixture was stirred for 30 min. The solid obtained was recrystallized from ethanol to give **7** (75%) as yellowish brown solid. M.p.: 232 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ = 4.3 (s, CH_2), 5.8, 6.6 (2 s, pyrazole-H), 7.0–8.3 (m, Ar–H + NH), 9.4 (s, CH=N), 10.8 (s, SO_2NH), 11.3 (s, NH_2) ppm; ^{13}C NMR (300 MHz, DMSO-d_6): δ = 29.76 (pyrrole-3-CH_2), 75.83 (pyrrole-4-C–CN), 91.58 (pyrazole-4-CH), 117.12 (C≡N), 121.78, 125.49, 127.20, 127.29, 129.18, 135.89, 138.20, 140.49, 141.13 (9C, aromatic carbons), 146.22 (pyrazole-3-CH), 162.78 (N=$CHNHNH_2$),

163.33 (pyrrole-5-C–N=CHNHNH$_2$), 169.35 (C=O) ppm; IR (KBr): $\bar{\nu}$ = 3,336, 3,310, 3,109 (NH + NH$_2$), 3,068 (CH-arom.), 2,919 (CH-aliph.), 2,203 (C≡N), 1,623 (C=O), 1,659 (C=N), 1,383, 1,156 (SO$_2$) cm^{-1}; MS (70 eV): m/z = 464 ([M + 2]$^+$, 2.65), 77 (100). Calcd. C 54.54, H 3.92, N 24.23, S 6.93. Found: C 54.34, H 3.80, N 23.83, S 6.83.

N-[3-Cyano-4,5-dihydro-5-oxo-1-[4-[N-[(1-phenyl-1H-pyrazol-5-yl)amino]sulfonyl]phenyl]-1H-pyrrol-2-yl]-acetamide (**9**, $C_{22}H_{18}N_6O_4S$)

Compound **5** (588 mg, 1.4 mmol) was refluxed in 50 cm^3 acetic anhydride for 3 h. The product was precipitated, collected, and recrystallized from benzene to give yellow needles of **9** (80%). M.p.: 202 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ = 2.45 (s, COCH$_3$), 5.9, 6.8 (2 s, pyrazole-H), 7.1–7.9 (m, Ar–H + NH), 9.9 (s, SO$_2$NH) ppm; ^{13}C NMR (300 MHz, DMSO-d_6): δ = 23.42 (CH$_3$, aliphatic), 30.25 (pyrrole-3-CH$_2$), 58.33 (pyrrole-4-C–CN), 91.65 (pyrazole-4-CH), 117.50 (C≡N), 121.72, 125.23, 127.50, 127.25, 129.32, 135.89, 138.45, 140.39, 141.02 (9C, aromatic carbons), 146.19 (pyrazole-3-CH), 162.78 (pyrrole-5-C–NHCOCH$_3$), 166.15, 169.95 (2 C=O) ppm; IR (KBr): $\bar{\nu}$ = 2,200 (C≡N), 1,710 (C=O), 1,320, 1,146 (SO$_2$) cm^{-1}. Calcd. C 57.13, H 3.92, N 18.17, S 6.93. Found: C 57.43, H 4.12, N 18.47, S 7.23.

4-[2-(3-Amino-2,5-dihydro-5-oxo-1H-pyrazol-4-yl)diazenyl]-N-(1-phenyl-1H-pyrazol-5-yl)benzenesulfonamide (**12**, $C_{18}H_{16}N_8O_3S$)

A mixture of 438 mg **11b** (1 mmol) and 500 mg hydrazine hydrate (10 mmol) in 50 cm^3 ethanol was heated under reflux for 2 h. The solid product which formed on heating was collected and recrystallized from ethanol to give **12** (64%) as a white powder. M.p.: 238 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ = 5.9, 6.5 (2 s, pyrazole-H), 6.4 (s, NH$_2$), 7.5–7.7 (m, Ar–H), 10.9 (s, SO$_2$NH) ppm; ^{13}C NMR (300 MHz, DMSO-d_6): δ = 77 (NH-N–C, 3-amino-pyrazole), 92.55 (pyrazole-4-CH), 120.58, 125.99, 127.69, 127.89, 129.78, 131.80, 139.05, 140.00, 140.89 (9C, aromatic carbons), 143.99 (pyrazole-3-CH), 166.5 (ketonic carbon), 173.10 (C-NH$_2$) ppm; IR (KBr): $\bar{\nu}$ = 3,310, 3,305 (NH$_2$/NH), 1,720 (C=O), 1,360, 1,187 (SO$_2$) cm^{-1}. Calcd. C 50.94, H 3.80, N 26.40, S 7.55. Found: C 50.74, H 3.70, N 26.00, S 7.65.

3-Amino-5-imino-4-[2-[4-[N-[(1-phenyl-1H-pyrazol-5-yl)-amino]sulfonyl]phenyl]diazenyl]-1H-pyrazole-2(5H)-carbothioamide (**13**, $C_{19}H_{18}N_{10}O_2S_2$)

A mixture of 391 mg hydrazone **11a** (1 mmol), 91 mg thiosemicarbazide (1 mmol), and 0.5 cm^3 triethylamine in 50 cm^3 dioxane was heated under reflux for 12 h. The solid product which formed on heating was collected and recrystallized from ethanol to give off-white crystals of **13** (82%). M.p.: 241 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ = 5.5, 6.0 (2 s, pyrazole-H), 6.54, 6.67 (2 s, 2NH$_2$), 7.0–7.7 (m, Ar–H + NH), 10.5 (s, SO$_2$NH) ppm; ^{13}C NMR (300 MHz, DMSO-d_6): δ = 60.32 (pyrazole-4-CH), 91.30, 121.33, 125.48, 127.35, 127.92, 129.50, 135.50, 138.25, 140.85, 141.32, 146.89, 164.04 (12C, aromatic carbons), 165.08 (pyrazole-5-C=NH), 177.00 (C=S) ppm; IR (KBr): $\bar{\nu}$ = 3,450, 3,354, 3,209 (NH$_2$), 3,031 (CH-arom.), 1,330, 1,167 (SO$_2$), 1,088 (C=S) cm^{-1}; MS (70 eV): m/z = 482 (M$^+$, 22), 164 (100). Calcd. C 47.29, H 3.76, N 29.03, S 13.29. Found: C 47.49, H 3.86, N 29.43, S 13.59.

4-[2-(7-Amino-3,4-dihydro-2-methyl-4-thioxopyrazolo-[1,5-a][1,3,5]triazin-8-yl)diazenyl]-N-(1-phenyl-1H-pyrazol-5-yl)benzenesulfonamide (**14**, $C_{21}H_{18}N_{10}O_2S_2$)

Pyrazole **13** (482 mg, 1 mmol) was refluxed in 80 cm^3 acetic anhydride for 3 h. The solid product was precipitated, collected, and recrystallized from *N*,*N*-dimethylformamide (DMF) to give **14** (62%) as a white powder. M.p.: 282 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ = 0.9 (s, CH$_3$), 5.8, 6.4 (2 s, pyrazole-H), 6.5 (s, NH$_2$), 7.4–7.7 (m, Ar–H + NH) ppm; ^{13}C NMR (300 MHz, DMSO-d_6): δ = 23.99 (CH$_3$, aliphatic), 88.6 (pyrazolotriazine-8-C), 91.32, 120.05, 121.20, 125.42, 127.27, 127.71, 129.61, 130.90, 135.55, 138.33, 140.79, 145.15, 152.89, 168.29 (14C, aromatic carbons), 188.12 (C=S) ppm; IR (KBr): $\bar{\nu}$ = 3,234, 3,159 (NH$_2$), 3,010 (CH-arom.), 1,342, 1,149 (SO$_2$), 1,085 (C=S) cm^{-1}; MS (70 eV): m/z = 506 (M$^+$, 34), 138 (100). Calcd. C 49.79, H 3.58, N 27.65, S 12.66. Found: C 49.99, H 3.88, N 28.05, S 12.86.

4-[2-(7-Amino-3,4-dihydro-4-thioxopyrazolo[1,5-a][1,3,5]-triazin-8-yl)diazenyl]-N-(1-phenyl-1H-pyrazol-5-yl)-benzenesulfonamide (**15**, $C_{20}H_{16}N_{10}O_2S_2$)

Pyrazole **13** (482 mg, 1 mmol) was refluxed with 70 cm^3 triethyl orthoformate for 10 h. The excess of reagent was removed and ether was added to the cold mixture. The obtained solid was filtered and recrystallized from ethanol to give **15** (86%) as a green powder. M.p.: >350 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ = 5.9, 6.2 (2 s, pyrazole-H), 6.9 (s, NH$_2$), 7.4–7.9 (m, Ar–H), 8.20 (s, CH triazine), 11.3 (s, SO$_2$NH) ppm; ^{13}C NMR (300 MHz, DMSO-d_6): δ = 88.9 (pyrazolotriazine-8-C), 91.20, 120.36, 121.28, 125.52, 127.47, 127.77, 129.65, 130.85, 135.55, 138.35, 140.81, 145.35, 145.89, 152.39 (14C, aromatic carbons), 188.02 (C=S) ppm; IR (KBr): $\bar{\nu}$ = 3,200, 3161 (NH$_2$), 3,020 (CH-arom.), 1,332, 1,146 (SO$_2$), 1,080 cm^{-1} (C=S); MS (70 eV): m/z = 492 (M$^+$, 16), 159 (100). Calcd. C 48.77, H 3.27, N 28.44, S 13.02. Found: C 49.17, H 3.37, N 28.54, S 13.32.

Antitumor activity (in vitro study)

The potential in vitro cytotoxic activity of the newly synthesized compounds was measured using the Sulfo-

Rhodamine-B stain (SRB) assay by the method of Skehan et al. [20]. The in vitro anticancer screening was done by the pharmacology unit at the National Cancer Institute, Cairo University. Cells were plated in 96-multiwell microtiter plates (10^4 cells/well) for 24 h before treatment with the compounds to allow attachment of cell to the wall of the plate. Test compounds were dissolved in DMSO and diluted with saline to the appropriate volume. Different concentrations of the compound under test (0, 1, 2.5, 5, and 10 mg cm^{-3}) were added to the cell monolayer. Triplicate wells were prepared for each individual dose. Monolayer cells were incubated with the compounds for 48 h at 37 °C and in an atmosphere of 5% CO_2. After 48 h, cells were fixed, washed, and stained for 30 min with 0.4% (wt/vol) with SRB dissolved in 1% acetic acid. Excess unbound dye was removed by four washes with 1% acetic acid and attached stain was recovered with Tris–EDTA buffer. Color intensity was measured in an ELISA reader. The relation between surviving fraction and drug concentration is plotted to get the survival curve for the breast tumor cell line after the specified time. The molar concentration required for 50% inhibition of cell viability (IC_{50}) was calculated and the results are given in Table 1.

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