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Synthesis and *in vitro*-Anticancer and Antimicrobial Evaluation of Some Novel Quinoxalines Derived from 3-Phenylquinoxaline-2(1*H*)-thione

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Two novel series derived from 3-phenylquinoxaline-2(1*H*)-thione **2** and 2-(hydrazinocarbonylmethylthio)-3-phenylquinoxaline **6** have been synthesized. Eight out of twenty six new compounds were selected at the National Cancer Institute for evaluation of their *in vitro*-anticancer activity. Among them, compounds **3b**, **3c**, **4b**, and **4c** displayed moderate to strong growth inhibition activity against most of the tested sub-panel tumor cell lines with GI_{50} 10^{-5} to 10^{-6} molar concentrations. Compound **4b** exhibited a significant value of percent tumor growth inhibition against breast cancer at concentration $<10^{-8}$ M. Compound **4c** showed moderate selectivity towards leukemia cell lines with GI_{50} of 1.8 to 3.8 μM (selectivity ratio = 5.7). Preliminary antimicrobial testing revealed that compounds **7a**, **7b**, **8a**, **11a**, and **11b** were as active as ampicillin against *B. subtilis* (MIC = 12.5 μg/mL). Compounds **7b** and **8a** were also nearly as active as ampicillin against *E. coli* (MIC = 12.5 μg/mL). In addition, compounds **4a**, **7b**, **10b**, and **11a** were as active as ampicillin against *P. aerugenosa* (MIC = 50 μg/mL). However, compounds **7b**, **8a**, and **10b** showed mild activity against *C. albicans* (MIC = 50 μg/mL). The values of minimum bactericidal concentrations indicated that compounds **4a** and **7b** were bactericidal against *B. subtilis* and *P. aerugenosa*, respectively, while compound **10b** was bactericidal against both organisms. However, compound **11a** was bactericidal against *E. coli*, *P. aerugenosa,* and *S. aureus.*

Keywords: Antitumor activities, Antimicrobial activities / 2-(Hydrazinocarbonylmethylthio)-3-phenylquinoxaline / *N*-Arylchloroacetamides / 3-Phenylquinoxaline-2(1*H*)-thione

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Introduction

Quinoxaline and quinoxalinone derivatives have received much attention due to their versatile biological properties, especially antitumor [1–6], anti-HIV [7–9], and antimicrobial [10–13] activities. Among these derivatives some found their way in clinical application. For example, the two known antineoplastic quinoxaline topoisomerase II inhibitors, 2-[4-(7-chloroquinoxalin-2-yl)-phenoxy]propionic acid (**XK469**) [5] and chloroquinoxalinesulfonamide (**CQS**) [6] (Fig. 1), in addition to the non-nucleoside reverse transcriptase inhibitors, (*S*)-3-ethyl-6-fluoro-4-isopropoxy-carbonyl-3,4-dihydroquinoxalin-2(1*H*)-one (**GW420867**) [9] and 4-isopropoxycarbonyl-6-methoxy-3-(methylthiomethyl)-3,4-dihyroquinoxalin-2-(1*H*)-thione (**HBY 097**) [8] (Fig. 1).

These findings prompted us to investigate some of our previously reported quinoxalines [14, 15] for their *in vitro*-antitumor effect. The results obtained from their screening have shown interesting tumor growth inhibition on various cell lines between 10^{-6}–10^{-5} M [16]. Some of these compounds exhibited significant values of percent growth inhibition at 10^{-7} M. For example: 1-(4-

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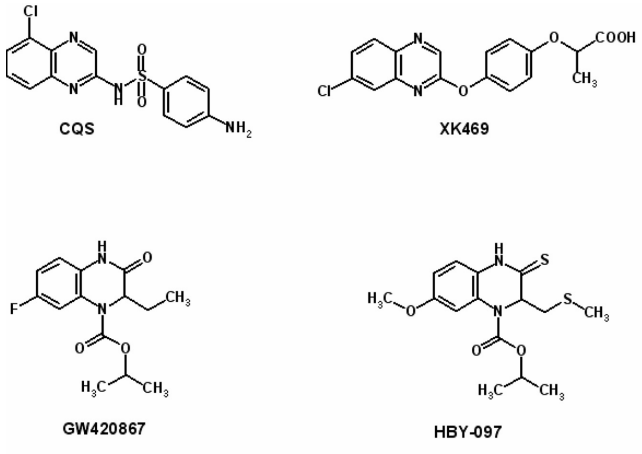


Figure 1. Chemical structure of antineoplastic quinoxaline topoisomerase II inhibitors, **XK469, CQS**, and non-nucleoside reverse transcriptase inhibitors, **GW420867**, **HBY 097**.

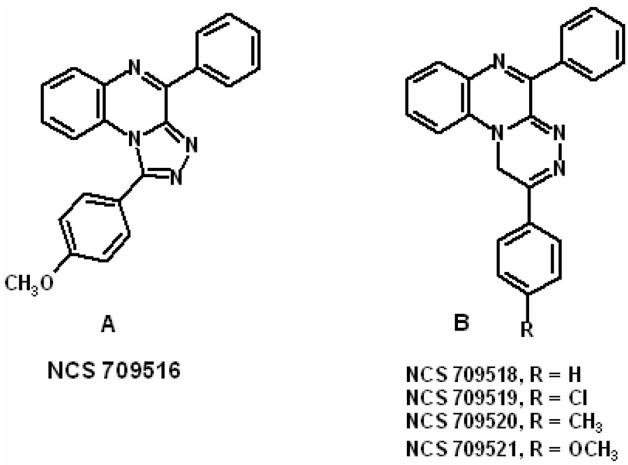
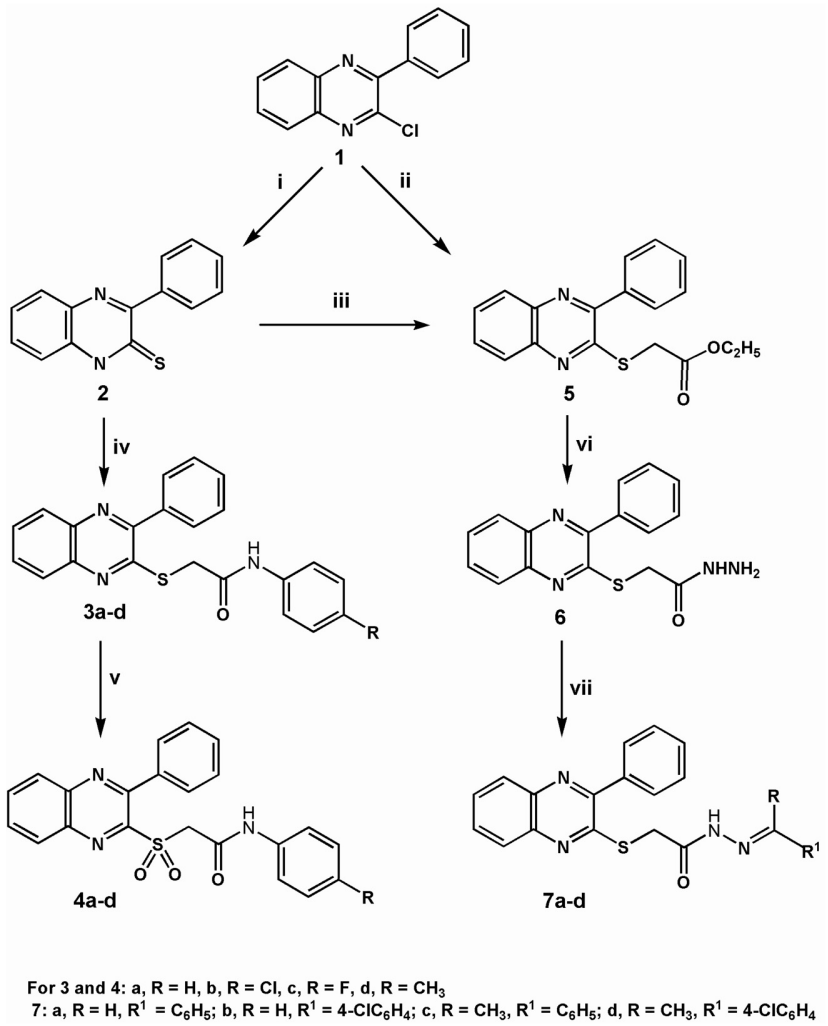
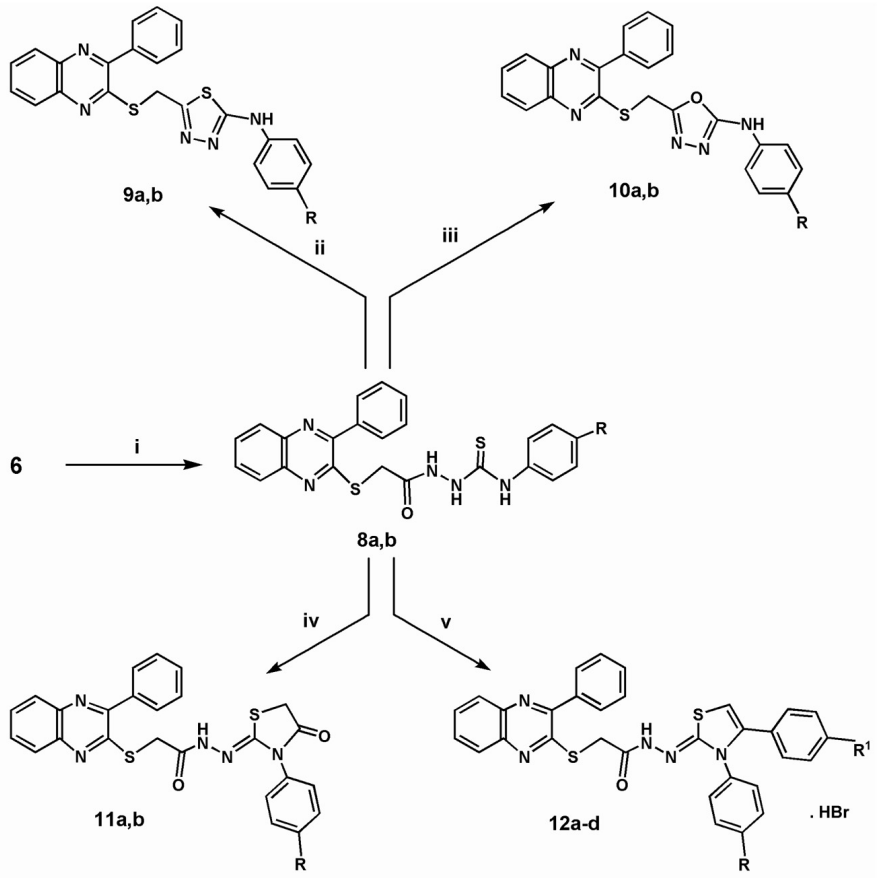


Figure 2. Chemical structure of 1-(4-methoxyphenyl)-4-phenyl-1,2,4-triazolo[4,3-*a*]quinoxaline (**A**) and 2-aryl-5-phenyl-*1H*-1,2,4-triazino-[4,3-*a*]quinoxalines (**B**).



Reagents: i = H_2N-CS-NH_2 / H_2SO_4; ii = $HSCH_2CO_2C_2H_5$ / K_2CO_3 / dry acetone;
iii = $ClCH_2CO_2C_2H_5$ / anhydrous CH_3CO_2Na / EtOH; iv = 4-$RC_6H_4NHCOCH_2Cl$ / anhydrous CH_3CO_2Na / EtOH;
v = $KMnO_4$ / gl. AcOH; vi = $NH_2NH_2 \cdot H_2O$ / EtOH; vii = R-CO-R^1 / EtOH.

Scheme 1. Synthesis route of 2-(*N*-arylcarbamoylmethylthio) and 2-(*N*-arylcarbonylmethylsulfonyl)-3-phenylquinoxalines (**3a – d** and **4a – d**) and 2-[arylidene and (1-substituted-ethylidene)-hydrazinocarbonyl-methylthio]-3-phenylquinoxalines **7a – d**.



8,9,10,11, a, R = H, b, R = CH_3; 12a, R = R^1 = H; 12b, R = H, R^1 = Cl;12c, R = CH_3, R^1 = H; 12d, R = CH_3,R^1 = Cl

Reagents: i = 4-RC_6H_4NCS / DMF, EtOH; ii = H_2SO_4; iii = HgO / dry dioxane; iv = $ClCH_2CO_2C_2H_5$ / abs. EtOH; v = 4-$R^1C_6H_4COCH_2Br$ / dry dioxane.

Scheme 2. Synthesis route of 2-(*N*-arylthiocarbamoyl-hydrazinocarbonylmethylthio)-3-phenylquinoxalines **8a, b** and derived ring systems (compounds **9a,b**; **10a,b**; **11a,b**; and **12a,b**).

methoxyphenyl)-4-phenyl-1,2,4-triazolo[4,3-*a*]quinoxaline (**A**) and 2-aryl-5-phenyl-*1H*-1,2,4-triazino-[4,3-*a*]quinoxalines (**B**) (Fig. 2).

In view of the above mentioned results and in continuation of our interest in biologically active quinoxalines [14 – 16], the aim of the present study was to synthesize and investigate the *in vitro*-anticancer and antimicrobial activity of some novel quinoxalines which were designed as structural relatives to the anticancer **CQS** and the non-nucleoside reverse transcriptase inhibitor **HBY 097** (Fig. 1). The new series comprised the compounds, namely: 2-(*N*-arylcarbamoylmethylthio) and 2-(*N*-arylcarbonylmethylsulfonyl)-3-phenylquinoxalines (**3a – d** and **4a – d**); 2-[arylidene and (1-substituted-ethylidene)-hydrazinocarbonylmethylthio]-3-phenylquinoxalines (**7a – d**, Scheme 1) and 2-(*N*-arylthiocarbamoyl-hydrazinocarbonylmethylthio)-3-phenylquinoxalines (**8a, b**; Scheme 2). The substitution pattern of these derivatives was selected to confer different electronic environment to the molecules.

Moreover, owing to the increasing biological importance of substituted 1,3,4-thiadiazoles [17 – 19], 1,3,4-oxadiazole [20 – 22], thiazoles and thiazolidinones [23 – 26] particularly in the field of chemotherapy, it was planed to synthesize additional derivatives. These derivatives comprise the quinoxaline backbone linked to the above mentioned heterocyclic ring systems by various atom spacers (compounds **9a, b**; **10a, b**; **11a, b**; and **12a – d**, Scheme 2), in order to investigate the effect of these structure variants on the anticipated antitumor and/or antimicrobial activity.

Results and Discussion

Chemistry

The preparation of target compounds was conducted according to the sequence of reactions depicted in Schemes 1 and 2. The starting compound 2-chloro-3-phenylquinoxaline **1** was obtained as previously described

[27]. 3-Phenyl-quinoxaline-2(1*H*)-thione **2** was obtained by reacting **1** with thio-urea as reported for the preparation of related compounds [28]. Treatment of **2** with the appropriate *N*-aryl substituted chloroacetamide afforded the respective 2-(*N*-arylcarbamoylmethylthio)-3-phenylquinoxalines **3a–d**. The target 2-[*N*-arylcarbamoylmethylsulfonyl)-3-phenylquinoxalines **4a–d** were obtained by oxidation of **3a–d** with potassium permanganate in glacial acetic acid. 2-(Ethoxycarbonymethylthio)-3-phenylquinoxaline **5** was obtained either by treatment of chloroquinoxaline **1** with ethyl thioglycolate in refluxing dry acetone containing anhydrous potassium carbonate (method A) or by reacting **2** with ethyl chloroacetate in refluxing ethanol containing anhydrous sodium acetate (method B). Treatment of **5** with ethanolic hydrazine hydrate at room temperature gave 2-(hydrazinocarbonylmethylthio)-3-phenylquinoxaline **6**. Reacting the latter with the selected aromatic aldehyde, acetophenone or 4-chloroacetophenone in refluxing ethanol yielded the corresponding 2-(arylidenehydrazinocarbonylmethylthio)-3-phenylquinoxalines **7a, b** and 2-[1-(arylethylidene)-hydrazinocarbonylmethylthio]-3-phenyquinoxaline derivatives **7c, d**. Refluxing **6** with the selected arylisothiocyanate in a mixture of ethanol and DMF resulted in the formation of the corresponding 2-(*N*-arylthiocarbamoylhydrazinocarbonylmethylthio)-3-phenylquinoxalines (**8a, b**). Treatment of the latter with cold concentrated sulfuric acid gave 2-[(5-substituted-1,3,4-thiadiazol-2-yl)methylthio]-3-phenylquinoxalines **9a, b**. Cyclodesulfurazation of **8a, b** with freshly prepared yellow mercuric oxide in boiling dioxane afforded the respective 2-[(5-substituted-1,3,4-oxadiazol-2-yl)methylthio]-3-phenylquinoxalines **10a, b**. Cyclocondensation of **8a, b** with ethyl chloroacetate in refluxing ethanol yielded 2-[(3-substituted-4-oxo-thiazolidin-2-ylidene)-hydrazinocarbonylmethylthio]-3-phenylquinoxalines **11a, b**. On the other hand, cyclocondensation of **8a, b** with phenacyl or 4-chlorophenacylbromide gave the corresponding 2-[(3,4-disubstituted-2,3-dihydrothiazol-2-ylidene)-hydrazinocarbonylmethylthio]-3-phenylquinoxaline hydrobromides (**12a–d**).

Biology

Antitumor activity

Antitumor activity tests were performed at the National Cancer Institute (NCI), Bethesda, MD, USA. Eight of the synthesized compounds (**3b**, **3c**, **4b**, **4c**, **7b**, **7d**, **12b**, and **12d**) were selected at the NCI and subjected to the NCI *in vitro*-disease-oriented human cells screening panel assay [29–31]. About 60 cell lines of nine tumor sub-panels were incubated with five concentrations (0.01–100 μM) for each compound and were used to create log-concentration *vs*.%-growth inhibition curves. Three response parameters (GI_{50}, TG1, and LC_{50}) were calculated for each cell line. The GI_{50} value corresponds for the compounds' concentration causing 50% decreases in net cell growth. The TG1 value is the compounds' concentration resulting in total growth inhibition and the LC_{50} value is the compounds' concentration causing a net 50% loss of initial cells at the end of the incubation period (48 h). Sub-panel and full panel mean-graph midpoint values (MG-MID) for certain agents are the average of individual real and default GI_{50}, TGI, or LC_{50} values of all cell lines in sub-panel and the full panel respectively [31].

In this study, the preliminary screening data indicated that four compounds showed antitumor activity namely: 2-(*N*-arylcarbamoylmethylthio)-3-phenylquinoxalines **3b, c**) and 2-(*N*-arylcarbamoylmethylsulfonyl)-3-phenylquinoxalines (**4b, c**) (Tables 1–4). Compounds **3b** and **4b** exhibited broad-spectrum antitumor activity against most of the tested sub-panel tumor cell lines while compounds **3c** and **4c** were of narrow spectrum of activity (Table 1). With regard to sensitivity against individual cell lines, compounds **3b** and **4b** proved to be sensitive against most of the tested cell lines. Compound **3b** showed high sensitivity against lung cancer HOP-62 and colon cancer HCT-116 with GI_{50} of 5.28 and 5.24 μM and TG1 values of 20.4 and 24.0 μM, respectively. However, compound **4b** exhibited a super sensitivity profile towards breast cancer HS-578T with GI_{50} value lying in the nanomolar range ($GI_{50} < 0.01$ μM and TG1 of 6.88 μM). The compound also showed significant activity against breast cancer B-549 ($GI_{50} = 1.33$ and TGI = 53.1 μM). On the other hand, compounds **3c** and **4c** showed remarkable activity against some of the tested cell lines. Compound **3c** exhibited high activity against ovarian cancer OVCAR-4 ($GI_{50} = 1.96$ and TG = 15.7 μM). In addition, compound **4c** showed moderate activity against all leukemia cell lines with GI_{50} values of 1.80 to 3.83 μM and TC1 values of 10.3 to 18.8 μM.

The LC_{50} (cytotoxicity) values were >100 μM for most tested cell lines. The ratio obtained by dividing the compounds' full panel MG-MID (mM) by its individual sub-panel MG-MID (μM) is considered as a measure of compound selectivity. Ratios between 3 and 6 refer to moderate selectivity, ratios >6 indicate high selectivity towards the corresponding cell line, while compounds meeting neither of these criteria are rated non-selective [31]. The tested compounds **3b, 3c**, and **4b** proved to be non-selective with a broad spectrum of activity, while compound **4c** showed moderate selectivity towards leukemia cell lines. Its selectivity ratio was 5.72 (Table 4).

The obtained screening results would indicate that the antitumor activity was only associated with 2-(*N*-arylcarbamoylmethylthio)-3-phenylquinoxalines (**3b, c**) and 2-(*N*-

Table 1. Growth inhibitory action (GI_{50}) of some selected *in vitro* tumor cell lines (μM)[a].

Compd. No.	NCS	Panel	Sub-panel cell lines (cytotoxicity GI_{50} μM)
3b	734278	Lung Cancer	HOP-62 (5.28), HOP-92 (15.40), NCI-H 226 (16.70), NCI-H460 (11.80), NCI-H 522 (15.30).
		Colon Cancer	HCT-116(5.24), HT29 (25.10).
		CNS cancer	SF-268 (15.50), SF-295 (17.50), SF-539 (16.30) SNB-75 (18.80), U 251 (23.60)
		Melanoma	SK-MEL-5 (19.30), UACC-62 (20.70), M 14 (23.70).
		Ovarian Cancer	OVCAR-3 (13.10), OVCAR-4 (13.60), OVCAR-8 (25.50).
		Renal Cancer	786-0 (16.00), ACHN (12.30), CAKI-1(12.50), RXF-393 (19.10), SN 12C (22.60).
		Breast Cancer	NCI/ADR-RES (24.90), HS 578T (16.10), T-47D (13.00).
3c	735806	Lung Cancer	HOP-92 (20.30), NCI-H522 (24.20).
		Colon Cancer	HTC-116 (14.10), HT29 (21.50).
		CNS Cancer	SF-539 (18.60), SNB-75 (21.60).
		Melanoma	MALME-3M (17.10), SK-MEL-5 (16.50).
		Ovarian Cancer	OVCAR-4 (1.96)
		Renal Cancer	RXF-393 (18.60)
		Breast Cancer	BT-549 (24.60), T-47D (19.20).
4b	734280	Leukemia	CCRF-CEM (23.40), MOLT-4 (24.40), RPMI-8226 (20.60), SR (18.70).
		Lung Cancer	HOP-92 (12.30), NCI-H460 (18.60), NCI-H522 (18.60), HOP-62(23.20).
		CNS Cancer	SF-295 (15.70), SF-539 (14.00), U251 (21.80).
		Melanoma	LOX IMVI (17.50), MALME-3M (19.10), SK-MEL-5 (15.20), UACC-62 (16.00).
		Ovarian Cancer	OVCAR-3 (21.50).
		Renal Cancer	A498 (19.00), ACHN (21.40), CAKI-1 (20.10), SN12C (18.20).
		Breast Caner	HS 578T (< 0.01), BT-549 (1.33),MDA-MB-231/ATCC (18.50), MDA-MB-435 (18.10).
		Colon Cancer	HCT-116 (16.00), HCT-15 (21.20), KM12 (21.70), COLO 205 (23.40).
4c	735807	Leukemia	CCRF-CEM (3.15), HL-60 (TB) (1.80), K-562 (3.83).
		Lung Cancer	HOP-92 (22.30)
		Colon Cancer	HCC-2998 (13.30), HCT-116 (25.70)
		CNS Cancer	SF-539 (18.70)
		Melanoma	SK-MEL-5 (23.90)
		Ovarian Cancer	OVCAR-4 (21.60).
		Renal Cancer	A498 (21.60)
		Prostate Caner	PC-3 (26.00)
		Breast Cancer	HS 578T (29.70), BT-549 (29.10).

[a] Data obtained from NCI *in vitro* disease-oriented human cell screen.

Table 2. Median growth inhibitory concentration (GI_{50}, μM) of *in vitro* sub-panel tumor cell lines.

Compd. No.	Sub-panel tumor cell lines[a]									MG-MID[b]
	I	II	III	IV	V	VI	VII	VIII	IX	
3b	82.40	30.72	50.25	21.27	42.70	46.25	25.65	43.00	27.44	41.08
3c	64.05	51.36	46.14	32.62	60.04	39.56	46.84	44.95	41.90	47.50
4b	21.78	23.78	26.60	35.80	25.90	39.53	23.55	25.90	19.39	26.91
4c	9.47	77.33	36.47	74.57	66.66	70.08	76.83	31.05	44.97	54.16

[a] I: Leukemia; II: non-small cell lung cancer; III: colon cancer; IV: CNS cancer; V: melanoma; VI: ovarian cancer; VII: renal cancer; VIII: prostate cancer; IX: breast cancer.

[b] GI_{50} full panel mean-graph midpoint (μM).

arylcarbamoylmethylsulfonyl)-3-phenylquinoxalines (**4b, c**). The results also revealed that the substitution at position 4 of the phenyl group of the *N*-arylcarbamoyl moiety with a chlorine atom yielded compounds with a broad-spectrum anticancer activity (**3b** and **4b**), while substitution with a fluorine atom yielded derivatives with narrow spectrum of activity (**3c** and **4c**, Table 1). Oxidation of 2-[(*N*-arylcarbamoyl)methylthio]quinoxalines **3a – d** to the corresponding methylsulfonyl derivatives **4a – d** increased activity and selectivity against some of the tested sub-panel cell lines. For example, compound **4b** exhibited significant activity against breast cancer

Table 3. Median total growth inhibitory (TGI) concentrations (μM) of the *in vitro* sub-panel tumor cell lines and TG1 full panel mean-graph midpoints (MG-MID).

Compd. No.	Sub-panel tumor cell lines[a]									MG-MID[b]
	I	II	III	IV	V	VI	VII	VIII	IX	
3b	–[c]#	71.79	89.14	66.68	97.25	90.05	72.43	–	94.50	86.87
3c	–	95.34	89.31	86.8	88.6	85.95	96.06	–	89.76	92.45
4b	58.31	72.70	68.49	83.08	61.83	91.78	65.50	–	70.53	75.80
4c	36.43	–	89.23	93.37	99.33	91.67	–	–	99.19	89.92

[a] For sub-panel tumor cell lines see foot note (a) of Table 2.
[b] TG1 (μM) full panel mean-graph midpoint (MG-MID) = the average sensitivity of all cell lines towards the test agent.
[c] Sub-panel TG1 value >100 μM.

Table 4. Selectivity ratios for the active compounds towards the nine tumor cell lines.

Compd. No.	Sub-panel tumor cell lines[a]								
	I	II	III	IV	V	VI	VII	VIII	IX
3b	0.50	1.34	0.82	1.93	0.96	0.89	1.60	0.96	1.50
3c	0.74	0.93	1.03	1.46	0.79	1.20	1.01	1.06	1.13
4b	1.24	1.13	1.01	0.75	1.04	0.68	1.14	1.04	1.39
4c	5.72	0.70	1.49	0.73	0.81	0.77	0.71	1.74	1.20

[a] For sub-panel tumor cell lines see footnote (a) of Table 2.

sub-panel cell lines especially HS 587T (GI_{50} < 0.01 μM) and compound **4c** showed moderate selectivity towards all leukemia sub-panel cell lines (GI_{50} values, 1.8–3.8 μM). Its selectivity ratio was 5.72 (Table 4).

Antimicrobial activity

All the newly synthesized compounds were preliminary evaluated for their *in vitro*-antibacterial activity against *S. aureus* and *B. subtilis* as Gram-positive bacteria; *E. coli* and *P. aerugenosa* as Gram-negative bacteria. The compounds were also evaluated for their *in vitro*-antifungal activity against *C. albicans*. Their inhibition zones using the cup diffusion technique [32] were measured, further evaluation was then carried out on compounds showing reasonable inhibition zones (>13 mm) to determine their minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) using the two-fold serial dilution method [33]. Ampicillin was used as standard antibacterial while clotrimazole was used as antifungal reference. Dimethylsulfoxide (DMSO) was used as a blank and showed no antimicrobial activity.

As revealed from Tables 5 and 6, eight compounds (**3d**, **4a**, **7a, b**, **8a**, **10b**, and **11a, b**) showed promising antimicrobial activity. Compounds **7a**, **7b**, **8a**, **11a**, and **11b** exhibited significant activity against *B. subtilis*. They were as active as ampicillin (MIC = 12.5 μg/mL). Compounds **7b** and **8a** also were nearly as active as ampicillin against *E. coli* (MIC = 12.5 μg/mL). In addition, **4a**, **7b**, **10b**, and **11a** were also as active as ampicillin against *P. aerugenosa* (MIC = 50 μg/mL). On the other hand, compounds **3d, 4a**, and **10b** showed moderate activity towards *B. subtilis*. They displayed half the activity of ampicillin (MIC = 25 μg/mL). Compounds **4a**, **10b**, and **11a** also exhibited half the activity of ampicillin towards *E. coli* (MIC = 25 μg/mL). However, the test compounds exhibited mild antimicrobial activity against *S. aureus* and *C. albicans*.

Table 5. The inhibition zones (IZ) in mm diameter of the most active compounds.

Compd. No.	*S. aureus*	*B. subtilis*	*P. aerugenosa*	*E. coli*	*C. albicans*
3d	12	12	15	13	16
4a	13	14	15	14	14
7a	14	14	18	14	21
7b	14	16	19	14	20
8a	19	14	16	14	14
10b	14	14	14	19	22
11a	15	14	15	23	13
11b	17	18	14	24	14

According to the MIC and MBC limits derived from the latest National Committee on Clinical Laboratory Standards (NCCLS), we can determine whether the test compound is bactericidal or bacteriostatic to the test organism. If the MBC = MIC, the test compound is considered a bactericidal but if MBC > MIC the test compound is con-

Table 6. Minimum inhibitory concentrations (MIC) and minimum bactericidal concentrations (MBC) of the most active compounds in μg/mL.

Compd. No.	*S. aureus*		*B. subtilis*		*P. aerugenosa*		*E. coli*		*C. albicans*	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
3d	100	100	25	50	100	100	50	100	100	200
4a	100	200	25	25	50	100	25	50	100	100
7a	50	50	12.5	25	100	100	50	100	100	100
7b	50	100	12.5	25	50	50	12.5	25	50	50
8a	50	50	12.5	25	100	100	12.5	25	50	100
10b	25	50	25	25	50	100	25	25	50	100
11a	25	25	12.5	25	50	50	25	25	100	200
11b	100	100	12.5	25	100	200	50	100	100	200
Ampicillin	5		12.5		50		10			
Clotrimazol									5	

sidered a bacteriostatic. Accordingly, as revealed from Table 6, compounds **7a**, **8a**, and **11a** were bactericidal against *S. aureus,* compounds **4a** and **10b** were bactericidal against *B. subtilis*; **10b** and **11a** were also bactericidal against *E. coli*. In addition, compound **7b** and **11a** were bactericidal against *P. aerugenosa*, while compound **7b** was fungicidal against *C. albicans*. The remaining compounds were bacteriostatic against the test organisms.

The above-mentioned results revealed that compounds **7b**, **10b** and **11a** exhibited a broad spectrum of antimicrobial activity and were devoid of cytotoxic activity (they were devoid of antitumor activity).

The activity of the test compounds could be correlated to the structure variations and modifications. The obtained screening results revealed that 2-[(*N*-arylcarbamoyl)methylthio]quinoxalines **3** exhibited antimicrobial activity. Maximum activity was observed when position 4 of the phenyl group was substituted with methyl group **3d**. The compound had half the activity of ampicillin against *B. subtilis* and *P. aerugenosa.* Oxidation of such compounds to the corresponding 2-(*N*-arylcarbamoyl)-methylsulfonyl analogs **4** increased the activity against the Gram-negative bacteria. Compound **4a** was the most active member. It had the same activity as ampicillin towards *P. aerugenosa* and half the activity against *E. coli*. 2-(Hydrazinocarbonylmethylthio)-3-phenylquinoxaline **6** did not show any antimicrobial activity. Condensation of **6** with aromatic aldehydes afforded the hydrazones **7a, b** with significant activity against *B. subtilis*, *P. aerugenosa*, *E. coli* and moderate activity towards *S. aureus* and *C. albicans*. When position 4 of the phenyl group of hydrazone was substituted with a chlorine atom **7b**, an increase in the activity was observed. This compound was as active as ampicillin against *B. subtilis*, *P. aerugenosa*, and *E. coli*. On the other hand, condensation of **6** with acetophenone or 4-chloroacetophenone gave inactive compounds **7c, d**. Reacting **6** with aryl isothiocyanates yielded active compounds **8,** of which compound **8a** exhibited significant activity. It was as active as ampicillin towards *B. subtilis*, *E. coli* and had half the activity against *P. aerugenosa*. Cyclization of **8a, b** to the substituted 1,3,4-thiadiazole derivatives **9a, b** abolished the antimicrobial activity. While cyclodesulfurization of **8a, b** afforded the corresponding 1,3,4-oxadiazole analogs **10a, b** with promising activity. Compound **10b** was the most active. It had the same activity as ampicillin against *P. aerugenosa* and half the activity towards *B. subtilis* and *E. coli*. Cyclocondensation of **8a, b** with ethyl bromoacetate gave the corresponding substituted 4-oxothiazolidin-2-ylidene derivatives (**11a, b**) with increased activity. Compound **11a** was as active as ampicillin towards *B. subtilis* and *P. aerugenosa* and had half the activity against *E. coli*. It exhibited moderate activity against *S .aureus* and *C. albicans*. On the other hand, cyclocondensation with phenacyl bromide or 4-chlorophenacylbromide yielded the respective 3,4-diaryl-substituted-2,3-dihydrothiazol-2-ylidene derivatives (**12a-d**) which were devoid of activity.

Experimental

Chemistry

All melting points were determined in open-glass capillaries on a Gallenkamp melting point apparatus (Sanyo) and are uncorrected. The IR spectra were recorded using KBr discs on a Perkin-Elmer 1430 spectrophotometer (Perkin-Elmer, Norwalk, CT, USA). ^{1}H-NMR (δ ppm) spectra were recorded on a Jeol spectrometer (JEOL, Tokyo, Japan) at 500 MHz using TMS as an internal standard and DMSO-d_6 as a solvent. The mass spectra (MS) were run on a Finnigan mass spectrometer (Thermo Electron Corp.) model SSQ/7000 (70 eV). The microanalyses were performed at the Microanalytical Laboratory, National Research Center, Cairo, Egypt and the data were within ± 0.4% of the theoretical values. Following up of the reactions and checking the homoge-

neity of the compounds were made by TLC on silica gel-protected aluminium sheets (Type 60 F_{254}, Merck; Darmstadt, Germany) and the spots were detected by exposure to UV-lamp at λ 254 nm for few seconds.

*2-(N-Arylcarbamoylmethylthio)-3-phenylquinoxalines **3a – d***

A mixture of 3-phenylquinoxaline-2(1*H*)-thione **2** (0.44 g, 2 mmol), the appropriate *N*-arylchloroacetamide (2 mmol) and anhydrous sodium acetate (0.49 g, 6 mmol) in absolute ethanol was refluxed for 1 h. The mixture was cooled, the white product separated was filtered, washed with water, dried, and crystallized from the appropriate solvent (Table 7). IR (KBr, cm^{-1}): 3242 (NH), 1691 – 1690 (C=O), 1648 (C=N), 1610, 1595 (C=C), 1533 δ NH, 1245 – 1244, 1085 (C-S-C). ^{1}H-NMR of **3d** (δ ppm): 2.2 (s, 3H, CH_3), 4.17 (s, 2H, CH_2), 7.06, 7.46 (two d, each 2H, J = 8.4 Hz, C_6H_4-CH_3), 7.55 – 7.56 (m, 3H, C_6H_5-$C_{3,4,5}$-H), 7.69 (t, 1H, J = 7.65 Hz, quinox. C_6-H), 7.74 – 7.77 (m, 3H, C_6H_5-$C_{2,6}$-H and quinox. C_7-H), 7.88 (d, J = 8.4 Hz, 1H, quinox. C_5-H), 8.01 (d, J = 7.65 Hz, 1H, quinox. C_8-H), 10.36 (s, 1H, NH, D_2O exchangeable).

*2-(N-Arylcarbamoylmethylsulfonyl)-3-phenylquinoxalines **4a – d***

A solution of 5% potassium permanganate was added dropwise to a stirred suspension of the appropriate **3a – d** (3 mmol) in glacial acetic acid (10 mL) till the pink color persisted. Stirring was continued at room temperature over night, then poured onto cooled sodium sulfite solution. The product was filtered, washed with water, dried, and crystallized from the appropriate solvent (Table 7). IR (KBr, cm^{-1}): 3330 – 3267 (NH), 1673 – 1659 (C=O), 1603 – 1601; 1515 – 1497 (C=C), 1548-1545 (δ NH), 1325 – 1319, 1157 – 1147 (SO_2), 1273 – 1262, 1096 – 1093 (C-S-C). ^{1}H-NMR of **4d** (δ ppm): 2.18 (s, 3H, CH_3), 4.85 (s, 2H, CH_2), 7.03, 7.28 (two d, each 2H, J = 8.4 Hz, C_6H_4-CH_3), 7.49 – 7.53 (m, 3H, C_6H_5-$C_{3,4,5}$-H), 7.74 (d, 2H, J = 6.9 Hz, C_6H_5-$C_{2,6}$-H), 8.01 (t, 1H, J = 7.65 Hz, quinox. C_6-H), 8.07 (t, 1H, J = 7.65 Hz, quinox. C_7-H), 8.19 (d, 1H, J = 7.65 Hz, quinox. C_5-H), 8.23 (d, 1H, J = 7.65 Hz quinox. C_8-H), 10.32 (s, 1H, NH, D_2O exchangeable). MS of **4b** m/z (relative abundance%): [$M^{+\bullet}$] at 437 absent, 375, 373 [M^+-SO_2] (2.6, 4.7), 330 (28.9), 247 (25.6), 204 (100), 127(39.6), 77 (88.3).

*2-Ethoxycarbonylmethylthio-3-phenylquinoxaline **5***

Method A

To a stirred mixture **1** (2.4 g, 10 mmol) and anhydrous potassium carbonate (1.4 g, 10 mmol) in dry acetone (40 mL), ethyl thioglycolate (1.2 g, 10 mmol) was added. The reaction mixture was refluxed for 6 h, cooled, and poured onto ice-water. The precipitate formed was filtered, dried, and crystallized from ethanol.

Method B

A mixture of **2** (2.38 gm, 10 mmol), ethyl chloroacetate (1.23 g, 10 mmol) and anhydrous sodium acetate (2.87 g, 30 mmol) in absolute ethanol (50 mL), was heated under reflux for 2 h. The mixture was cooled and poured onto ice-water. The product was filtered, washed with water, dried, and crystallized from ethanol. IR (KBr, cm^{-1}): 1739 (C=O), 1642 (C=N), 1607, 1480 (C=C), 1243, 1151, 1030 (C-O-C), 1088 (C-S-C). ^{1}H-NMR (δppm): 1.17 (t, 3H, J = 7.6 Hz, $CH_{H_2CH_3}$), 4.08 (s, 2H, CH_2CO), 4.12 (q, 2H, J = 7.6 Hz, CH_2CH_3), 7.55-7.56 (m, 3H, C_6H_5-$C_{3,4,5}$-H), 7.72 (t, 1H, J = 8.4 Hz, quinox. C_6-H), 7.73 – 7.75 (m, 2H, C_6H_5-$C_{2,6}$-H), 7.78 (t, 1H, J = 8.4 Hz, quinox. C_7-H), 7.84 (d, 1H, J = 8.4 Hz, quinox. C_5-H), 8.02 (d, 1H, J = 8.4 Hz, quinox. C_8-H).

Table 7. Physical and analytical data of the synthesized compounds **3 – 12**.

Compd. No.	R	R[1]	Mp. (°C) cryst. solvent	Yield (%)	Mol. Formula[a] Mol. Wt.
3a	H	–	211 – 212 DMF/EtOH	96	$C_{22}H_{17}N_3OS$ 371.46
3b	Cl	–	216 – 217 DMF/EtOH	92	$C_{22}H_{16}ClN_3OS$ 405.91
3c	F	–	202 – 203 EtOH	78	$C_{22}H_{16}FN_3OS$ 389.45
3d	CH_3	–	202 – 203 DMF/EtOH	68	$C_{23}H_{19}N_3OS$ 385.49
4a	H	–	189 – 190 EtOH	67	$C_{22}H_{17}N_3O_3S$ 403.46
4b	Cl	–	214 – 215 EtOH	85	$C_{22}H_{16}ClN_3O_3S$ 437.91
4c	F	–	191 – 192 EtOH	72	$C_{22}H_{16}FN_3O_3S$ 421.45
4d	CH_3	–	201 – 202 EtOH	67	$C_{23}H_{19}N_3O_3S$ 417.49
5	–	–	138 – 139 EtOH	82	$C_{18}H_{16}N_2O_2S$ 324.40
6	–	–	195 – 196 EtOH	92	$C_{16}H_{14}N_4OS$ 310.38
7a	H	C_6H_5	222 – 223 DMF-EtOH	72	$C_{23}H_{18}N_4OS$ 398.49
7b	H	4-ClC_6H_4	236 – 237 DMF-EtOH	93	$C_{23}H_{17}ClN_4OS$ 432.49
7c	CH_3	C_6H_5	201 – 202 DMF-EtOH	92	$C_{24}H_{20}N_4OS$ 412.52
7d	CH_3	4-ClC_6H_4	223 – 224 DMF-EtOH	72	$C_{24}H_{19}ClN_4OS$ 446.96
8a	H	–	209 – 210 DMF/EtOH	97	$C_{23}H_{19}N_5OS_2$ 445.57
8b	CH_3	–	208 – 209 DMF/EtOH	95	$C_{24}H_{21}N_5OS_2$ 459.60
9a	H	–	246 – 247 DMF/H_2O	96	$C_{23}H_{17}N_5S_2$ 427.55
9b	CH_3	–	236 – 237 DMF-H_2O	98	$C_{24}H_{19}N_5S_2$ 441.58
10a	H	–	206 – 208 DMF-EtOH	52	$C_{23}H_{17}N_5OS$ 411.49
10b	CH_3	–	207 – 208 DMF-H_2O	97	$C_{24}H_{19}N_5OS$ 425.52
11a	H	–	151 – 152 EtOH	40	$C_{25}H_{19}N_5O_2S_2$ 485.59
11b	CH_3	–	234 – 235 EtOH	48	$C_{26}H_{21}N_5O_2S_2$ 499.62
12a	H	H	234 – 235 EtOH	82	$C_{31}H_{23}N_5OS_2 \cdot HBr$ 626.61
12b	H	Cl	237 – 238 EtOH	73	$C_{31}H_{22}ClN_5OS_2 \cdot HBr$ 661.05
12c	CH_3	H	236 – 237 DMF-EtOH	86	$C_{32}H_{25}N_5OS_2 \cdot HBr$ 640.05
12d	CH_3	Cl	244 – 245 DMF-EtOH	60	$C_{32}H_{24}ClN_5OS_2 \cdot HBr$ 675.08

[a] Analyzed for C, H, N; the results are within ±0.4% of the theoretical values.

2-(Hydrazinocarbonylmethylthio)-3-phenylquinoxaline ***6***

To a suspension of **5** (3.24 g, 10 mmol) in absolute ethanol (50 mL), hydrazine hydrate (98%) (5 g, 100 mmol) was added and the mixture was stirred at room temperature for 24 h. The product was filtered, washed with water, dried, and crystallized from ethanol. IR (KBr, cm^{-1}): 3298, 3257, 3115 (NH), 1639 (C=O, C=N), 1534 (δ NH), 1499, 1483 (C=C), 1242, 1085 (C-S-C). ^{1}H-NMR (δ ppm): 3.93 (s, 2H, CH_2), 4.25 (s, 2H, NH_2, D_2O exchangeable), 7.52 – 7.57 (m, 3H, C_6H_5-$C_{3,4,5}$-H), 7.71 (t, 1H, J = 7.65 Hz, quinox. C_6-H), 7.72 – 7.76 (m, 2H, C_6H_5-$C_{2,6}$-H), 7.79 (t, 1H, J = 7.65 Hz, quinox. C_7-H), 7.95 (d, 1H, J = 7.65 Hz, quinox. C_5-H), 8.04 (d, 1H, J = 7.65 Hz, quinox. (C_8-H), 9.34 (s, 1H, NH, D_2O exchangeable).

2-(N-Arylidenehydrazinocarbonylmethylthio)-3-phenylquinoxalines ***7a, b*** *and 2-[(1-arylethylidene)hydrazinocarbonylmethylthio]-3-phenylquinoxalines* ***7c, d***

To a suspension of **6** (0.31 g, 1 mmol) in ethanol (10 mL), the appropriate aldehyde or ketone (1 mmol) was added. The mixture was refluxed for 1 h. then cooled, filtered, dried, and crystallized from the proper solvent (Table 7). IR (KBr, cm^{-1}): 3183 – 3172 (NH), 1675 – 1670 (C=O), 1616 – 1606 (C=N), 1568, 1519 – 1518, 1490 – 1485 (C=C), 1535-1534 (δ NH), 1224 – 1221, 1089 – 1088 (C-S-C). ^{1}H-NMR of **7b** (δ ppm): 4.55 (s, 2H, CH_2), 7.43 (d, 2H, J = 8.4 Hz, C_6H_4-Cl $C_{3,5}$-H), 7.45 – 7.79 (m, 7H, C_6H_5 and quiox. $C_{6,7}$-H), 7.81 (d, 1H, J = 7.65 Hz, quinox. C_5-H), 7.91 (d, 1H, J = 8.4, quinox. C_8-H), 8.02 (d, 2H, J = 8.4 Hz, C_6H_4-Cl $C_{2,6}$-H), 8.24 (s, 1H, CH=N), 11.68 (s, $^1/_2$ H, NH, D_2O exchangeable), 11.88 (s, $^1/_2$ H, OH, enolic). ^{1}H-NMR of **7d** (δ ppm): 2.25 (s, 3H, CH_3), 4.59 (s, 2H, CH_2), 7.39, 7.79 (two d, each 2H, J = 8.4 Hz C_6H_4-Cl), 7.41 – 7.81 (m, 7H, C_6H_5 and quiox. $C_{6,7}$-H), 7.92 (d, 1H, J = 7.65 Hz, quinox. C_5-H), 8.01 (d, 1H, J = 7.65 Hz, quinox. C_8-H), 10.81 (s, $^1/_2$ H, NH enolic, D_2O exchangeable), 10.89 (s, $^1/_2$ H, OH, D_2O exchangeable). MS of **7d** m/z (relative abundance%): [M^+] at 446 absent, 279 [M^+-$C_6H_{11}N_2OS$, (75.4)], 278 (100), 250 (60.1), 204 (24.6), 151 (10.4), 102 (27.2), 77 (49).

2-(N-Arylthiocarbamoylhydrazinocarbonylmethylthio)-3-phenylquinoxalines ***8a, b***

A mixture of equimolar amounts of **6** (3.1 gm, 10 mmol) and the appropriate arylisothiocyanate in absolute ethanol and DMF (3 : 1, 4 mL) was heated under reflux for 3 h. The reaction mixture cleared, then a yellow crystalline product was separated. The mixture was cooled, filtered, washed with ethanol, dried, and recrystallized from the proper solvent (Table 7). IR (KBr, cm^{-1}): 3316 – 3312, 3270 – 3236, 3186 – 3179 (NH), 1654 – 1649 (C=O), 1620 – 1613 (C=N), 1513 – 1498 (C=C), 1566 – 1557, 1272 – 1268, 1086 – 1085, 987 – 986 (N-C=S), 1238 – 1235, 1046 – 1030 (C-S-C). ^{1}H-NMR of **8b** (δ ppm): 2.24 (s, 3H, CH_3), 4.09 (s, 2H, CH_2), 7.08 (d, 2H, J = 8.4 Hz, CH_3-C_6H_4-$C_{3,5}$-H), 7.18 (dist. d, 2H, $CH_3C_6H_4$ $C_{2,6}$-H), 7.55-7.56 (m, 3H, C_6H_5-$C_{3,4,5}$-H), 7.73 (t, 1H, J = 6.9 Hz, quinox.C_6-H), 7.74 – 7.76 (m, 3H, C_6H_5, $C_{2,6}$-H and quinox. C_7-H), 8.01, 8.02 (two d, 2H, J = 8.0 Hz, quinox. $C_{5,8}$-H), 9.47, 9.67, 10.33 (three s, 3H, 3 NH, D_2O exchangeable).

2-[(5-Arylamino-1,3,4-thiadiazol-2-yl)methylthio]-3-phenylquinoxalines ***9a, b***

A solution of **8a** or **8b** (1 mmol) in cold conc. H_2SO_4 (3 mL) was stirred at room temperature. The mixture was poured onto crushed ice, the product was filtered, washed with water, dried, and crystallized from the proper solvent (Table 7). IR (KBr, cm^{-1}): 3254 – 3244, 3194 – 3188 (NH), 1613 – 1602, (C=N), 1516 – 1502 (C=C), 1549 – 1534 (δ NH), 1244 – 1241, 1087 – 1085 (C-S-C). ^{1}H-NMR of **9b** (δ ppm): 2.19 (s, 3H, CH_3), 4.77 (s, 2H, CH_2), 7.06, 7.40 (two d, each 2H, J = 8.4 Hz, C_6H_4-CH_3), 7.51 – 7.79 (m, 5H, C_6H_5), 7.87 (t, 1H, J = 8.4 Hz, quinox. C_6-H), 8.00 (t, 1H, J = 8.4 Hz, quinox. C_7-H), 8.07 (d, 1H, J = 8.4 Hz quinox. C_5-H), 8.08 (d, 1H, J = 7.65 Hz, quinox. C_8-H), 10.07 (s, 1H, NH, D_2O exchangeable). MS of **9b** m/z (relative abundance%): 441 [M^+, (38.6)], 407 (42.0), 333 (29.5), 237 (100), 204 (53.4), 150 (50.0), 91 (100), 77 (94.3).

2[-(5-Arylamino-1,3,4-oxadiazol-2-yl)methylthio]-3-phenylquinoxalines ***10a, b***

A mixture of **8a** or **8b** (2 mmol) and freshly prepared yellow HgO (0.42 g, 1 mmol) in dry dioxane (20 mL) was heated under reflux for 4 h. The mixture was filtered, the filtrate was evaporated under reduced pressure, and the residue was crystallized from the proper solvent (Table 7). IR (KBr, cm^{-1}): 3228, 3178, 3122 (NH), 1642 (C=N), 1575 (δ NH), 1517, 1482 (C=C), 1273, 1083 (C-S-C), 1247, 1058 (C-O-C). ^{1}H-NMR of **10b** (δ ppm): 2.18 (s, 3H, CH_3), 4.73 (s, 2H, CH_2), 7.03, 7.32 (two d, each 2H, J = 8.4 Hz, C_6H_4-CH_3), 7.54-7.73 (m, 5H, C_6H_5), 7.74 (t, 1H, J = 8.4 Hz, quinox.C_6-H), 7.81 (t, 1H, J = 8.4 Hz, quinox. C_7-H), 7.97 (d, 1H, J = 8.4 Hz, quinox. C_5-H), 8.04 (d, 1H, J = 8.4 Hz, quinox. C_8-H), 10.28 (s, 1H, NH, D_2O exchangeable). MS of **10b** m/z (relative abundance%): 425 [M^+, (14.0)], 318 (100), 276 (18.7), 236 (70.8), 134 (56.2), 77 (86.2).

2-[(3-Aryl-4-oxothiazolidin-2-ylidene)hydrazinocarbonylmethylthio]-3-phenylquinoxalines ***11a, b***

A mixture of equimolar amounts of **8a** or **8b** (1 mmol) and ethyl chloroacetate in absolute ethanol (10 mL) was heated under reflux for 6 h. The mixture was cooled to room temperature, the crystalline product was filtered, dried, and recrystallized from ethanol (Table 7). IR (KBr, cm^{-1}) of **11a**: 3188 (NH), 1755 (C=O), 1661 (C=O), 1644 (C=N), 1608, 1517 (C=C), 1531 (δ NH), 1276, 1087 (C-S-C). ^{1}H-NMR of **11a** (δ ppm): 4.02 (s, 2H-thiazolidinone C_5-H), 4.63 (s, 2H, CH_2), 7.22, 7.36 (two t, each 1H, J = 7.65 Hz, quinox. $C_{6,7}$-H), 7.41, 7.97 (two d, each 1H, J = 7.65 Hz, quinox. $C_{5,8}$-H), 7.51 – 7.68 (m, 10H, Ar-H), 10.40 (s, 1H, NH, D_2O exchangeable). IR of **11b** (KBr, cm^{-1}): 3168 (NH), 1731 (C=O), 1678 (C=O), 1660 (C=N), 1602, 1513 (C=C), 1535 (δ NH), 1242, 1084 (C-S-C). ^{1}H-NMR of **11b** (δ ppm): 1.9 (s, 3H, CH_3), 4.02 (s, 2H, thiazolidinone C_5-H), 4.67 (s, 2H, CH_2), 7.01, 7.2 (two d, each 2H, J = 8.00 Hz, C_6H_4-CH_3), 7.5 – 8.0 (m, 9H, C_6H_5-H^+ quinox. $C_{5,6,7,8}$-H); 10.41 (s, 1H, NH, D_2O exchangeable).

2-[(3,4-Disubstituted-2,3-dihydrothiazol-2-ylidene)-hydrazinocarbonyl-methylthio]-3-phenylquinoxaline hydrobromides ***12a-d***

A mixture of equimolar amounts of **8a** or **8b** (1 mmol) and phenacyl bromide or 4-chlorophenacyl bromide in dry dioxane (10 mL) was heated under reflux for 2 h. The reaction mixture cleared, then a white fluffy product was formed. The mixture was then cooled, filtered by suction, dried, and crystallized from the proper solvent (Table 7). IR (KBr, cm^{-1}): 3391 – 3390 (NH), 1723 – 1706 (C=O), 1643 – 1642 (C=N), 1612 – 1597, 1573 – 1570, 1519 – 1509 (C=C), 1537 – 1535 (δ NH), 1282 – 1252, 1086 – 1084 (C-S-C). ^{1}H-NMR of **12c** (δ ppm): 2.25 (s, 3H, CH_3), 4.04, 4.21 (two d, each 1H, J = 15.3 Hz, CH_2), 6.89, 6.98 (two t, each 1H, J = 7.60 Hz,

quinox. $C_{6,7}$-H), 6.94 (s, 1H, thiazolidin-C_5H), 7.28 – 7.74 (m, 15 H, two C_6H_5, *p*-tolyl and quiox. C_5-H), 8.00 – 8.02 (dist d, 1H, quinox. C_8-H), 11.94 (s, 1H, NH, D_2O-exchangeable). ^{1}H-NMR of **12d** (δ ppm): 2.33 (s, 3H, CH_3), 4.01, 4.25 (two d, each 1H, J = 14.5 Hz, CH_2), 6.87, 7.33 (two d, each 2H, J = 8.4 Hz, *p*-tolyl), 6.88 (s, 1H, thiazolidene C_5-H), 7.28 – 7.74 (m, 12H, C_6H_5, 4-ClC_6H_4 and quinox. $C_{5,6,7}$-H), 8.01 (dist d, 1H, quinox. C_8-H), 11.97 (s, 1H, NH, D_2O exchangeable). MS of **12d** m/z (relative abundance%): 594 [M^+, (2.5)], 593 (4.3), 592 (4.9), 343 (15), 341 (38.9), 299 (100), 238 (13.1), 236 (48.7), 204 (42.1), 167 (22.5), 133 (35.8), 77 (57.5).

Biology

Antitumor activity

Eight of the prepared compounds were selected and tested for their *in vitro*-antitumor activity against 60 human tumor cell lines, derived from nine clinically isolated types of cancer (leukemia, lung, brain, melanoma, colon, ovarian, renal, breast, and prostate) following the National Cancer Institute (NCI) preclinical antitumor drug discovery screen. Each compound was tested at five, ten-fold dilutions, a 48 h continuous drug exposure protocol was used and with a sulforodamine B (SKB) protein assay the cell viability or growth was estimated [29 – 31]. The results are presented in Tables 1 – 4.

Antimicrobial activity

Inhibition zones measurement

All the synthesized compounds were evaluated by the agar cup diffusion technique [32] using a 1mg/mL solution in DMSO. The test organisms were *Staphylococcus aureus* (DSM 1104) and *Bacillus substilis* (ATCC 6633) as Gram-positive bacteria; *Escherichia coli* (ATCC 11775) and *Pseudomonas aerugenosa* (ATCC 10145) as Gram-negative bacteria. *Candida albicans* (DSM 70014) was also used as a representative for fungi. Each 100 mL of sterile molten agar (at 45°C) received 1 mL of 6 h-broth culture and then the seeded agar was poured into sterile Petri-dishes. Cups (8 mm in diameter) were cut in the agar. Each cup received 0.1 mL of the 1 mg/mL solution of the test compounds. The plates were then incubated at 37°C for 24 h or for 48 h for *C. albicans*. A control using DMSO without the test compound was included for each organism. Ampicillin was used as standard antibacterial, while clotrimazole was used as antifungal reference. The resulting inhibition zones are recorded (Table 5).

Minimal inhibitory concentration (MIC) measurement

The minimal inhibitory concentrations (MIC) of the most active compounds were measured using the two-fold serial broth dilution method [33]. The test organisms were grown in their suitable broth for 24 h for bacteria and 48 h for fungi at 37°C. Two-fold serial dilutions of solutions of the test compounds were prepared using 200, 100, 50, 25, and 12.5 μg/mL. The tubes were then inoculated with the test organisms; each 5 mL received 0.1 mL of the above inoculum and were incubated at 37°C for 48 h. Then, the tubes were observed for the presence or absence of microbial growth. The MIC values of the prepared compounds are listed in Table 6.

Minimal bactericidal concentration (MBC) measurement

MIC tests were always extended to measure the MBC as follows: A loop-full from the tube not showing visible growth (MIC) was spread over a quarter of Müller – Hinton agar plate. After 18 h of incubation, the plates were examined for growth. Again, the tube containing the lowest concentration of the test compound that failed to yield growth on sub-culture plates was judged to contain the MBC of that compound for the respective test organism (Table 6).

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