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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

[1,2,4]Triazolo[4,3-*a*]quinoxaline: synthesis, antiviral, and antimicrobial activities

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Abstract A series of novel [1,2,4]triazolo[4,3-*a*]quinoxaline derivatives and their isosteres, pyrimido-quinoxaline, were synthesized as potential antiviral and antimicrobial agents. The new compounds were synthesized via aromatic nucleophilic substitution of 4-chloro-8-methyl[1,2,4]triazolo[4,3-*a*]quinoxaline-1-amine with different amines and triazole-2-thiol. Some of the synthesized compounds were subjected to antiviral and cytotoxicity screening using plaque-reduction assay. Most of the tested compounds exhibited cytotoxicity at concentration 160 μg/ml and compound **8b** showed promising antiviral activity. In vitro antimicrobial screening against different pathogenic organisms using agar diffusion method demonstrated that compounds **4d**, **6c**, **7b**, and **8a** exhibit antibacterial and/or antifungal activities.

Keywords [1,2,4]-triazolo[4,3-*a*]quinoxaline · Pyrimidotriazoloquinoxaline · Antiviral agents · Antimicrobial agents

Introduction

The antifungal drugs that have triazole moiety, such as voriconazole and posaconazole, play the leading role in combating fungal infections. In addition, several literatures have pointed out the value of triazoles as antimicrobial agents (Suresh Kumar *et al*., 2010; Creaven *et al*., 2010; Sangshetti and Shinde, 2010) On the other hand, quinoxalines show broad spectrum antibacterial activity (Khan *et al*., 2007). These findings encouraged us for further exploration of [1,2,4]triazolo[4,3-*a*]quinoxaline as antimicrobial agents.

It was reported that the presence of piperazine subunit or its isosteres enhances the antimicrobial activity of the fused triazoles ring systems (Wei *et al*., 2006). Furthermore, the highly potent fluoroquinolone antibiotics, such as norfloxacin and ciprofloxacin contain piperazine moiety (Fig. 1). Based on this hypothesis, [1,2,4]triazolo[4,3-*a*]quinoxaline **2a**, **b** and **4a–d** were deliberated to contain piperazine or piperidine in order to investigate their antimicrobial properties.

Moreover, to increase the bioactivity of [1,2,4]triazolo[4,3-*a*]quinoxaline as antiviral agents, thioamide group is another structural modification that was achieved in this study (Zachariadis *et al*., 2003). Therefore, compounds **8a**, **b** were designed and synthesized as potential antiviral agents.

Furthermore, it was reported that [1,3,4]oxadiazole moiety enhances the antimicrobial activity (Abbas *et al*., 1994). Therefore, We proposed to incorporate different [1,3,4]-oxadiazole and [1,2,4]-triazole subunits onto the [1,2,4]-triazolo[4,3-*a*]quinoxaline ring. Finally, it is known that the arylidene derivatives have antimicrobial properties (Periyasami *et al*., 2008; Pandeya *et al*., 1998). Therefore, certain Schiff's bases were synthesized by the reaction of amino[1,2,4]triazolo[4,3-*a*]quinoxalines with certain aromatic aldehydes. Therefore, this study was aimed at synthesizing [1,2,4]triazolo[4,3-*a*]quinoxalines substituted with piperazine, piperidine, thioamide, [1,3,4]oxadiazole, and [1,2,4]triazole as potential antimicrobial agents.

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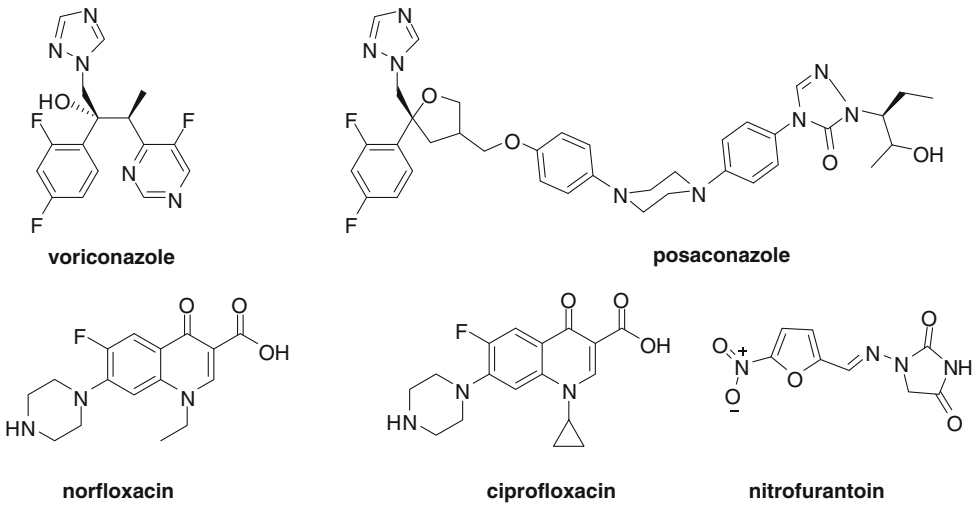


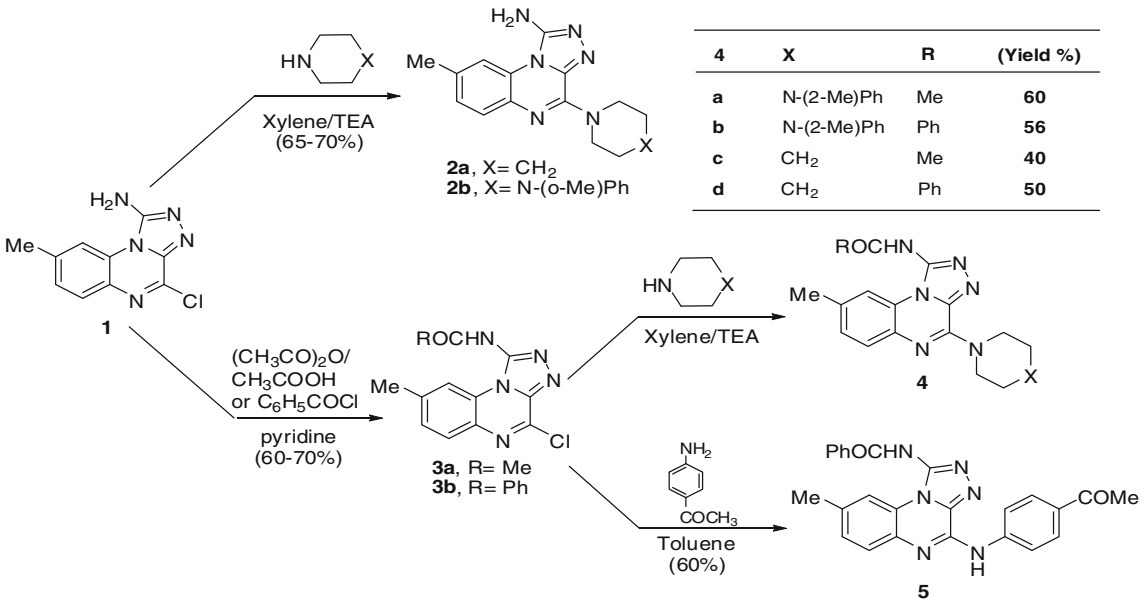
Fig. 1 Fluoroquinolones antibiotics and antifungal drugs

Chemistry

We initiated this study by the reaction of 4-chloro-8-methyl[1,2,4]triazolo[4,3-*a*]quinoxaline-1-amine (**1**) (Sarges *et al*., 1990; Sastry *et al*., 1988) with 1-(*o*-tolyl)piperazine or piperidine in the presence of Et_3N as a base and xylene as a solvent to afford [1,2,4]-triazolo[4,3-*a*]quinoxaline (**2a**, **b**), respectively. Acetylation of compound **1** was achieved by a mixture of acetic anhydride and acetic acid to give compound **3a**. Furthermore, benzoylation of compound **1** was carried out using benzoyl chloride in pyridine to give the compound **3b**.

Compounds **2a**, **b** and **4a**–**d** were prepared by refluxing compounds **3a**, **b** with 1-(*o*-tolyl)piperazine or piperidine in the presence of Et_3N as a base and xylene, respectively. Treatment of compound **3b** with *p*-aminoacetophenone in the presence of Et_3N in toluene afforded compound **5** (Scheme 1).

Several methods were reported for the preparation of thioethers from the reaction of aromatic halides and mercapto derivatives in EtOH/KOH (Muhi-Eldeen *et al*., 1991), $CHCl_3$/TEA (Hirai *et al*., 1991), or DMF/K_2CO_3 (De La Rosa *et al*., 2006). In this investigation, the synthesis of compounds **6a**–**c** was achieved by reacting compound **1** with the appropriate mercapto-1,3,4-oxadiazoles and mercapto-1,2,4-triazole in DMF containing anhydrous K_2CO_3. In a similar way, the title compounds **7a**, **b** were prepared by reaction of compound **1** with the appropriate 3-(substituted



4	X	R	(Yield %)
a	N-(2-Me)Ph	Me	60
b	N-(2-Me)Ph	Ph	56
c	CH_2	Me	40
d	CH_2	Ph	50

Scheme 1 *N*-substitution products of 4-chloro-8-methyl[1,2,4]triazolo[4,3-*a*]quinoxaline-1-amine

benzylideneamino)-5-mercapto-1*H*-1,2,4-triazoles in DMF/K_2CO_3 (Scheme 2).

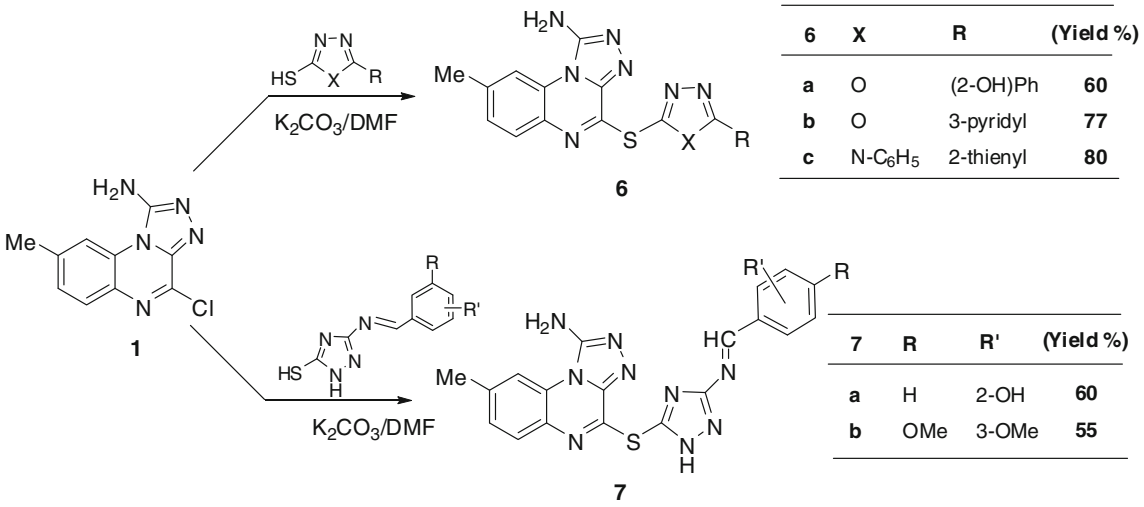
The synthesis of the thioamide derivatives **8a**, **b** was accomplished by the reaction of the amine **1** with the appropriate isothiocyanate derivatives in ethanol. The formation of Schiff's bases **9a–c** was carried out by the reaction of the amine **1** and aromatic aldehydes in EtOH (Love and Ren, 1993; Khalil, 2006; Novikova *et al*., 2005), containing catalytic amount of acetic acid (Scheme 3).

Cyclocondensation reaction of amino[1,2,4]triazolo[4,3-*a*]quinoxaline with dimethyl acetylene dicarboxylate (DMAD) in methanol gave the corresponding methyl 4-oxo-4*H*-pyrimido[2′,1′:5,1][1,2,4]triazolo[4,3-*a*]quinoxaline-2-carboxylate (**10**). Finally, the title compound **11** was prepared via reacting 1,2,4-triazolo[4,3-*a*]quinoxaline-1-amine(**1**) with diethyl ethoxymethylenemalonate (DEEM) in glacial acetic acid (Scheme 4).

Biology

Antiviral and cytotoxicity screening

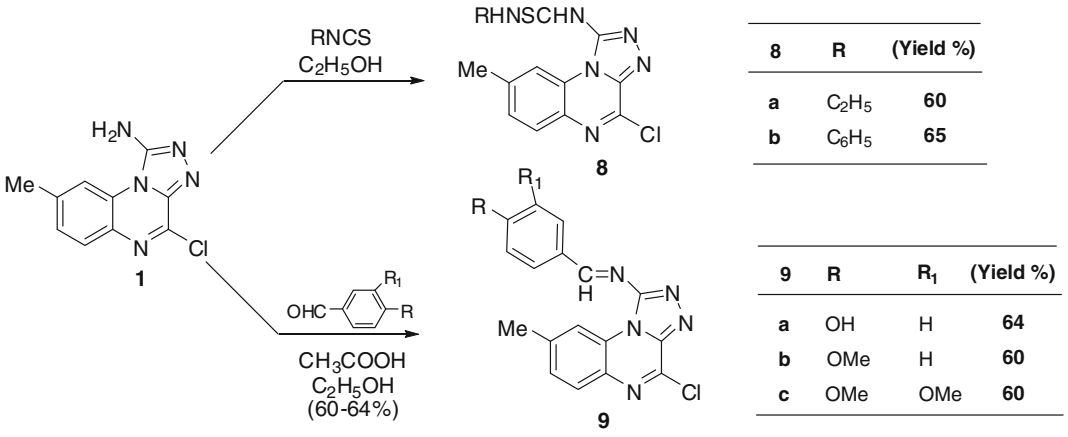
Potential antiviral activity and cytotoxicity using an improved plaque-reduction assay against *Herpes simplex* virus grown on *Vero* African monkey kidney cells (Schmidtke *et al*., 2001; Badria *et al*., 2003) were tested for eleven [1,2,4]triazolo[4,3-*a*]quinoxaline derivatives. The antiviral and cytotoxicity data for these compounds are summarized in Table 1. The greatest antiviral activity among the [1,2,4]triazolo[4,3-*a*]quinoxalines compounds was found for **8b** which showed reduction of the number of the plaques by 25% at 20 mg/ml. On the other hand, the other ten compounds reduced the number of plaques by less than 25% at relatively high concentrations. Moreover, compound **4a** showed the lowest cytotoxicity among the



6	X	R	(Yield %)
a	O	(2-OH)Ph	60
b	O	3-pyridyl	77
c	N-C_6H_5	2-thienyl	80

7	R	R'	(Yield %)
a	H	2-OH	60
b	OMe	3-OMe	55

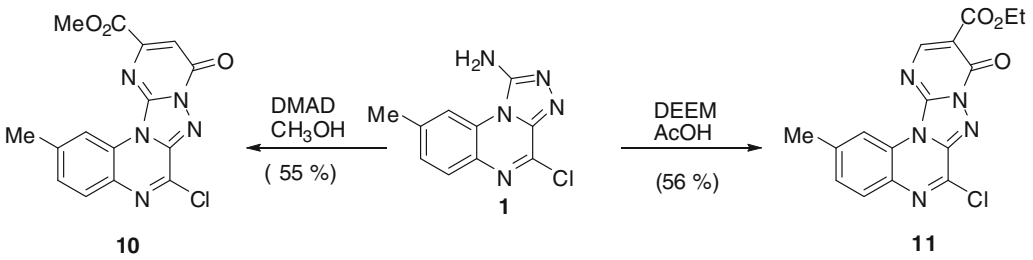
Scheme 2 *S*-substitution products of 4-chloro-8-methyl[1,2,4]triazolo[4,3-*a*]quinoxaline-1-amine



8	R	(Yield %)
a	C_2H_5	60
b	C_6H_5	65

9	R	R_1	(Yield %)
a	OH	H	64
b	OMe	H	60
c	OMe	OMe	60

Scheme 3 Thioamides and Schiff's base formation



Scheme 4 Formation 4-oxo-4*H*-pyrimido[1,2,4]triazolo[4,3-*a*]quinoxaline-2-carboxylate

tested compounds. It can be concluded that the antiviral activity may be due to thiourea moiety and this indicates the importance of the selected moieties for the development of antiviral activity (Table 1).

Antimicrobial screening

[1,2,4]Triazolo[4,3-*a*]quinoxaline derivatives were screened for their potential in vitro antimicrobial activity against Gram-positive (*Bacillus subtilis* and *Staphylococcus aureus*), Gram-negative bacteria (*Escherichia coli*), and fungi (*Candida albicans*) using disk diffusion assay (Smith *et al*., 2006; Espinel-Ingroff, 2007). Ciprofloxacin (50 μg/ml) was used as a reference standard for antibacterial screening, while clotrimazole (1000 μg/ml) was used as a reference standard for antifungal screening. Compounds exhibited moderate activity (inhibition zone $>$ 15 mm) were subjected to further quantitative assay to determine their minimum inhibitory concentrations (MICs) using the agar dilution method (Table 1) (Wiegand *et al*., 2008) against *B. subtilis*, *S. aureus*, *E. coli*, and *C. albicans*, respectively. The antimicrobial data for these compounds are summarized in Table 1.

The greatest antibacterial activity among the [1,2,4]triazolo[4,3-*a*]quinoxaline compounds was found for **4d**, and **6c** which showed strong in vitro activity against *S. aureus and E. coli*, respectively. Compounds **6c**, **7b**, and **8a** showed the greatest activity against *C. albicans* (20–25 mm) with MIC from 78 to 112 μg/l. It could be concluded that compound **8b** which showed the highest antiviral activity did not have good activity as antibacterial or antifungal agent which is expected because of the unique structure of viruses.

In addition, among the [1,2,4]triazolo[4,3-*a*]quinoxaline compounds, five compounds (**2b**, **5**, **6c**, **7b**, and **8a**) showed a good activity. Compound **6c** has comparable activity as ciprofloxacin against *B. subtilis* at MIC levels of 91 μg/ml. Good activity was also demonstrated by the compounds **5**, **6c**, and **8a** against *S. aureus* at MIC from 94 to 124 μg/ml. Compounds **2b**, **5**, **7b**, and **8a** displayed good activity against *E. coli* at MIC from 67 to 102 μg/ml. Finally, it is of interest to note that the piperidine (**2a**, **4d**) or thiotriazole (**6c**, **7b**) substituted [1,2,4]triazolo[4,3-*a*]quinoxaline exhibited the greatest antimicrobial activity.

Experimental

Chemistry

Melting points (°C) were recorded using Fisher-John apparatus and are uncorrected. Elemental analyses were performed at the microanalytical unit, Cairo University, Egypt. IR spectra were recorded on Mattason 5000FT-IR spectrometer (υ in cm^{-1}) using KBr disk. The ^1H NMR and ^{13}C NMR spectrums were recorded on Bruker Ac 250 FT NMR spectrometer (250 MHz) in DMSO-d_6, microanalytical unit, Cairo University. The chemical shifts in ppm are expressed in δ units using DMSO-d_6 as solvent and tetramethylsilane (TMS) as internal reference. Mass spectra were performed on JOEL JMS-600H spectrometer at Cairo University, Egypt. 4-Chloro-8-methyl-1,2,4-triazolo[4,3-*a*]quinoxaline-1-amine (**1**) was prepared previously in the literature (Sarges *et al*., 1990; Sastry *et al*., 1988).

*Synthesis of 4-chloro-8-methyl-[1,2,4]triazolo-[4,3-a]quinoxaline-1-amine (**1**)*

A mixture of 2,3-dichloro-6-methylquinoxaline (1.1 g, 0.005 mol) and thiosemicarbazide (0.9 g, 0.01 mol) in *n*-butanol (20 ml) was heated under reflux for 6 h. The solid product was filtered, washed with water, dried, and recrystallized from ethanol to give **1**. Yield, 75%, mp: 225–230 °C, IR (KBr): ν_{max}, cm^{-1}: 3100 (NH_2), ^1H NMR (DMSO-d_6): δ 2.3 (s, 3H, CH_3), 6.6 (s, 2H, NH_2, D_2O-exchangeable), 7.4–7.8 (m, 3H, Ar–H). MS FAB: *m/z* 234 (31, M^++1), 151 (16.5), 82 (100), Anal. Calcd for $C_{10}H_8ClN_5$ (233.66): C, 51.40; H, 3.45; N, 29.97. Found: C, 51.32; H, 3.72; N, 30.20.

General method of nucleophilic substitution reaction of 4-chloro-8-methyl-[1,2,4]-triazolo-[4,3-*a*]-quinoxaline-1-amine.

A mixture of 1-amino-4-chloro-8-methyl-[1,2,4]-triazolo-[4,3-*a*]-quinoxaline (**1**) (2.3 g, 0.01 mol), 1-*o*-Tolylpiperazine or piperidine (0.01 mol), and Et_3N (1 ml) in xylene (15 ml) was heated under reflux for 24 h. The precipitate

Table 1 Antiviral, cytotoxicity, MIC of [1,2,4]triazolo[4,3-*a*]quinoxalines compounds

Serial no.	Compd. no.	Antiviral		Cytotoxicity	Gram-positive				Gram-negative		Fungi	
		Plaque reduction			*B. subtilis*		*S. aureus*		*E. coli*		*C. albicans*	
		% reduction	MIC (μg/ml)	50CD (μg/ml)[a]	Inhibition zone[a] (mm)	MIC (μg/ml)	Inhibition zone[a] (mm)	MIC (μg/ml)	Inhibition zone[a] (mm)	MIC (μg/ml)	Inhibition zone[a] (mm)	MIC (μg/ml)
1	**2b**	NT		NT	(+)	ND	NT		(+)	(85)	(+)	ND
2	**4a**	NT		>200	NT		NT		NT		NT	
3	**4b**	<25	80	160	NT		NT		NT		NT	
4	**4d**	<25	80	160	NT		(+++)	84	(+)	ND	(+)	ND
5	**5**	NT		NT	(+)	ND	20–15	94	(++)	67	NT	
6	**6a**	<25	80	160	NT		NT		NT		NT	
7	**6b**	<25	80	160	NT		NT		(+)	ND	NT	
8	**6c**	<25	80	160	(++)	91	(++)	124	(+++)	98	(+++)	78
9	**7b**	NT		–	(+)	ND	(+)	ND	(++)	102	(+++)	112
10	**8a**	<25	80	160	(+)	ND	(++)	115	(++)	87	(+++)	89
11	**8b**	25	20	200	NT		NT		NT		NT	
12	**9a**	<25	80	160	NT		NT		NT		NT	
13	**9b**	NT		–	NT		NT		NT		NT	
14	**9c**	<25	80	160	NT		NT		NT		NT	
15	**10**	<25	80	160	NT		NT		NT		NT	
16	**11**	NT		–	NT		15–10	ND	NT		NT	
	Aphidicolin	100	5	20	NT				NT		NT	
	Ciprofloxacin	NT		NT	(++)	62	(++++)	76	(++++)	57	NT	
	Clotrimazole	NT		NT	NT		NT		NT		(++++)	48

NT not tested; *ND* not determined

(+) Inhibition zone (10–15 mm) is slightly active; (++) inhibition zone (15–20 mm) is moderately active; (+++) inhibition zone (20–25 mm) is highly active; (++++) inhibition zone (>25 mm) is strongly active

[a] Degree of activity

was collected by filtration, washed with water, dried, and recrystallized from EtOH/DMF mixture to afford **2a**, **b**.

*Synthesis of 8-methyl-4-(piperidin-1-yl)-[1,2,4]-triazolo[4,3-a]quinoxaline-1-amine (**2a**)*

Yield, 65%, mp: 237–239°C; IR (KBr): ν_{max}, cm^{-1}: 2950 (CH), 3400 (NH_2), ^1H NMR (DMSO-d_6): δ 2.2 (s, 3H, CH_3), 3.2–3.7 (m, 10H, piperidine-H), 6.3 (s, 2H, NH_2, D_2O-exchangeable), 7.1–7.6 (m, 3H, Ar–H). ^{13}C NMR (DMSO-d_6): δ 17.9, 21.2, 48.6, 49.3, 118.6, 121.7, 135.4, 151, 155, 167.1. MS FAB: *m/z* 283 (16, M^++1), 198 (100), 175 (34), Anal. Calcd for $C_{21}H_{23}N_7$ (282.34): C, 63.81; H, 6.43; N, 29.77. Found: C, 64.11; H, 6.26; N, 29.97.

*Synthesis of 8-methyl-4-(4-o-tolylpiperazin-1-yl)-[1,2,4]-triazolo-[4,3-a]-quinoxaline-1-amine (**2b**)*

Yield, 70%, mp: 215–217°C, IR (KBr): ν_{max}, cm^{-1}: 2920 (CH), 3450 (NH_2), ^1H NMR (DMSO-d_6): δ 1.9 (s, 3H, tolyl-CH_3), 2.5 (s, 3H, CH_3), 3.2–3.6 (m, 8H, piperazine-H), 6.8 (s, 2H, NH_2, D_2O-exchangeable), 6.9–7.2 (m, 4H, Ar–H), 7.8–8.1 (m, 3H, Ar–H). ^{13}C NMR (DMSO-d_6): δ 21.3, 24.5, 25.3, 52, 127.4, 129, 135.1, 135.8, 155.9. MS FAB: *m/z* 273 (36, M^+), 198 (100), 175 (25), Anal. Calcd for $C_{21}H_{23}N_7$ (373.45): C, 67.54; H, 6.21; N, 26.25. Found: C, 67.83; H, 6.50; N, 25.97.

*Synthesis of N-(4-chloro-8-methyl[1,2,4]triazolo-[4,3-a]-quinoxalin-1-yl)acetamide (**3a**)*

4-Chloro-8-methyl-[1,2,4]-triazolo-[4,3-*a*]-quinoxaline-1-amine (**1**) (2.3 g, 0.01 mol) was added to a mixture of acetic anhydride (20 ml) and acetic acid (10 ml). The mixture was refluxed for 2 h, cooled, and poured onto ice-water. The formed solid was collected by filtration, dried, and recrystallized from absolute EtOH to yield compound **3a**. Yield, 70%, mp: 122–125°C, IR (KBr): ν_{max}, cm^{-1}: 1652 (CO, amide), 3600 (NH, amide), ^1H NMR (DMSO-d_6): δ 2.1 (s, 3H, $COCH_3$), 2.4 (s, 3H, CH_3), 7.4–7.8 (m, 3H, Ar–H), 9.2 (s, 1H, NH, D_2O-exchangeable), ^{13}C NMR (DMSO-d_6): δ 21.3, 24, 125.7, 127.4, 132.8, 135.1, 145.9, 149.7, 157.2. MS FAB: *m/z* 277 (58, M^++2), 151 (11), 124 (100), Anal. Calcd for $C_{12}H_{10}ClN_5O$ (275.69): C, 52.28; H, 3.66; N, 25.40. Found: C, 52.73; H, 3.60; N, 25.20.

*Synthesis of N-(4-chloro-8-methyl-[1,2,4]-triazolo[4,3-a]quinoxalin-1-yl)benzamide (**3b**)*

To a mixture of 4-chloro-8-methyl-[1,2,4]-triazolo[4,3-*a*]quinoxaline-1-amine (**1**) (2.3 g, 0.01 mol) and pyridine (0.79 g, 0.01 mol), benzoyl chloride (1.4 g, 0.01 mol) was added and the mixture was stirred at 0°C over a period of 30 min. The mixture was extracted with chloroform, and the organic layer was washed with water and 5% HCl. The chloroform layer was dried over anhydrous magnesium sulfate and evaporated to afford **3b**. Yield, 60%, mp: 110°C, IR (KBr): ν_{max}, cm^{-1}: 1640 (CO, amide), 3320 (NH, amide), ^1H NMR (DMSO-d_6): δ 2.5 (s, 3H, CH_3), 7.4–7.6 (m, 3H, Ar–H), 7.6–8.1 (m, 5H, Ar–H), 9.4 (s, 1H, NH, D_2O-exchangeable), ^{13}C NMR (DMSO-d_6): δ 21.7, 125.7, 127.5, 132, 134.9, 138.6, 145.4, 149.3, 157, 165.1. MS FAB: *m/z* 339 (41, M^++2), 217 (100), 120 (21), Anal. Calcd for $C_{17}H_{12}ClN_5O$ (337.76): C, 60.45; H, 3.58; N, 20.73. Found: C, 60.23; H, 3.90; N, 21.01.

General method of nucleophilic substitution reaction of *N*-acyl-4-chloro-8-methyl-[1,2,4]-triazolo[4,3-a]quinoxaline-1-amine (**3a**, **b**)

A mixture of *N*-acyl-4-chloro-8-methyl[1,2,4]triazolo[4,3-a]quinoxaline-1-amine (**3a**, **b**) (0.005 mol), 1-*o*-Tolylpiperazine or piperidine (0.005 mol), and Et_3N (0.5 ml) in xylene (10 ml) was refluxed for 24 h. The solid product was collected by filtration, washed with water, dried, and recrystallized from EtOH to furnish **4a–d**.

*Synthesis of N-[8-methyl-4-(4-o-tolylpiperazin-1-yl)[1,2,4]triazolo[4,3-a]quinoxalin-1-yl]acetamide (**4a**)*

Yield, 60%, mp: 250–252°C, IR (KBr): ν_{max}, cm^{-1}: 1640 (CO, amide), 2910 (C–H), 3315 (NH, amide), ^1H NMR (DMSO-d_6): δ 2.1 (s, 3H, COCH3), 2.2 (s, 3H, tolyl-CH_3), 2.5 (s, 3H, CH_3), 3.1 (m, 8H, piperazine-H), 6.9–7.2 (m, 4H, Ar–H), 7.7–7.9 (m, 3H, Ar–H), 9.5 (s, 1H, NH, D_2O-exchangeable). ^{13}C NMR (DMSO-d_6): δ 17.9, 21.3, 24.9, 52.6, 121.9, 128.4, 135.4, 142.6, 167.1. MS FAB: *m/z* 416 (1.54, M^++1), 341 (100), 325 (8.71), 240 (2.30), 175 (4.26), 91 (24.43), 58 (3.57). Anal. Calcd for $C_{23}H_{25}N_7O$ (415.49): C, 66.49; H, 6.06; N, 23.60. Found: C, 66.53; H, 5.91; N, 23.56.

*Synthesis of N-[8-methyl-4-(4-o-tolylpiperazin-1-yl)[1,2,4]triazolo[4,3-a]quinoxalin-1-yl]benzamide (**4b**)*

Yield, 56%, mp: >300°C, IR (KBr): ν_{max}, cm^{-1}: 1630 (CO, amide), 2920 (C–H), 3315 (NH, amide), ^1H NMR (DMSO-d_6): δ 2.3 (s, 3H, tolyl-CH_3), 2.4 (s, 3H, CH_3), 3.4 (m, 8H, piperazine-H), 6.8–7.2 (m, 4H, Ar–H), 7.4–7.6 (m, 5H, Ar–H), 7.7–7.9 (m, 3H, Ar–H), 9.1 (s, 1H, NH, D_2O-exchangeable), ^{13}C NMR (DMSO-d_6): δ 17.2, 24, 48.9, 49.9, 121.4, 135.7, 142.6, 151.3, 157.8. MS FAB: m/z 477 (27.8, M +), 241 (100), 174 (13), Anal. Calcd for $C_{28}H_{27}N_7O$ (477.56): C, 70.42; H, 5.70; N, 20.53. Found: C, 70.52; H, 5.32; N, 20.96.

*Synthesis of N-[8-methyl-4-(piperidin-1-yl)[1,2,4]triazolo[4,3-a]quinoxalin-1-yl]acetamide (**4c**)*

Yield, 40%, mp: 231–233°C, IR (KBr): ν_{max}, cm^{-1}: 1650 (CO, amide), 2910 (C–H), 3300 (NH, amide), ^1H NMR (DMSO-d_6): δ 2.2 (s, 3H, $COCH_3$), 2.6 (s, 3H, CH_3), 3.8 (m, 10H, piperidine-H), 7.5–7.7 (m, 3H, Ar–H), 9.3 (s, 1H, NH, D_2O-exchangeable), ^{13}C NMR (DMSO-d_6): δ 24.5, 25.5, 52.7, 121.5, 127.4, 128.2, 132.4, 135.8, 142.6, 157.6, 167.1. MS FAB: *m/z* 326 (4.13, M^++2), 325 (4.39, M^++1), 170 (5.68), 104 (100), 84 (7.24), 58 (5.68). Anal. Calcd for $C_{17}H_{20}N_6O$ (324.38): C, 62.95; H, 6.21; N, 25.91. Found: C, 62.52; H, 6.05; N, 26.11.

*Synthesis of N-[8-methyl-4-(piperidin-1-yl)[1,2,4]triazolo[4,3-a]quinoxalin-1-yl]benzamide (**4d**)*

Yield, 50%, mp: 240–242°C, IR (KBr): ν_{max}, cm^{-1}: 1653 (CO, amide), 2910 (C–H), 3304 (NH, amide), ^1H NMR (DMSO-d_6): δ 2.7 (s, 3H, CH3), 3.1–3.3 (m, 10H, piperidine-H), 7.4–7.6 (m, 3H, Ar–H), 7.7–8.2 (m, 5H, Ar–H), 9.5 (s, 1H, NH, D_2O-exchangeable), ^{13}C NMR (DMSO-d_6): δ 21.3, 48.2, 49.4, 118.7, 123.1, 128.4, 129, 132.4, 135.6, 165.7, 167. MS FAB: *m/z* 386 (3.55, M^+), 264 (5.67), 120 (5.51), 84 (34.20), 69 (100). Anal. Calcd for $C_{22}H_{22}N_6O$ (386.45): C, 68.38; H, 5.74; N, 21.75. Found: C, 68.56; H, 5.71; N, 21.94.

*Synthesis of N-[4-(4-actophenylamino)-8-methyl[1,2,4]triazolo[4,3-a]quinoxalin-1-yl]benzamide (**5**)*

A mixture of *N*-(4-chloro-8-methyl[1,2,4]triazolo[4,3-*a*]quinoxalin-1-yl)benzamide (**3b**) (1.7 g, 0.005 mol), 4-Aminoacetophenone (0.7 g, 0.005 mol), and Et_3N (0.5 ml) in toluene (12 ml) was heated under reflux for 7 h. The solvent was evaporated, and the remaining solid was washed with water, dried, and recrystallized from EtOH to afford **5**. Yield, 60%, mp: >300°C, IR (KBr): ν_{max}, cm^{-1}: 1653 (CO, amide), 3214 (NH), 3304 (NH, amide), ^1H NMR (DMSO-d_6): δ 2.5 (s, 3H, CH_3), 2.6 (s, 3H, $CO\underline{CH_3}$), 7.5–7.9 (m, 12H, Ar–H), 8.3 (s, 1H, NH, D_2O-exchangeable), 8.4 (s, 1H, NH, D_2O-exchangeable). ^{13}C NMR (DMSO-d_6): δ 21.2, 26.4, 111.8, 125.3, 127.4, 131, 133.8, 140.1, 145.9, 157.1, 165, 196.7. MS FAB: *m/z* 437 (32, M^++1), 304 (42), 133 (100). Anal. Calcd for $C_{25}H_{20}N_6O_2$ (436.47): C, 68.80; H, 4.62; N, 19.25. Found: C, 68.54; H, 4.50; N, 19.41.

General method of thioether formation (**6a–c**)

A mixture of 4-chloro-8-methyl-[1,2,4]-triazolo[4,3-*a*] quinoxaline-1-amine (**1**) (2.3 g, 0.01 mol), the appropriate mercapto oxadiazole or mercapto triazole (0.01 mol), and anhydrous K_2CO_3 (1.4 g, 0.01 mol) in DMF (30 ml) was stirred for overnight. The reaction mixture was poured onto ice-water. The solid was collected by filtration, washed with water, and recrystallized from DMF/water mixture afforded **6a–c**.

*Synthesis of 1-amino-8-methyl-4-(5-(2-hydroxyphenyl)-1,3,4-oxadiazol-2-yl)-1,2,4-triazolo[4,3-a]quinoaxalines (**6a**)*

Yield, 60%, mp: 230–232°C, IR (KBr): ν_{max}, cm^{-1}:3224 (OH); 3502 (NH_2), ^1H NMR (DMSO-d_6): δ 2.3 (s, 3H, CH_3), 6.9 (s, 2H, NH_2, D_2O-exchangeable), 7.1–7.3 (m, 4H, Ar–H), 7.5–7.8 (m, 3H, Ar–H), 10.5 (s, 1H, OH, D_2O-exchangeable). ^{13}C NMR (DMSO-d_6): δ 21.4, 108, 117.8, 121.4, 126.8, 138.7, 142, 150.7, 156.9, 164.7. MS FAB: *m/z* 393 (12, M^++2), 198 (100), 197 (31). Anal. Calcd for $C_{18}H_{13}N_7O_2S$ (391.41): C, 55.23; H, 3.35; N, 25.05. Found: C, 54.96; H, 3.80; N, 25.32.

*Synthesis of 8-methyl-4-[5-(pyridin-3-yl)-1,3,4-oxadiazol-2-ylthio)[1,2,4]triazolo[4,3-a]quinoaxaline-1-amine (**6b**)*

Yield, 77%, mp: 250–252°C, IR (KBr): ν_{max}, cm^{-1}: 3513 (NH_2), ^1H NMR (DMSO-d_6): δ 2.5 (s, 3H, CH_3), 6.9 (s, 2H, NH_2, D_2O-exchangeable), 7.6–7.8 (m, 3H, Ar–H), 8.1–8.5 (m, 4H, pyridine-H). ^{13}C NMR (DMSO-d_6): δ 21.4, 108.2, 117, 121.7, 126.2, 138.7, 140, 150.2, 157.4, 164.9. MS FAB: *m/z* 378 (24, M^++2), 300 (100), 198 (33), 180 (23), 78 (12.3). Anal. Calcd for $C_{17}H_{12}N_8OS$ (376.40): C, 54.25; H, 3.21; N, 29.77. Found: C, 54.21; H, 3.56; N, 30.12.

*Synthesis of 8-methyl-4-[(4-phenyl-5-(thien-2-yl)-4H-1,2,4-triazol-3-yl)thio][1,2,4]triazolo[4,3-a] quinoxaline-1-amine (**6c**)*

Yield, 80%, mp: 210–212°C, IR (KBr): ν_{max}, cm^{-1}: 3514 (NH_2), ^1H NMR (DMSO-d_6): δ 2.3 (s, 3H, CH_3), 6.7 (s, 2H, NH_2, D_2O-exchangeable), 7.5–7.9 (m, 8H, Ar–H; 3H, thiophen-H). ^{13}C NMR (DMSO-d_6): δ 21.7, 125.6, 128.3, 129.6, 138.4, 140, 143.8, 150.4, 152.1, 155.9, 161.1. MS FAB: m/z 458 (24, M^++2), 260 (100), 199 (32.5). Anal. Calcd for $C_{22}H_{16}N_8S_2$ (456.55): C, 57.88; H, 3.53; N, 24.54. Found: C, 57.98; H, 3.77; N, 24.90.

General method of thioether formation (**7a–b**)

A mixture of 4-chloro-8-methyl-1,2,4-triazolo[4,3-*a*]quinoxaline-1-amine (**1**) (2.3 g, 0.01 mol), the appropriate 3-(substituted benzylideneamino)-5-mercapto-1*H*-1,2,4-triazoles (0.01 mol), and anhydrous potassium carbonate (1.4 g, 0.01 mol) in DMF (50 ml) was stirred overnight.

The reaction mixture was poured onto ice-water. The formed solid was collected by filtration, washed with water, and recrystallized from DMF/water mixture to furnish **7a**, **b**.

*Synthesis of 8-methyl-4-[(3-(2-hydroxy)benzylideneamino)-1H-[1,2,4]-triazolo-5-yl)thio]-1,2,4-triazolo[4,3-a]quinoxaline-1-amine (**7a**)*

Yield, 60%, mp: 295–298°C, IR (KBr): ν_{max}, cm^{-1}:1665 (C=N), 2690 (=CH), 3200 (OH), 3415 (NH_2), ^1H NMR (DMSO-d_6): δ 2.9 (s, 3H, CH_3), 6.8 (s, 2H, NH_2, D_2O-exchangeable), 7.2–7.9 (m, 7H, Ar–H), 8.6 (s, 1H, N=CH), 12.2 (s, 1H, OH, D_2O-exchangeable), 12.5 (s, 1H, triazole-H). ^{13}C NMR (DMSO-d_6): δ 21.1, 117.4, 121, 127.4, 128.3, 132.1, 132.7, 134.4, 138.7, 150.8, 155.9, 158.1, 159.3, 160.8. MS FAB: *m/z* 419 (31, M^++2), 221 (15), 198 (100). Calcd for $C_{19}H_{15}N_9OS$ (417.45): C, 54.67; H, 3.62; N, 30.20. Found: C, 54.97; H, 3.55; N, 30.23.

*Synthesis of 8-methyl-4-{[3-(3,4-dimethoxy)benzylideneamino]-1H-[1,2,4]-triazolo-5-yl)thio]-[1,2,4]-triazolo[4,3-a]quinoxaline-1-amine (**7b**)*

Yield, 55%, mp: 281–283°C, IR (KBr): ν_{max}, cm^{-1}: 1659 (C=N), 2693 (=CH), 3510 (NH_2), ^1H NMR (DMSO-d_6): δ 2.5 (s, 3H, CH_3), 3.4 (s, 6H, OCH_3), 6.9 (s, 2H, NH_2, D_2O-exchangeable), 7.2–7.7 (m, 6H, Ar–H), 8.4 (s, 1H, N = CH), 12.3 (s, 1H, triazole-H). ^{13}C NMR (DMSO-d_6): δ 21.7, 56.1, 56.4, 109.6, 11, 125.1, 128.3, 128.9, 130.1, 138.7, 150.1, 155.8, 158.1, 159.6, 161. MS FAB: m/z 462 (9.5, M^++1), 232 (0.36), 230 (100). Anal. Calcd for $C_{21}H_{19}N_9O_2S$ (461.50): C, 54.65; H, 4.15; N, 27.32. Found: C, 55.05; H, 4.10; N, 27.43.

General method of preparation of thioureas derivatives (**8a**, **b**)

A mixture of 4-chloro-8-methyl[1,2,4]triazolo[4,3-*a*]quinoxaline-1-amine (**1**) (2.3 g, 0.01 mol) and appropriate isothiocyante derivatives (0.01 mol) in EtOH (15 ml) was refluxed for 6 h. On cooling, the separated solid was collected by filtration, dried, and recrystallized.

*Synthesis of 1-(4-chloro-8-methyl[1,2,4]triazolo[4,3-a]quinoxalin-1-yl)-3-ethyl thiourea (**8a**)*

Yield, 60%, mp: 160–162°C, IR (KBr): ν_{max}, cm^{-1}: 1050 (C=S), 3415,3490 (NH), ^1H NMR (DMSO-d_6): δ 1.2 (t, 3H, CH_3-ethyl), 2.0 (s, 1H, NH), 2.3 (s, 3H, CH_3), 4.1 (s, 1H, NH), 4.3 (q, 2H, CH_2-ethyl), 7.4–7.8 (m, 3H, Ar–H), ^{13}C NMR (DMSO-d_6): δ 15.2, 21.7, 40.6, 127.5, 128.8, 132.4, 136.1, 145.3, 148.9, 157.9, 179.2. MS FAB: *m/z* 323 (0.53, M^++2), 232 (0.56), 217 (0.92), 205 (100), 103 (7.38), 88 (5.40). Anal. Calcd for $C_{13}H_{13}ClN_6S$ (320.80): C, 48.67; H, 4.08; N, 26.20. Found: C, 48.54; H, 3.96; N, 26.71.

*Synthesis of 1-(4-chloro-8-methyl[1,2,4]triazolo[4,3-a]quinoxalin-1-yl)-3-phenyl thiourea (**8b**)*

Yield, 65%, mp: 192–193°C, IR (KBr): ν_{max}, cm^{-1}: 1050 (C=S), 3435, 3512 (NH), ^1H NMR (DMSO-d_6): δ 2.4 (s, 3H, CH_3), 3.5 (s, 1H, NH), 6.8–7.2 (m, 5H, Ar–H), 7.4–7.8 (m, 3H, Ar–H), 8.2 (s, 1H, NH), ^{13}C NMR (DMSO-d_6): δ 22, 126.4, 127.4, 128.9, 129.4, 133.1, 138.1, 138.9, 145.2, 149.4, 179.4. MS FAB: *m/z* 368 (3.34, M^+), 217 (32.56), 191 (2.03), 151 (100), 136 (12.78). Anal. Calcd for $C_{17}H_{13}ClN_6S$ (368.84): C, 55.36; H, 3.55; N, 22.78. Found: C, 55.76; H, 3.92; N, 22.20.

General method of Schiff's base formation (**9a–c**)

A mixture of 4-chloro-8-methyl[1,2,4]triazolo[4,3-*a*]quinoxaline-1-amine (**1**) (0.01 mol) and the appropriate benzaldehyde derivatives (0.01 mol) were dissolved in EtOH (30 ml) containing catalytic amount of acetic acid (1 ml). The reaction mixture was refluxed for 4 h, and the organic solvent was evaporated. The resulted solid was dried and recrystallized from EtOH to afford **9a–c**.

*Synthesis of 4-chloro-1-(4-hydroxybenzylideneamino)-8-methyl[1,2,4]triazolo[4,3-a]quinoxalines (**9a**)*

Yield, 64%, mp: 194–196°C, IR (KBr): ν_{max}, cm^{-1}: 1675 (C=N), 2709 (=CH), 3224 (OH), ^1H NMR (DMSO-d_6): δ 2.4 (s, 3H, CH_3), 5.3 (s, 1H, OH), 6.8–7.4 (m, 4H, Ar–H), 7.5–7.8 (m, 3H, Ar–H), 8.6 (s, 1H, N=CH), ^{13}C NMR (DMSO-d_6): δ 21.7, 116.7, 127.4, 128.3, 129.6, 130.9, 135.5, 138.1, 145.3, 149, 153.5, 160.6. MS FAB: *m/z* 337 (22.02, M^+), 217 (9.01), 168 (0.23), 146 (100), 120 (21.60). Anal. Calcd for $C_{17}H_{12}ClN_5O$ (337.76): C, 60.45; H, 3.58; N, 20.73. Found: C, 60.23; H, 3.67; N, 20.79.

*Synthesis of 4-chloro-8-methyl-1-(4-methoxybenzylideneamino)[1,2,4]triazolo[4,3-a]quinoxalines (**9b**)*

Yield, 60%, mp: 215–217°C, IR (KBr): ν_{max}, cm^{-1}: 1663 (C=N), 2686 (=CH), ^1H NMR (DMSO-d_6): δ 2.5 (s, 3H, CH_3), 3.3 (s, 3H, OCH_3), 7.1–7.7 (m, 7H, Ar–H), 9.8 (s, N=CH). ^{13}C NMR (DMSO-d_6): δ 20.9, 55.7, 113, 125.6, 128.4, 135.7, 132, 145.6, 154. MS FAB: *m/z* 351 (22, M^+), 218 (1.2), 134 (100), Anal. Calcd for $C_{18}H_{14}ClN_5O$ (351.79): C, 61.46; H, 4.01; N, 19.91. Found: C, 61.35; H, 3.97; N, 19.47.

*Synthesis of 4-chloro-8-methyl-1-(3,4-dimethoxybenzylideneamino)-[1,2,4]-triazolo[4,3-a]quinoxalines (**9c**)*

Yield, 60%, mp: >300°C, IR (KBr): ν_{max}, cm^{-1}: 1673 (C=N), 2680 (=CH), ^{1}H NMR (DMSO-d_6): δ 2.2 (s, 3H, CH_3), 3.1 (s, 6H, OCH_3), 7.1–7.4 (m, 3H, Ar–H), 7.6–7.9 (m, 3H, Ar–H), 9.5 (s, N=CH). ^{13}C NMR (DMSO-d_6): δ 21.4, 56.4, 56.8, 109.1, 111.1, 128, 131.1, 132.7, 135.4, 138.4, 145.1, 149.2, 152.3. MS FAB: *m/z* 381 (40.7, M^+), 217 (3), 164 (100), Anal. Calcd for $C_{19}H_{16}ClN_5O_2$ (381.82): C, 59.77; H, 4.22; N, 18.34. Found: C, 60.06; H, 4.25; N, 18.01.

*Synthesis of methyl 7-chloro-11-methyl-4-oxo-4H-pyrimido[2′,1′:5,1][1,2,4]triazolo[4,3-a]quinoxaline-2-carboxylate (**10**)*

Dimethyl acetylenedicarboxylate (1.4 g, 0.01 mol) was added dropwise to a stirred solution of 4-chloro-8-methyl-1,2,4-triazolo[4,3-*a*]quinoxaline-1-amine (**1**) (2.3 g, 0.01 mol) in methanol (20 ml). The reaction mixture was refluxed for 5 h, and the solvent was evaporated. The product was dried and recrystallized from EtOH to give **10**. Yield, 55%, mp: 160–162°C, IR (KBr): ν_{max}, cm^{-1}: 1760 (C=O, ester), 1731 (C=O; ketone), ^{1}H NMR (DMSO-d_6): δ 2.3 (s, 3H, CH_3), 3.8 (s, 3H, OCH_3), 6.9–7.1 (m, 3H, Ar–H), 8.8 (s, 1H, pyrimidine-H), ^{13}C NMR (DMSO-d_6): δ 21.7, 52.4, 102.4, 117.2, 134, 137.1, 139, 142.6, 148.8, 153.1, 169, 174.1. MS FAB: *m/z* 344 (18, M^++1), 179 (100), 169 (41). Anal. Calcd for $C_{15}H_{10}ClN_5O_3$ (343.72): C, 52.41; H, 2.93; N, 20.37. Found: C, 52.75; H, 2.67; N, 20.17.

*Synthesis of ethyl 7-chloro-11-methyl-4-oxo-4H-pyrimido[2′,1’:5,1][1,2,4]triazolo[4,3-a]quinoxaline-3-carboxylate (**11**)*

Diethyl ethoxymethylenemalonate (2.1 g, 0.01 mol) was added dropwise to a stirred solution of 4-chloro-8-methyl-1,2,4-triazolo[4,3-*a*]quinoxaline-1-amine (**1**) (2.3 g, 0.01 mol) in glacial acetic acid (25 ml). The reaction mixture was refluxed for 3 h and left to attain room temperature. The precipitated solid was filtered, dried, and recrystallized from acetic acid to afford the desired compound **11**. Yield, 56%, mp: 210–213°C, IR (KBr): ν_{max}, cm^{-1}: 1767 (C=O, ester), 1742 (C=O; ketone), ^{1}H NMR (DMSO-d_6): δ 1.4 (t, 3H, CH_3-ethyl), 2.3 (s, 3H, CH3), 4.2 (q, 2H, CH_2-ethyl), 6.9–7.2 (m, 3H, Ar–H), 9.2 (s, 1H, pyrimidine-H), ^{13}C NMR (DMSO-d_6): δ 14.1, 21.6, 61.9, 113, 118.1, 135.1, 136.4, 137.2, 141, 143.1, 150.1, 152.8, 163.4, 167.1. MS FAB: *m/z* 357 (32.02, M^+), 284 (16.09), 219 (7.17), 138 (100), 73 (34.17). Anal. Calcd for $C_{16}H_{12}ClN_5O_3$ (357.75): C, 53.72; H, 3.38; N, 19.58. Found: C, 53.63; H, 3.12; N, 19.91.

Biology

Antiviral and cytotoxicity screening

Preparation of synthetic drug solutions Samples were prepared by dissolving in 50 µl of DMSO and diluting aliquots into sterile culture medium. These solutions were sub-diluted in sterile medium and the two solutions used as stocks to test samples at 2–200 µg/ml in triplicate in the wells of micotiter plates.

Virus and cell culture *H. simplex* type I (HS-I) stock was prepared as aliquots of culture medium from *Vero* cells infected at multiplicity of 1 virion per 10 cells and cultured 3 days. They were stored at −80°C.

Working stocks were prepared by titrating virus by serial dilution in culture medium and assayed in triplicate on *Vero* monolayers in the wells of microtiter trays. Virus suspensions that gave about 30 plaques per well were stored at 4°C until used. *Vero* African green monkey kidney cells were purchased from Viromed Laboratories, Minnetonka, MN, and grew in Dulbeccois modified Eagle's medium supplemented with 10% (v/v) calf serum (HyClone Laboratories, Ogden, UT), 60 µg/ml streptomycin sulfate maintained at 37°C in a humidified atmosphere containing about 15% (v/v) CO_2 in air.

All medium components were obtained from Sigma Chemical Co., St. Louis, MO, unless otherwise indicated. *Vero* stocks were maintained at 34°C in culture flasks filled with medium supplemented with 1% (v/v) calf serum. Subculture for virus titration or antiviral screening were grown in the wells of microtiter trays (Falcon Microtest III 96-wells tray, Becton–Dickinson Labware, Lincolin Park, NJ) by suspending *Vero* cells in medium following trypsin–EDTA treatment, counting the suspension with a hemocyto-meter, diluting in medium containing 10% calf serum to 2×10^4 cells per 200 ml culture, aliquoting into each well of a tray, and culturing until confluent.

Antiviral screening procedures Microtiter trays with confluent monolayer culture of *Vero* cells were inverted. The medium was shaken out and replaced with serial dilutions of sterile extracts of samples in triplicate in 100 µl medium followed by tittered virus in 100 µl medium containing 10% (v/v) calf serum in each well. Aphidicolin was used as positive control.

In each tray, the last row of wells was reserved for controls that were not treated with compounds nor with virus. The trays were cultured for 66 h then inverted into a pad of paper towels. The remaining cells rinsed carefully

with medium and fixed with 3.7% (v/v) formaldehyde in saline for at least 20 min. The fixed cells were rinsed with water and examined visually.

Antiviral activity is identified as confluent, relatively unaltered monolayers of stained *Vero* cells treated with HS-1. Furthermore, cytotoxicity was estimated as the concentration that caused approximately 50% loss of the monolayer present around the plaques caused by HSV-1. Observed data are shown in Table 1.

Antimicrobial screening

Whatman No. 1 filter paper disks of 5 mm diameter sterilized by autoclaving for 15 min at 121°C. The sterile disks were impregnated with the DMSO-solutions of the test compounds (500 μg/disk). Agar plates (Muller Hinton agar for bacteria and Sabouraud dextrose agar medium for fungi) were prepared by pouring a suitable volume of autoclaved melted agar into each 75 mm Petri plates. The volume of nutrient agar was enough to keep its depth at approximately 6 mm. The agar plates were allowed to cool at room temperature and then surface inoculated with standard inoculums (10^5 cells/ml broth) of the test organisms (local strains) namely; *B. subtilis*, *S. aureus*, *E. coli*, and *C. albicans*.

The impregnated disks were placed on the agar plates media, suitably spaced apart, and the plates were incubated at 5°C for 1 h to permit good diffusion and then transferred to an incubator at 37°C for 24 h for bacteria and at 28°C for 72 h for fungi, then examined for the inhibition zones caused by various compounds on the tested microorganisms. The zones of inhibition were measured using a caliber, to the nearest mm. Ciprofloxacin (50 μg/ml) was used as a reference standard for antibacterial screening, while clotrimazole (1000 μg/ml) was used as a reference standard for antifungal screening. Data are shown in Table 1.

MIC

MIC of the compounds was determined by agar dilution method. Stock solutions of the synthesized compounds (100 μg/ml) in DMSO were prepared, and graded quantities were incorporated in specified quantity of molten sterile agar (Muller Hinton agar for anti-bacterial activity and Sabouraud dextrose agar medium for anti-fungal activity). A specified quantity of the medium (40–50°C) containing each compound was poured into a Petri dish to give a depth of 3-4 mm and allowed to solidify. Suspension of the micro-organism was prepared to contain approximately 105 cfu/ml and applied to plates with serially diluted compounds in DMSO to be tested and incubated at 37°C for 24 and 48 h for bacteria and fungi, respectively. The MIC was considered to be the lowest concentration of the test substance exhibiting no visible growth. Data are shown in Table 1.

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