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ORIGINAL ARTICLE

Design and synthesis of some novel 2-(3-methyl-2-oxoquinoxalin-1(2*H*)-yl)-*N*-(4-(substituted)phenyl) acetamide derivatives for biological evaluation as anticonvulsant agents

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2-(3-Methyl-2-oxoquinoxalin-1(2*H*)-yl)acetamide;
Quinoxaline;
Acetamide;
Anticonvulsant agent

Abstract A new series of 2-(3-methyl-2-oxoquinoxalin-1(2*H*)-yl)-*N*-(4-substitutedphenyl)acetamides (**2**–**15**$_{a–c}$) were designed and synthesized in order to evaluate their anticonvulsant activity. The structure of the synthesized compounds was confirmed by elemental analysis and spectral data (IR, ^{1}H NMR and Mass). The data obtained from biological screening revealed that; compounds **9**$_c$ and **8**$_c$ showed the highest anticonvulsant activities in experimental mice.

1. Introduction

Epilepsy, a common chronic neurological disorder that is characterized by recurrent unprovoked seizures, inflicts more than 60 million people worldwide according to epidemiological studies.[1]

Every year approximately 250,000 new cases are added to this figure. It is roughly estimated that 28–30% of patients are resistant to the available medical therapies.[2] It is important to emphasize that epileptic patients should use the anticonvulsant drugs continuously for years. Hence the chronic toxicity of such a long term is of particular importance.[3] The goal of an anticonvulsant is to suppress the rapid and excessive firing of neurons that start a seizure.[4]

Despite the development of several new anticonvulsants, the treatment of epilepsy remains still inadequate, and the patients suffer from a lot of specific problems like neurotoxicity, depression and other CNS related diseases. Moreover many antiepileptic drugs have serious side effects and lifelong medication may be required. Therefore, it is essential to search for newer and potent chemical entities for the treatment of epilepsy.[5]

Drugs in each therapeutic classification frequently show some common basic chemical structure, it is the hope of the chemist that, by the addition of various side chains or a combination of side chains or by somewhat altering the basic

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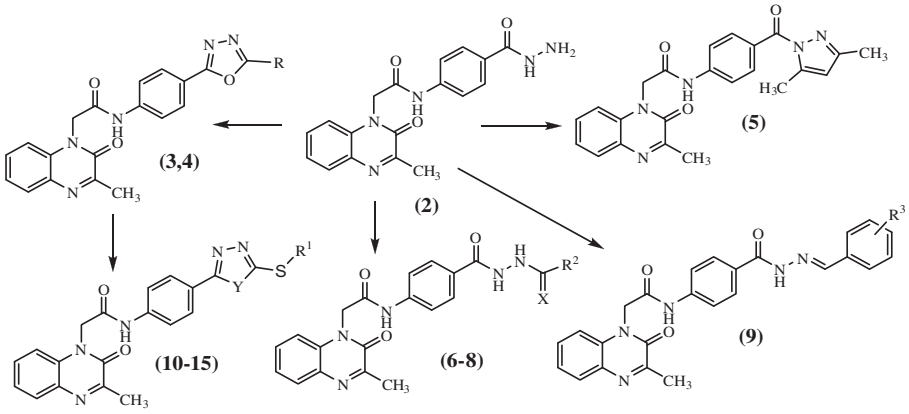


structure itself, he will find compounds better suited for therapeutic use.

Quinoxaline derivatives have received much attention in recent years due to their biological significance and pharmaceutical applications.[6–8] Many quinoxaline derivatives have been reported to possess anticonvulsant activity.[9,10] Some quinoxaline derivatives showed a highly significant effect as an anticonvulsant in comparing to diazepam as a reference drug.[9,11] Many currently prescribed antiepileptic drugs (AEDs) act via voltage-gated sodium channels, voltage-gated calcium channels, voltage-gated potassium channels, activation of γ-aminobutyric acid (GABA) receptor, or by inhibition of the glutamate receptor.[12,13] Glutamate receptors are classified into two major subtypes, *N*-methyl-D-aspartate (NMDA) and α-amino-3-hydroxy-5-methyl-4-isoxazole-propionic acid (AMPA) receptors. AMPA receptor antagonists may have greater potential clinical utility than do the NMDA antagonists[14] due to the fact that the latter may produce schizophrenia-like symptoms.[15]

Extensive studies have been conducted on quinoxaline derivatives to possess central nervous system (CNS) depressant action with potent (AMPA) receptor antagonist activity, which in some cases inhibit AMPA-induced lethal convulsions in mice.[10,11,16–18] Several members of this family were found to exhibit moderate to significant anticonvulsant activity in comparing to Phenobarbital as the standard drug.[17,18] Furthermore, Compound I (Figure 1) was reported as a potent AMPA receptor antagonist.[16]

On the other hand it has been reported that a lot of compounds containing acid, ester, amide, acid hydrazide,[17–21] oxadiazole, pyrazole,[20,21] semicarbazide, thiosemicarbazide[20] and/or arylidene[17–21] moieties possess good anticonvulsant activity.

Stimulated by the successful applications of quinoxaline derivatives as anticonvulsant agents, our objective was to synthesize novel derivatives of quinoxaline endowed with the previously mentioned moieties, hoping that the hybrid of these pharmacophoric features would produce enhanced anticonvulsant activity and compare the difference in the pharmacological effect.

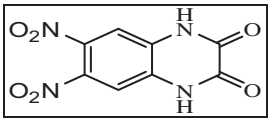


Figure 1 Compound I potent AMPA receptor antagonist.

Therefore a new series of 2-(3-methyl-2-oxoquinoxalin-1(2*H*)-yl)-*N*-(4-(substituted)phenyl)-acetamides were designed and synthesized starting with ortho-phenylenediamine by its reaction with sodium pyruvate to afford 3-methylquinoxalin-2(1*H*)-one which underwent different reactions following the reported procedures[17–26] to afford ethyl ester (**1**), acid hydrazide (**2**), 5-sulphanyl-oxadiazole (**3**), 5-phenyl-oxadiazole (**4**$_{a–c}$), pyrazole (**5**), acetyl (**6**), semicarbazide (**7**$_{a–c}$), thiosemicarbazide (**8**$_{a–c}$), Schiff's bases (**9**$_{a–e}$), triazole (**10**), S-alkyl (**12**$_{a–f}$), S-acetic acid (**13**), S-alkyl acetate (**14**$_{a–g}$) and thioester (**15**$_{a–c}$) derivatives in order to explore the influence of incorporating these groups on the anticonvulsant activity.

The data obtained from the biological screening of the synthesized compounds revealed that; the incorporation of Schiff's bases (compound **9**$_{c}$), thiosemicarbazide (compound **8**$_{c}$), semicarbazide (compounds **7**$_{b}$ and **7**$_{c}$) and 5-benzylsulphanyloxadiazole (compound **12**$_{f}$) moieties exhibited the highest anticonvulsant potency in comparing to Phenobarbital sodium as the reference drug (Table 1).

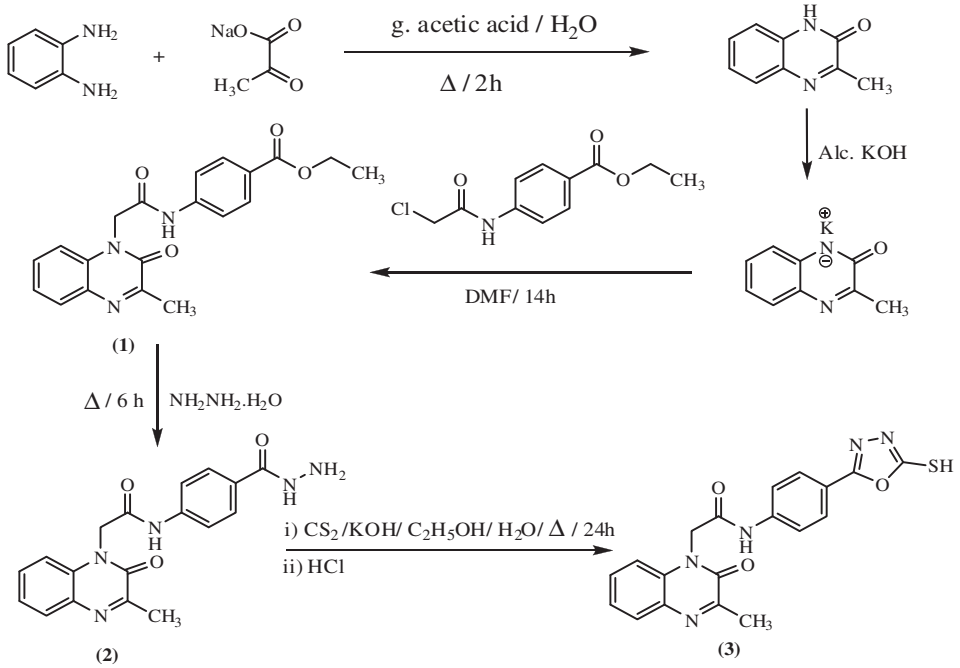
2. Chemistry

The sequence of reactions followed in the syntheses of the target compounds is illustrated in Schemes 1–3.

A new series of 2-(3-methyl-2-oxoquinoxalin-1(2*H*)-yl)-*N*-(4-(substituted)phenyl)acetamides were designed and synthesized starting with ortho-phenylenediamine by its reaction with sodium pyruvate to afford 3-methylquinoxalin-2(1*H*)-one, following the reported procedures,[22–26] which was then treated with alcoholic potassium hydroxide to afford the corresponding potassium salt.[13] Heating of the obtained potassium salt with ethyl-4-(2-chloroacetamido)-benzoate afforded the corresponding ethyl ester (**1**). The reaction of (1) with hydrazine hydrate afforded the intermediate compound *N*-(4-(hydrazinecarbonyl)phenyl)-2-(3-methyl-2-oxoquinoxalin-1(2*H*)-yl)acetamide (**2**) which underwent cyclization by refluxing with carbon disulfide in the presence of alcoholic potassium hydroxide followed by acidification with hydrochloric acid to give the corresponding 5-sulfanyloxadiazole (**3**) (Scheme 1).

Table 1 Anticonvulsant activity of the chosen compounds.

Test comp.	Dose mg/kg	Protection	ED_{50} mg/kg	ED_{50} mmol/kg	Relative potency
3	200	66.67	150	**0.38**	**0.13**
	100	33.33			
	50	16.67			
4_a	200	66.67	100	**0.23**	**0.21**
	100	50.00			
	50	16.67			
4_c	200	83.33	100	**0.22**	**0.22**
	100	50.00			
	50	33.33			
5	200	83.33	75	**0.18**	**0.27**
	100	50.00			
	50	16.67			
7_a	200	66.67	150	**0.31**	**0.16**
	100	33.33			
	50	16.67			
7_b	200	83.33	75	**0.16**	**0.30**
	100	50.00			
	50	33.33			
7_c	200	100.00	75	**0.16**	**0.30**
	100	83.33			
	50	33.33			
8_a	200	66.67	100	**0.23**	**0.21**
	100	33.33			
	50	16.67			
8_c	200	83.33	75	**0.15**	**0.32**
	100	50.00			
	50	16.67			
9_b	200	100.00	100	**0.22**	**0.22**
	100	50.00			
	50	33.33			
9_c	200	100.00	75	**0.15**	**0.33**
	100	66.67			
	50	33.33			
9_d	200	66.67	150	**0.32**	**0.15**
	100	33.33			
	50	16.67			
12_b	200	83.33	75	**0.18**	**0.27**
	100	50.00			
	50	33.33			
12_d	200	100.00	75	**0.17**	**0.29**
	100	50.00			
	50	33.33			
12_f	200	83.33	75	**0.16**	**0.30**
	100	50.00			
	50	16.67			
14_a	200	50.00	200	**0.43**	**0.11**
	100	16.67			
	50	16.67			
14_e	200	50.00	200	**0.39**	**0.13**
	100	33.33			
	50	16.67			
14_g	200	50.00	150	**0.30**	**0.16**
	100	33.33			
	50	16.67			
15_a	200	66.67	150	**0.30**	**0.16**
	100	33.33			
	50	16.67			
15_b	200	83.33	150	**0.28**	**0.18**
	100	50.00			
	50	16.67			
Ph.	25	16.67	12.5	**0.049**	**1.00**
	12.5				
	6.25				



Scheme 1 Synthesis of compounds **1–3**.

Cyclization of the acid hydrazide (**2**) with the appropriate 4-substituted benzoic acids and/or acetyl acetone afforded the corresponding 5-phenyloxadiazole (**4**$_{a–c}$) and pyrazole (**5**) derivatives respectively. The reaction of 2 with acetic anhydride, the appropriate isocyanates and/or isothiocyanates afforded the corresponding acetyl (**6**), semicarbazide (**7**$_{a–c}$), and thiosemicarbazide (**8**$_{a–c}$) derivatives respectively. Condensation of the acid hydrazide derivative (**2**) with certain aromatic aldehydes yielded the corresponding Schiff's bases (**9**$_{a–c}$) (Scheme 2).

Reaction of 5-sulfanyloxadiazole derivative (**3**) with hydrazine hydrate yielded the corresponding triazole derivative (**10**). The treatment of 5-sulfanyloxadiazole derivative (**3**) with alcoholic potassium hydroxide resulted in the corresponding S-potassium salt (**11**) which was then reacted with alkyl bromides, 2-chloroacetic acid, alkyl-2-chloroacetates and/or 4-substitutedbenzoylchlorides to give the corresponding S-alkyl (**12**$_{a–f}$), S-acetic acid (**13**), S-alkyl acetate (**14**$_{a–g}$) and thioester (**15**$_{a–c}$) derivatives respectively (Scheme 3).

2.1. Experimental

All melting points were carried out by open capillary method on a Gallen kamp melting point apparatus at the faculty of pharmacy, Al-Azhar University and were uncorrected. The infrared spectra were recorded on pye Unicam SP 1000 IR spectrophotometer at the Pharmaceutical analytical Unit, Faculty of Pharmacy, Al-Azhar University using potassium bromide disc technique. Proton magnetic resonance ^{1}H NMR spectra were recorded on a jeol 400 MHZ-NMR spectrometer at the Microanalytical Center, Asuit University. TMS was used as internal standard and chemical shifts were measured in δ scale (ppm). The mass spectra were recorded on Varian MAT 311-A (70 eV) at the Regional Center for Mycology and Biotechnology, Al-Azhar University. Elemental analyses (C, H, N) were performed on a CHN analyzer at the Regional Center for Mycology and Biotechnology, Al-Azhar University. All compounds were within ±0.4 of the theoretical values. The reactions were monitored by thin-layer chromatography (TLC) using TLC sheets precoated with UV fluorescent silica gel Merck 60 F254 plates and were visualized using UV lamp and different solvents as mobile phases.

3-Methylquinoxalin-2(1*H*)-one and its potassium salt were obtained according to the reported procedures.[18,22–26]

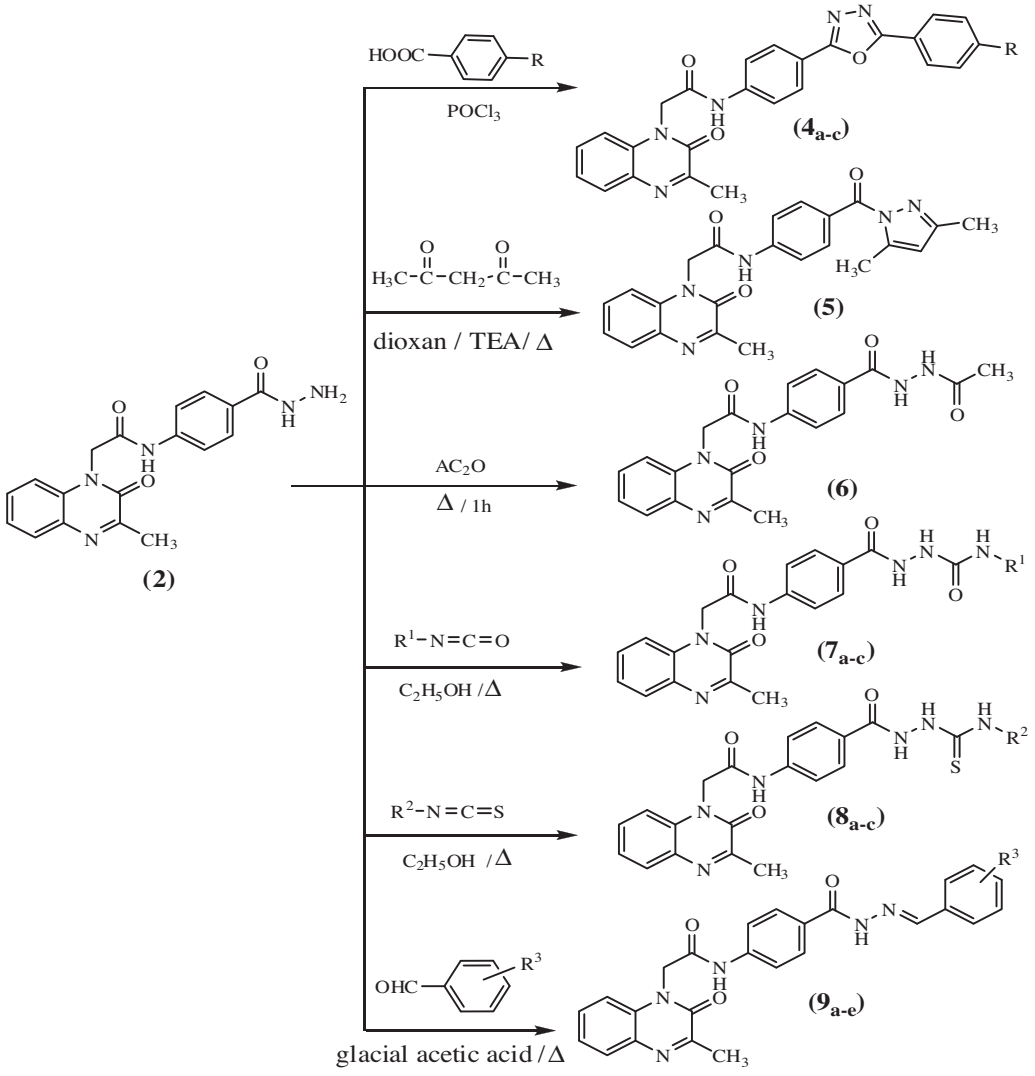
2.1.1. Ethyl-4-(2-(3-methyl-2-oxoquinoxalin-1(2H)-yl)-acetamido)benzoate (1)

A mixture of the potassium salt of 3-methylquinoxalin-2(1*H*)-one (19.80 g, 0.1 mol) and ethyl 4-(2-chloroacetamido)benzoate (24.1 g, 0.01 mol) in DMF (50 ml) was heated on a water-bath for 14 h. After cooling to room temperature, the reaction mixture was poured onto ice-water (500 ml) and stirred for 30 min. The formed precipitate was filtered, washed with water and crystallized from ethanol to give white crystals of **(1)**.

Yield, 75%; mp: 213–214 °C. Analysis for $C_{20}H_{19}N_3O_4$ (mw 365); Calcd.: C, 65.74; H, 5.24; N, 11.50. Found: C, 65.89; H, 5.32; N, 11.62. IR (KBr, cm^{-1}): 3278 (NH), 3050 (C–H aromatic), 2985 (C–H aliphatic), 1740 (C≡O ester), 1667 (C≡O amide), 1601 (C≡O quinoxaline). ^{1}H NMR ($CDCl_3$, ppm): 1.36 (t, 3H, CH_2CH_3, J = 6.8 Hz), 2.61 (s, 3H, CH_3-quinox.), 4.32 (q, 2H, CH_2CH_3, J = 6.8 Hz), 5.03 (s, 2H, CH_2), 7.26–8.03 (m, 8H, Ar–H), 9.04 (s, 1H, NH), (D_2O exchangeable). MS (*m*/*z*): 365 (M^+, 5.12%), 321 (6.34%), 201 (80.12%), 173 (20.33%) 145 (100%, base beak).

2.1.2. N-(4-(Hydrazinecarbonyl)phenyl)-2-(3-methyl-2-oxoquinoxalin-1(2H)-yl)acetamide (2)

A mixture of ester (3.65 g, 0.01 mol) and hydrazine hydrate (10 ml, 85%) in ethanol (20 ml) was stirred well and refluxed



(4) a) R= H **b) R=** Cl **c) R=** F

(7) a) R^1 = $(CH_2)_5CH_3$ **b) R^1 =** C_6H_{11} **c) R^1 =** C_6H_5

(8) a) R^2= CH_2CH_3 **b) R^2=** $CH_2CH{=}CH_2$ **c) R^2=** C_6H_5

(9) a) R^3= 4- Cl **b) R^3=** 4-F **c) R^3=** 2,4-diCl
d) R^3= OCH_3 **e) R^3=** $N(CH_3)_2$

Scheme 2 Synthesis of compounds **4–9**.

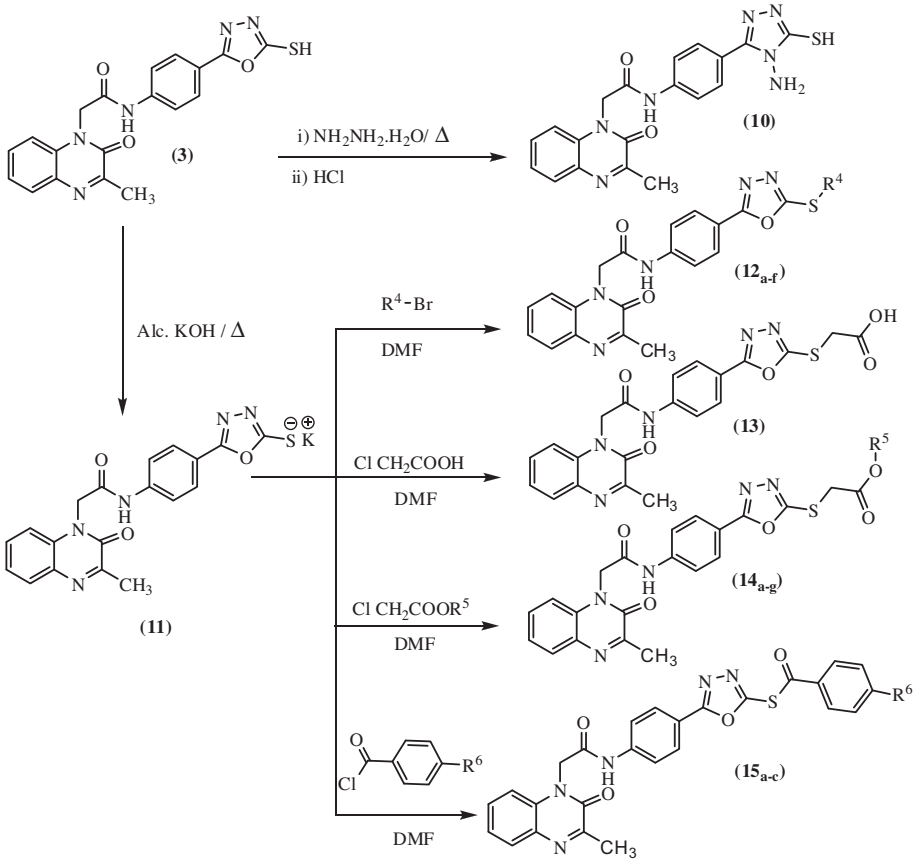
for 6 h. The reaction mixture was cooled and the crude product was collected by filtration, washed with water and recrystallized from ethanol.

Yield, 74%; mp: 295–297 °C. Analysis for $C_{18}H_{17}N_5O_3$ (mw 351); Calcd.: C, 61.53; H, 4.88; N, 19.93. Found: C, 61.84; H, 4.80; N, 20.32. IR (KBr, cm^{-1}): 3342, 3300 (NH–NH_2), 3250 (NH), 3040 (C–H aromatic), 2975 (C–H aliphatic), 1672 (C≡ONHNH$_2$), 1626 (C≡O amide), 1600 (C≡O quinox.). ^{1}H NMR (DMSO-d_6, ppm): 2.50 (s, 3H, CH_3,) 4.44 (s, 2H, NH_2) (D_2O exchangeable), 5.14 (s, 2H, CH_2), 7.36–7.80 (m, 8H, Ar–H), 9.63 (s, 1H, *NH*–NH_2), (D_2O exchangeable), 10.63 (s, 1H, NH-phenyl) (D_2O exchangeable). MS (*m*/*z*): 351(M^+, 2.01%), 320 (7.23%), 201 (20.43%), 159 (3.25%), 145 (96.20%), 131 (13.57%), 119 (100%, base beak).

*2.1.3. N-(4-(5-Salfanyl-1,3,4-oxadiazol-2-yl)phenyl)-2-(3-methyl-2-oxoquinoxalin-1(2H)-yl)-acetamide (**3**)*

Compound (**2**) (3.51 g, 0.01 mol) was dissolved in a solution of potassium hydroxide (0.56 g, 0.01 mol) in ethanol (40 ml) and water (4 ml). Carbon disulfide (3 ml) was then added while stirring and the reaction mixture was refluxed for 24 h. The reaction mixture was concentrated, cooled to room temperature and acidified with diluted hydrochloric acid. The obtained precipitate was filtered, washed with water and recrystallized from ethanol to give compound (**3**).

Yield, 71%; mp: 305–307 °C. Analysis for $C_{19}H_{15}N_5O_3S$ (mw 393); Calcd.: C, 58.01; H, 3.84; N, 17.80. Found: C, 58.14; H, 4.11; N, 17.92. IR (KBr, cm^{-1}): 3264 (NH), 3100 (C–H aromatic), 2940 (C–H aliphatic), 2590 (SH), 1649



(12) **a)** R^4= -CH_3 **b)** R^4= -CH_2CH_3 **c)** R^4= -$(CH_2)_2CH_3$ **d)** R^4= -$(CH_2)_3CH_3$
e) R^4= -$CH_2CH{=}CH_2$ **f)** R^4= -$CH_2C_6H_5$

(14) **a)** R^5= -CH_3 **b)** R^5= -CH_2CH_3 **c)** R^5= -$(CH_2)_2CH_3$ **d)** R^5= -$(CH_2)_3CH_3$
e) R^5= -$CH(CH_3)_2$ **f)** R^5= -$CH_2CH(CH_3)_2$ **g)** R^5= -$C(CH_3)_3$

(15) **a)** R^6= H **b)** R^6= Cl **c)** R^6= F

Scheme 3 Synthesis of compounds **10–15**.

(C≡O amide), 1611 (C≡O quinox.). ^{1}H NMR (DMSO-d_6, ppm): 2.49 (s, 3H, CH_3), 5.15 (s, 2H, CH_2), 7.34–7.93 (m, 8H, Ar–H), 10.84 (s, 1H, NH), (D_2O exchangeable), 14.16 (s, 1H, SH) (D_2O exchangeable). MS (*m*/*z*): 393 (M^+, 6.50%), 281 (5.22%), 215 (8.20%), 200 (53.19%), 174 (15.64%), 145 (100%, base beak).

*2.1.4. 2-(3-Methyl-2-oxoquinoxalin-1(2H)-yl)-N-(4-(5-(4-substitutedphenyl)-1,3,4-oxadiazol-2-yl)phenyl)-acetamide (**4**$_{a–c}$)*

2.1.4.1. General method. A mixture of equimolar quantities of the acid hydrazide (**2**) (0.70 g, 0.002 mol) and the appropriate 4-(un) substituted benzoic acid namely, benzoic acid, 4-chlorobenzoic acid and/or 4-flourobenzoic acid (0.002 mol) in phosphorous oxychloride (10 ml) was heated under reflux at 110°C for 6 h. The reaction mixture was cooled to room temperature, poured carefully onto an ice-water (300 ml), and then neutralized with solid sodium bicarbonate. The formed precipitate, after standing for 1 h, was filtered, washed with water, dried and crystallized from ethanol to afford compounds (**4**$_{a–c}$), respectively.

*2.1.4.2. Compound **4**$_a$.* Yield, 60%; mp: 260–262 °C. Analysis for $C_{25}H_{19}N_5O_3$ (mw 437); Calcd.: C, 68.64; H, 4.38; N, 16.01. Found: C, 68.88; H, 4.44; N, 16.14. IR (KBr, cm^{-1}): 3240 (NH), 3053 (C–H aromatic), 2923 (C–H aliphatic), 1651 (C≡O amide), 1611 (C≡O quinox.). MS (*m*/*z*): 437 (M^+, 2.13%), 377 (2.02%), 236 (5.32%), 201 (27.82%), 173 (5.48%), 145 (100% base beak), 105 (75.70%).

*2.1.4.3. Compound **4**$_b$.* Yield, 65%; mp: 263–265 °C. Analysis for $C_{25}H_{18}ClN_5O_3$ (mw 472); Calcd.: C, 63.63; H, 3.84; N, 14.84. Found: C, 64.02; H, 3.99; N, 14.11. IR (KBr, cm^{-1}): 3273 (NH), 3067 (C–H aromatic), 2940 (C–H aliphatic), 1666 (C≡O amide), 1599 (C≡O quinox.). MS (*m*/*z*): 473 ($M^+ + 2$, 0.55%), 472 (M^+, 1.40%), 201 (25.12%), 145 (49.32%), 139 (100% base beak), 111 (77.930%), 74 (74.78%).

*2.1.4.4. Compound **4**$_c$.* Yield, 70%; mp: 245–247 °C. Analysis for $C_{25}H_{18}FN_5O_3$ (mw 455); Calcd.: C, 65.93; H, 3.98; N, 15.38. Found: C, 66.08; H, 4.12; N, 15.49. IR (KBr, cm^{-1}): 3275 (NH), 3079 (C–H aromatic), 2944 (C–H aliphatic), 1647 (C≡O amide), 1609 (C≡O quinox.). ^{1}H NMR ($CDCl_3$,

ppm): 2.49 (s, 3H, CH_3), 5.17 (s, 2H, CH_2), 7.35–8.15 (m, 12H, Ar–H), 10.91 (s, 1H, NH), (D_2O exchangeable).

*2.1.5. N-(4-(3,5-Dimethyl-1H-pyrazol-1-ylcarbonyl)phenyl)-2-(3-methyl-2-oxoquinoxalin-1(2H)-yl)acetamide (**5**)*

Acetylacetone (1.00 g, 0.01 mol) was added to a solution of compound (**2**) (3.51 g, 0.01 mol) in dioxan (20 ml) and a few drops of TEA. The reaction mixture was refluxed for 4 h, concentrated, cooled to room temperature and the formed precipitate was filtered and crystallized from ethanol to give compound (**5**).

Yield, 65%; mp: 150–151 °C. Analysis for $C_{23}H_{21}N_5O_3$ (mw 415); Calcd.: C, 66.49; H, 5.09; N, 16.86. Found: C, 66.63; H, 5.23; N, 17.08. IR (KBr, cm^{-1}): 3253 (NH), 3052 (C–H aromatic), 2940 (C–H aliphatic), 1663 (2C≡O overlapped), 1608 (C≡O quinox.). MS (*m*/*z*): 415 (M^+, 1.17%), 201 (4.61%), 186 (5.39%), 159 (7.11%), 145 (100% base beak), 118 (90.34%).

*2.1.6. N-(4-(2-Acetylhydrazinecarbonyl)phenyl)-2-(3-methyl-2-oxoquinoxalin-1(2H)-yl)acetamide (**6**)*

Compound (**2**) (0.70 g, 0.002 mol) was refluxed with acetic anhydride (5 ml) for 1 h. The reaction mixture was allowed to attain room temperature, and then poured carefully onto ice-water (100 ml). The formed precipitate was filtered and crystallized from ethanol to give compound (**6**).

Yield, 65%; mp: 258–259 °C. Analysis for $C_{20}H_{19}N_5O_4$ (mw 393); Calcd.: C, 61.06; H, 4.87; N, 17.80. Found: C, 61.14; H, 4.98; N, 17.93. IR (KBr, cm^{-1}): 3319, 3270, 3209 (NH_2, 2NH), 3040 (C–H aromatic), 2947 (C–H aliphatic), 1710 (C≡O acetyl), 1664 (2C≡ONH overlapped), 1607 (C≡O quinox.). MS (*m*/*z*): 393 (M^+, 2.10%), 375 (5.02%), 320 (15.11%), 201 (100% base beak), 173 (45.70%), 161 (20.11%), 131 (22.33%), 119 (91.35%), 103 (25.71%), 91 (40.22%).

*2.1.7. 2-(3-Methyl-2-oxoquinoxalin-1(2H)-yl)-N-(4-(4-substitutedsemicarbazidecarbonyl)phenyl)-acetamide (**7a–c**)*

2.1.7.1. General method. A mixture of the acid hydrazide (**2**) (0.70 g, 0.002 mol) and the appropriate isocyanate namely *n*-hexyl, cyclohexyl and/or phenylisocyanate (0.015 mol) was refluxed in ethanol (25 ml) for 12 h. the reaction mixture was cooled and the formed solid was filtered and recrystallized from ethanol to give compounds (**7a–c**) respectively.

*2.1.7.2. Compound **7a**.* Yield, 61%; mp: 160–2 °C. Analysis for $C_{25}H_{30}N_6O_4$ (mw 478); Calcd.: C, 62.75; H, 6.32; N, 17.56. Found: C, 62.89; H, 6.51; N, 17.68. IR (KBr, cm^{-1}): 3297, 3126 (4NH overlapped), 3060 (C–H aromatic), 2936 (C–H aliphatic), 1674 (3C≡O overlapped), 1605 (C≡O quinox.).

*2.1.7.3. Compound **7b**.* Yield, 74%; mp: 227–229 °C. Analysis for $C_{25}H_{28}N_6O_4$ (mw 476); Calcd.: C, 63.01; H, 5.92; N, 17.64. Found: C, 63.19; H, 6.13; N, 17.91. IR (KBr, cm^{-1}): 3307, 3110 (4NH overlapped), 3058 (C–H aromatic), 2932 (C–H aliphatic), 1655 (3C≡O overlapped), 1605 (C≡O quinox.). ^{1}H NMR (DMSO-d_6, δ, ppm): 1.07–1.92 (m, 10H, cyclohexyl), 2.52 (s, 3H, CH_3), 3.54 (m, 1H, cyclohexyl), 5.17 (s, 2H, CH_2), 6.28 (s, 1H, NH-cyclohexyl) (D_2O exchangeable), 6.30 (s, 1H, *NH*CONH-cyclohexyl) (D_2O exchangeable), 7.36–7.88 (m, 8H, Ar–H), 10.02 (s, 1H, NH-phenyl) (D_2O exchangeable), 10.76 (s,1H, *NH*NHCONH-cyclohexyl) (D_2O exchangeable).

*2.1.7.4. Compound **7c**.* Yield, 75%; mp: 181–183 °C. Analysis for $C_{25}H_{22}N_6O_4$ (mw 470); Calcd.: C, 63.82; H, 4.71; N, 17.86. Found: C, 64.08; H, 4.93; N, 17.97. IR (KBr, cm^{-1}): 3308, 3203, 3133 (4NH overlapped), 3069 (C–H aromatic), 2941 (C–H aliphatic), 1653 (3C≡O overlapped), 1605 (C≡O quinox.). MS (*m*/*z*): 470 (M^+, 13.83%), 120 (25.90%), 92 (92.70%), 77 (51.50%), 65 (100% base beak).

*2.1.8. 2-(3-Methyl-2-oxoquinoxalin-1(2H)-yl)-N-(4-(4-substitutedthiosemicarbazidecarbonyl)-phenyl)acetamide (**8a–c**)*

2.1.8.1. General method. A mixture of the acid hydrazide (**2**) (0.70 g, 0.002 mol) and the appropriate isothiocyanate namely ethyl, allyl and/or phenylisothiocyanate (0.015 mol) was refluxed in ethanol (25 ml) for 12 h. the reaction mixture was cooled and the formed solid was filtered and recrystallized from ethanol to give compounds (**8a–c**) respectively.

*2.1.8.2. Compound **8a**.* Yield, 80%; mp: 205–207 °C. Analysis for $C_{21}H_{22}N_6O_3S$ (mw 438); Calcd.: C, 57.52; H, 5.06; N, 19.17. Found: C, 57.73; H, 5.17; N, 19.42. IR (KBr, cm^{-1}): 3285, 3114 (4NH overlapped), 3052 (C–H aromatic), 2984 (C–H aliphatic), 1652 (2C≡O amide), 1608 (C≡O quinox.), 1251 (C≡S). MS (*m*/*z*): 437 (M^+-1, 3.73%), 359 (2.97%), 295 (3.79%), 201 (6.02%), 172 (7.48), 146 (18.50%), 120 (23.36%), 118 (94.11%), 90 (68.72%), 77 (21.59%), 63 (40.31%), 51 (100%, base beak).

*2.1.8.3. Compound **8b**.* Yield, 72%; mp: 242–244 °C. Analysis for $C_{22}H_{22}N_6O_3S$ (mw 450); Calcd.: C, 58.65; H, 4.92; N, 18.65. Found: C, 58.82; H, 5.08; N, 18.89. IR (KBr, cm^{-1}): 3278, 3129 (4NH overlapped), 3053 (C–H aromatic), 2926 (C–H aliphatic), 1660 (2C≡O amide), 1608 (C≡O quinox.), 1264 (C≡S).

*2.1.8.4. Compound **8c**.* Yield, 75%; mp: 230–232 °C. Analysis for $C_{25}H_{22}N_6O_3S$ (mw 486); Calcd.: C, 61.71; H, 4.56; N, 17.27. Found: C, 61.86; H, 4.83; N, 17.43. IR (KBr, cm^{-1}): 3228, 3125 (4NH overlapped), 3050 (C–H aromatic), 2970 (C–H aliphatic), 1649 (2C≡O amide), 1604 (C≡O quinox.), 1246 (C≡S). ^{1}H NMR (DMSO-d_6, δ, ppm): 2.49 (s, 3H, CH_3), 5.17 (s, 2H, SCH_2), 6.98–7.89 (m, 13H, Ar–H), 9.61 (s, 1H, C≡S*NH*NH) (D_2O exchangeable), 10.44 (s, 1H, C≡SNH-Ph) (D_2O exchangeable), 10.66 (s, 1H, C≡O*NH*NH) (D_2O exchangeable), 10.88 (s, 1H, C≡ONH-Ph) (D_2O exchangeable).

*2.1.9. 2-(3-Methyl-2-oxoquinoxalin-1(2H)-yl)-N-(4-(2-substitutedbenzylidenehydrazinecarbonyl)-phenyl)acetamide (**9a–d**)*

2.1.9.1. General method. Equimolar amounts of (**2**) (0.70 g, 0.002 mol) and the appropriate aromatic aldehyde namely, 4-chlorobenzaldehyde, 4-flourobenzaldehyde, 2,4-dichlorobenzaldehyde, 4-methoxybenzaldehyde, and/or 4-dimethylaminobenzaldehyde (0.002 mol) were refluxed in glacial acetic acid (25 ml) for 3 h. The mixture was cooled and the formed solid was filtered and crystallized from ethanol to afford **9a–d**, respectively.

2.1.9.2. Compound ***9a***. Yield, 74%; mp: 295–296 °C. Analysis for $C_{25}H_{20}ClN_5O_3$ (mw 474); Calcd.: C, 63.36; H, 4.25; N, 14.78. Found: C, 63.48; H, 4.33; N, 14.93. IR (KBr, cm^{-1}): 3275, 3202 (2NH), 3071 (C–H aromatic), 2950 (C–H aliphatic), 1665 (2C≡O overlapped), 1612 (C≡O quinox.). MS (*m*/*z*): 475 ($M^+ + 1$, 0.65%), 474 (M^+, 2.02%), 336 (5.13%), 173 (12.32%), 145 (100% base beak).

2.1.9.3. Compound ***9b***. Yield, 85%; mp: 280–282 °C. Analysis for $C_{25}H_{20}FN_5O_3$ (mw 457); Calcd.: C, 65.64; H, 4.41; N, 15.31. Found: C, 65.59; H, 4.63; N, 15.66. IR (KBr, cm^{-1}): 3275, 3206 (2NH), 3048 (C–H aromatic), 2940 (C–H aliphatic), 1658 (2C≡O overlapped), 1608 (C≡O quinox.). ^{1}H NMR (DMSO-d_6, δ, ppm): 2.51 (s, 3H, CH_3,), 5.17 (s, 2H, CH_2), 7.26–8.71 (m, 13H, Ar–H, N≡CH overlapped with aromatic protons), 10.79 (s, 1H, NH-phenyl) (D_2O exchangeable), 11.75 (s, 1H, N≡*NH*CO) (D_2O exchangeable).

2.1.9.4. Compound ***9c***. Yield, 80%; mp: 201–203 °C. Analysis for $C_{25}H_{19}Cl_2N_5O_3$ (mw 508); Calcd.: C, 59.07; H, 3.77; N, 13.78. Found: C, 59.24; H, 3.62; N, 13.92. MS (*m*/*z*): 511 ($M^+ + 3$, 0.54%), 509 ($M^+ + 1$, 1.42%), 508 (M^+, 2.22%), 388 (2.03%), 200 (7.14%), 159 (34.86%), 131 (20.02%), 118 (60.32), 104 (30.23), 90 (25.44%), 74 (25.21%), 63 (40.15%), 50 (100% base beak).

2.1.9.5. Compound ***9d***. Yield, 81%; mp: 330–332 °C. Analysis for $C_{26}H_{23}N_5O_3$ (mw 469); Calcd.: C, 66.51; H, 4.94; N, 14.92. Found: C, 66.87; H, 5.30; N, 15.23. MS (*m*/*z*): 469 (M^+, 2.03%), 320 (1.17%), 200 (7.14%), 145 (53.09%), 131 (14.42%), 119 (100% base beak), 104 (22.63), 91 (97.03%).

2.1.9.6. Compound ***9e***. Yield, 67%; mp: 273–275 °C. Analysis for $C_{27}H_{26}N_6O_3$ (mw 482); Calcd.: C, 67.21; H, 5.43; N, 17.42. Found: C, 67.837 H, 5.58; N, 17.37. IR (KBr, cm^{-1}): 3259, 3185 (2NH), 3049 (C–H aromatic), 2955 (C–H aliphatic), 1658 (2C≡O overlapped), 1608 (C≡O quinox.).

*2.1.10. N-(4-(4-Amino-5-sulfanyl-4H-1,2,4-triazol-3-yl)phenyl)-2-(3-methyl-2-oxoquinoxalin-1(2H)-yl)acetamide (**10**)*

Compound (**3**) (3.93 g, 0.01 mol) was dissolved in ethanol (50 ml), hydrazine hydrate (10 ml, 85%) was added and the reaction mixture was heated under reflux on an oil-bath at 110°C for 8 h. The reaction mixture was evaporated to dryness under reduced pressure; the obtained solid was dissolved in water (300 ml) and acidified with conc. HCl to pH 1.00. The precipitate was filtered, washed with water and crystallized from chloroform/ethanol (2/1) mixture to afford the desired compound (**9**).

Yield, 71%; mp: 222–224 °C. Analysis for $C_{19}H_{17}N_7O_2S$ (mw 407); Calcd.: C, 56.01; H, 4.21; N, 24.06. Found: C, 56.12; H, 4.29; N, 24.18. IR (KBr, cm^{-1}): 3270, 3212 (NH_2, NH), 3023 (C–H aromatic), 2928 (C–H aliphatic), 2625 (SH), 1639 (C≡O amide), 1603 (C≡O quinox.). MS (*m*/*z*): 406 ($M^+ - 1$, 1.10%), 390 (1.05%), 259 (1.15), 201 (2.31%), 173 (3.06%), 146 (35.31%), 130 (20.43%), 116 (15.11%), 100 (17.98), 85 (18.00%), 73 (16.30%), 58 (100% base beak).

*2.1.11. Potassium salt of N-(4-(5-salfanyl-1,3,4-oxadiazol-2-yl)phenyl)-2-(3-methyl-2-oxoquinoxalin-1(2H)-yl)-acetamide (**11**)*

A mixture of (**4**) (3.93 g, 0.01 mol) and potassium hydroxide (0.56 g, 0.01 mol) in absolute ethanol (20 ml) was refluxed for 0.5 h. Upon cooling to room temperature, a yellow precipitate was obtained which was collected and washed with diethyl ether to afford the corresponding potassium salt (**11**).

Yield, 95%; mp: >300 °C. Analysis for $C_{19}H_{14}KN_5O_3S$ (mw 431); Calcd.: C, 52.88; H, 3.27; N, 16.23. Found: C, 52.50; H, 3.67; N, 16.55.

*2.1.12. 2-(3-Methyl-2-oxoquinoxalin-1(2H)-yl)-N-(4-(5-(substitutedsulfanyl)-1,3,4-oxadiazol-2-yl)-phenyl)acetamide (**12a–f**)*

2.1.12.1. General method. A mixture of the potassium salt (**11**), (0.86 g, 0.002 mol) and the appropriate alkyl bromide, namely methyl, ethyl, propyl, butyl, allyl and/or benzyl bromide (0.002 mol) in DMF (20 ml) was heated on a water-bath for 2 h. After cooling to room temperature, the reaction mixture was poured onto ice-water (150 ml) while stirring. The formed precipitate was filtered, washed with water and crystallized from ethanol to yield the corresponding compounds (**12a–f**) respectively.

2.1.12.2. Compound ***12a***. Yield, 77%; mp: 264–266 °C. Analysis for $C_{20}H_{17}N_5O_3S$ (mw 407); Calcd.: C, 58.96; H, 4.21; N, 17.19. Found: C, 59.28; H, 4.38; N, 17.31. IR (KBr, cm^{-1}): 3274 (NH), 3055 (C–H aromatic), 2933 (C–H aliphatic), 1660 (C≡O amide), 1602 (C≡O quinox.). MS (*m*/*z*): 407 (M^+, 2.02%), 371 (2.55%), 339 (2.28%), 286 (1.98%), 258 (2.13%), 243 (2.22%), 201 (3.71%), 186 (2.82%), 145 (100%, base beak), 131 (18.17%), 119 (58.12%), 90 (28.78%), 75 (74.70%), 58 (74.50%).

2.1.12.3. Compound ***12b***. Yield, 76%; mp: 272–274 °C. Analysis for $C_{21}H_{19}N_5O_3S$ (mw 421); Calcd.: C, 59.84; H, 4.54; N, 16.62. Found: C, 59.89; H, 4.61; N, 16.63. IR (KBr, cm^{-1}): 3266 (NH), 3080 (C–H aromatic), 2979 (C–H aliphatic), 1652 (C≡O amide), 1607 (C≡O quinox.).

2.1.12.4. Compound ***12c***. Yield, 70%; mp: 267–269 °C. Analysis for $C_{22}H_{21}N_5O_3S$ (mw 435); Calcd.: C, 60.67; H, 4.86; N, 16.08. Found: C, 60.85; H, 4.97; N, 16.16. MS (*m*/*z*): 435 (M^+, 1.19%), 345 (1.02%), 234 (5.20%), 201 (15.01%), 173 (10.03%), 145 (100%, base beak), 131(25.11%), 118 (40.32%).

2.1.12.5. Compound ***12d***. Yield, 73%; mp: 280–282 °C. Analysis for $C_{23}H_{23}N_5O_3S$ (mw 449); Calcd.: C, 61.45; H, 5.16; N, 15.58. Found: C, 61.59; H, 5.39; N, 15.69. IR (KBr, cm^{-1}): 3282 (NH), 3055 (C–H aromatic), 2990 (C–H aliphatic), 1670 (C≡O amide), 1606 (C≡O quinox.). ^{1}H NMR (DMSO-d_6, δ, ppm): 0.95 (t, 3H, CH_2CH_3, J = 8 Hz), 1.47 (m, 2H, CH_2CH_2 CH_2CH_3, J = 8 Hz), 1.79 (m, 2H, $CH_2CH_2CH_2CH_3$, J = 7.2 Hz), 2.66 (s, 3H, CH_3 of quinox.), 3.28 (t, 2H, $CH_2CH_2CH_2CH_3$, J = 7.2 Hz), 5.05 (s, 2H, CH_2CO), 7.27–7.92 (m, 8H, Ar–H), 9.06 (s, 1H, NH) (D_2O exchangeable).

2.1.12.6. Compound ***12e***. Yield, 75%; mp: 290–292 °C. Analysis for $C_{22}H_{19}N_5O_3S$ (mw 433); Calcd.: C, 60.96; H, 4.42; N,

16.16. Found: C, 60.88; H, 4.51; N, 16.34. MS (*m*/*z*): 433 (M^+, 2.73%), 396 (3.30%), 363 (4.11%), 296 (2.48%), 232 (4.76), 218 (2.71), 186 (2.84%), 173 (5.14%), 158 (4.88), 145 (100% base beak), 131 (16.49%), 103 (9.13%), 91 (35.16), 76 (28.34%), 63 (22.19%), 51 (35.68%).

2.1.12.7. Compound ***12f***. Yield, 67%; mp: 194–196 °C. Analysis for $C_{26}H_{21}N_5O_3S$ (mw 483); Calcd.: C, 64.58; H, 4.38; N, 14.48. Found: C, 64.45; H, 4.45; N, 14.54. IR (KBr, cm^{-1}): 3258 (NH), 3055 (C–H aromatic), 2933 (C–H aliphatic), 1659 (C≡O amide), 1608 (C≡O quinox.). ^{1}H NMR ($CDCl_3$, δ, ppm): 2.65 (s, 3H, CH_3), 4.72 (s, 2H, $PhCH_2$), 5.03 (s, 2H, $COCH_2$), 7.23–8.09 (m, 13H, Ar–H), 9.07 (s, 1H, NH), (D_2O exchangeable).

*2.1.13. 2-(5-(4-(2-(3-Methyl-2-oxoquinoxalin-1(2H)-yl)-acetamido)phenyl)-1,3,4-oxadiazol-2-yl-sulfanyl)acetic acid (**13**)*

A mixture of the potassium salt (**11**), (0.86 g, 0.002 mol), 2-chloroacetic acid (0.19 g, 0.002 mol) in DMF (20 ml) was heated on a water-bath for 2 h. After cooling to room temperature, the reaction mixture was poured onto ice-water (150 ml) while stirring. The formed precipitate was filtered, washed with water, and crystallized from ethanol.

Yield, 67%; mp: 194–196 °C. Analysis for $C_{21}H_{17}N_5O_5S$ (mw 451); Calcd.: C, 55.87; H, 3.80; N, 15.51. Found: C, 55.98; H, 3.93; N, 15.63. MS (*m*/*z*): 451 (M^+, 11.22%), 400 (10.72%), 330 (11.32%), 271 (23.51%), 242 (14.73%), 201 (33.23), 173 (23.76%), 153 (21.62), 145 (100%, base beak), 120 (61.50%), 111 (62.90%), 102 (56.97%), 92 (54.85), 75 (75.68), 63 (57.65), 51 (81.20).

*2.1.14. Alkyl-2-(5-(4-(2-(3-methyl-2-oxoquinoxalin-1(2H)-yl)-acetamido)phenyl)-1,3,4-oxadiazol-2-ylsalfanyl)acetate (**14a–g**)*

2.1.14.1. General method. A mixture of the potassium salt (**11**), (0.86 g, 0.002 mol) and the appropriate alkyl chloroacetate, namely methyl, ethyl, propyl, isopropyl, butyl, isobutyl and/or ter.butyl chloroacetate (0.012 mol) in DMF (20 ml) was heated on a water-bath for 2 h. After cooling to room temperature, the reaction mixture was poured onto an ice-water (150 ml) while stirring. The formed precipitate was filtered, washed with water and crystallized from ethanol to afford the corresponding ester derivatives (**13a–g**) respectively.

2.1.14.2. Compound ***14a***. Yield, 71%; mp: 226–228 °C. Analysis for $C_{22}H_{19}N_5O_5S$ (mw 465); Calcd.: C, 56.77; H, 4.11; N, 15.05. Found: C, 56.89; H, 4.24; N, 15.13. IR (KBr, cm^{-1}): 3273 (NH), 3049 (C–H aromatic), 2964 (C–H aliphatic), 1733 (C≡O ester), 1659 (C≡O amide), 1610 (C≡O quinox.). ^{1}H NMR (DMSO-d_6, δ, ppm): 2.59 (s, 3H, CH_3), 2.64 (s, 3H, OCH_3), 2.73 (s, 2H, SCH_2), 5.01 (s, 2H, $CH_2C{\equiv}O$), 7.24–7.90 (m, 8H, Ar–H), 9.00 (s, 1H, NH) (D_2O exchangeable).

2.1.14.3. Compound ***14b***. Yield, 72%; mp: 194–196 °C. Analysis for $C_{23}H_{21}N_5O_5S$ (mw 479); Calcd.: C, 57.61; H, 4.41; N, 14.61. Found: C, 57.67; H, 4.52; N, 14.82. IR (KBr, cm^{-1}): 3282 (NH), 3089 (C–H aromatic), 2984 (C–H aliphatic), 1721 (C≡O ester), 1651 (C≡O amide), 1610 (C≡O quinox.). ^{1}H NMR (DMSO-d_6, δ, ppm): 1.24 (t, 3H, CH_2CH_3), 2.63 (s, 3H, CH_3), 4.06 (q, 2H, CH_2CH_3), 4.21 (s, 2H, SCH_2), 5.01 (s, 2H, $CH_2C{\equiv}O$), 7.24–7.99 (m, 8H, Ar–H), 9.01 (s, 1H, NH) (D_2O exchangeable).

2.1.14.4. Compound ***14c***. Yield, 70%; mp: 190–192 °C. Analysis for $C_{24}H_{23}N_5O_5S$ (mw 493); Calcd.: C, 58.41; H, 4.70; N, 14.19. Found: C, 58.52; H, 4.81; N, 14.59. IR (KBr, cm^{-1}): 3293 (NH), 3057 (C–H aromatic), 2962 (C–H aliphatic), 1720 (C≡O ester), 1654 (C≡O amide), 1608 (C≡O quinox.). ^{1}H NMR (DMSO-d_6, δ, ppm): 0.93 (t, 3H, CH_2CH_3), 1.68 (m, 2H, $CH_2CH_2CH_3$), 2.64 (s, 3H, CH_3 of quinox.), 4.12 (t, 2H, $CH_2CH_2CH_3$), 4.27 (s, 2H, SCH_2), 5.06 (s, 2H, CH_2CO), 7.27–7.85 (m, 8H, Ar–H), 9.14 (s, 1H, NH) (D_2O exchangeable).

2.1.14.5. Compound ***14d***. Yield, 71%; mp: 123–124 °C. Analysis for $C_{24}H_{23}N_5O_5S$ (mw 493); Calcd.: C, 58.41; H, 4.70; N, 14.19. Found: C, 58.54; H, 4.79; N, 14.58. MS (*m*/*z*): 494 ($M^+ + 1$, 2.13%), 421 (1.34%), 393 (6.97%), 201 (50.19%), 173 (20.70%), 145 (100%, base beak), 118 (70.37%), 91 (55.10%).

2.1.14.6. Compound ***14e***. Yield, 65%; mp: 157–158 °C. Analysis for $C_{25}H_{25}N_5O_5S$ (mw 507); Calcd.: C, 59.16; H, 4.96; N, 13.80. Found: C, 59.30; H, 5.04; N, 13.91. MS (*m*/*z*): 507 (M^+, 3.06%), 391 (2.75%), 307 (3.07%), 293 (5.87%), 202 (8.80%), 201 (97.91%), 173 (27.67%), 145 (100%, base beak).

2.1.14.7. Compound ***14f***. Yield, 63%; mp: 164–165 °C. Analysis for $C_{25}H_{25}N_5O_5S$ (mw 507); Calcd.: C, 59.16; H, 4.96; N, 13.80. Found: C, 59.38; H, 5.10; N, 13.90. IR (KBr, cm^{-1}): 3301 (NH), 3063 (C–H aromatic), 2957 (C–H aliphatic), 1721 (C≡O ester), 1643 (C≡O amide), 1611 (C≡O quinox.). ^{1}H NMR (DMSO-d_6, δ, ppm): 0.89 (d, 6H, $CH(CH_3)_2$), 1.93 (m, 1H, $CH(CH_3)_2$), 2.57 (s, 3H, CH_3 of quinox.), 3.92 (d, 2H, OCH_2CH), 4.07 (s, 2H, SCH_2), 5.03 (s, 2H, CH_2CO), 7.24–7.81 (m, 8H, Ar–H), 9.12 (s, 1H, NH) (D_2O exchangeable).

2.1.14.8. Compound ***14g***. Yield, 60%; mp: 118–120 °C. Analysis for $C_{25}H_{25}N_5O_5S$ (mw 507); Calcd.: C, 59.16; H, 4.96; N, 13.80. Found: C, 59.39; H, 5.12; N, 13.91. IR (KBr, cm^{-1}): 3291 (NH), 3067 (C–H aromatic), 2979 (C–H aliphatic), 1723 (C≡O ester), 1660 (C≡O amide), 1608 (C≡O quinox.). ^{1}H NMR (DMSO-d_6, δ, ppm): 1.43 (s, 9H, $C(CH_3)_3$), 2.57 (s, 3H, CH_3 of quinox.), 4.02 (s, 2H, SCH_2), 5.02 (s, 2H, CH_2CO), 7.07–8.12 (m, 8H, Ar–H), 9.14 (s, 1H, NH) (D_2O exchangeable).

*2.1.15. 2-(3-Methyl-2-oxoquinoxalin-1(2H)-yl)-N-(4-(5-(4-(un)substitutedbenzoylsulfanyl)-1,3,4-oxadiazol-2-yl)-phenyl)acetamide (**15a–c**)*

2.1.15.1. General method. A mixture of the potassium salt (**11**), (0.86 g, 0.002 mol) and the appropriate 4-(un)substituted benzoyl chloride, namely benzoyl chloride, 4-chlorobenzoyl chloride and/or 4-flourobenzoyl chloride (0.002 mol) in DMF (20 ml) was heated on a water-bath for 1 h. After cooling to room temperature, the reaction mixture was poured onto ice-water (150 ml) while stirring. The formed precipitate was filtered, washed with water and crystallized from ethanol to afford the corresponding thioester derivatives (**15a–c**) respectively.

*2.1.15.2. Compound **15$_a$**.* Yield, 61%; mp: 163–165 °C. Analysis for $C_{26}H_{19}N_5O_4S$ (mw 497); Calcd.: C, 62.77; H, 3.85; N, 14.08. Found: C, 62.84; H, 4.02; N, 14.20. IR (KBr, cm^{-1}): 3261 (NH), 3049 (C–H aromatic), 2940 (C–H aliphatic), 1646 (2 C≡O overlapped), 1609 (C≡O quinox.). ^{1}H NMR (DMSO-d_6, δ, ppm): 2.55 (s, 3H, CH_3), 5.13 (s, 2H, CH_2), 7.33–8.11 (m, 13H, Ar–H), 9.15 (s, 1H, NH) (D_2O exchangeable).

*2.1.15.3. Compound **15$_b$**.* Yield, 65%; mp: 255–257 °C. Analysis for $C_{26}H_{18}ClN_5O_4S$ (mw 531); Calcd.: C, 58.70; H, 3.41; N, 13.16. Found: C, 58.89; H, 3.30; N, 13.19. IR (KBr, cm^{-1}): 3282 (NH), 3081 (C–H aromatic), 2934 (C–H aliphatic), 1653 (2C≡O overlapped), 1606 (C≡O quinox.). ^{1}H NMR (DMSO-d_6, δ, ppm): 2.25 (s, 3H, CH_3), 5.17 (s, 2H, $COCH_2$), 7.35–8.12 (m, 12H, Ar–H), 10.9 (s, 1H, NH) (D_2O exchangeable).

*2.1.15.4. Compound **15$_c$**.* Yield, 64%; mp: 171–173 °C. Analysis for $C_{26}H_{18}FN_5O_4S$ (mw 515); Calcd.: C, 60.58; H, 3.52; N, 13.59. Found: C, 60.42; H, 3.81; N, 13.67. IR (KBr, cm^{-1}): 3203 (NH), 3078 (C–H aromatic), 2999 (C–H aliphatic), 1687 (2C≡O overlapped), 1604 (C≡O quinox.). MS (*m*/*z*): 516 ($M^+ + 1$, 2.21%), 515 (M^+, 5.11%), 362 (5.07%), 319 (5.94%), 285 (6.33%), 256 (4.97%), 233 (5.15%), 190 (5.82%), 111 (17.27%), 91 (23.56%), 65 (20.93%), 57 (100%, base beak).

3. Anticonvulsant Screening

In the present study, 20 compounds of the newly synthesized quinoxaline derivatives were selected to be screened *in vivo* for their anticonvulsant activity against pentylenetetrazole-induced convulsions in mice following a reported procedure.[27,28] The results were compared with Phenobarbital sodium as a standard anticonvulsant.

The animal studies were undertaken with approval from the Ethics Committee (approval # 23PD/3/12/8R) of Al-Azhar University, Nasr City, Cairo, Egypt. All the trials were carried out according to the respective internationally guidelines. Swiss albino adult male mice, weighing 20–25 g, were used as experimental animals. They were obtained from an animal facility (Animal house, Department of Pharmacology and Toxicology, Faculty of Pharmacy, Al-Azhar University). Mice were housed in stainless steel wire-floored cages without any stressful stimuli. Animals were kept under well-ventilated conditions at room temperature (25–30 °C). They were fed on an adequate standard laboratory chow (El-Nasr Co., Abou-Zabal, Egypt) and allowed to acclimatize with free access to food and water for a 24 h period before testing except during the short time when they were removed from the cages for testing. Albino mice were randomly arranged in groups, each of 6 animals. Phenobarbital sodium (Sigma–Aldrich Chemical Co, Milwaukee, WI, USA) was used as a reference drug for comparison. Pentylenetetrazole (Sigma–Aldrich Chemical Co, Milwaukee, WI, USA) was used to induce convulsions in the experimental animals.

The test compounds were solublized in DMSO. Test compounds were orally administered in a dose ranging from 50 to 200 mg/kg animal weight using the same dosing volume of 0.2 ml per 20 g. Pentylenetetrazole (PTZ, Sigma) was dissolved in normal saline in 2% concentration and was given intraperitoneally (i.p.) in a dose of 60 mg/kg body weight (dose that could induce convulsions in at least 80% of the animals without death during the following 24 h). Phenobarbital sodium (Sigma, USA) was dissolved in normal saline in 2% concentration and it was i.p. given in doses of 6.25, 12.5 and 25 mg/kg using the same dosing volume. All drugs were freshly prepared to the desired concentration just before use.

Groups of 6 mice were administered the graded doses of the test compounds orally. Control animals received an equal volume of saline (10 ml/kg). After 1 h, the animals were subcutaneously injected with the convulsive dose of pentylenetetrazole (60 mg/kg). The criterion of anticonvulsant activity is complete protection against convulsions of any kind. Observations were made at least 60 min after the administration of pentylenetetrazole. Doses that gave full protection against the induced convulsions and that which exhibited 50% protection in addition to the relative potencies of the test compounds to Phenobarbital sodium were recorded.

The percentage protection per dose and the ED_{50} of each compound (in mg/kg and millimole) were calculated and presented in (Table 1). Finally the relative potencies of the test compounds compared to Phenobarbital sodium was calculated and used for comparison among compounds under test as shown in (Table 1).

The data presented in (Table 1) showed that: all the tested compounds (**3**, **4$_a$**, **4$_c$**, **5**, **7$_a$**, **7$_b$**, **7$_c$**, **8$_a$**, **8$_c$**, **9$_b$**, **9$_c$**, **9$_d$**, **12$_b$**, **12$_d$**, **12$_f$**, **14$_a$**, **14$_e$**, **14$_g$**, **15$_a$** and **15$_b$**) showed anticonvulsant activities lesser than that of the reference drug. Their potencies range from 0.33 to 0.11 of that of Phenobarbital sodium. Compounds **9$_c$** and **8$_c$** showed the highest anticonvulsant activities in experimental mice with anticonvulsant potency in comparing to Phenobarbital sodium as the reference drug of 0.33 and 0.32 respectively. Compounds **7$_b$**, **7$_c$** and **12$_f$** exhibited the same relative potency of 0.30. Compound 2 exhibited the lowest relative potency of 0.09. The other compounds showed variable relative potencies of 0.11–0.29. Compounds **7$_c$**, **9$_b$**, **9$_c$** and **12$_d$** caused 100% protection in a dose of 200 mg/kg body weight, compounds **4$_c$**, **5**, **7$_b$**, **8$_c$**, **12$_b$**, **12$_f$** and **15$_b$** caused 83.33%, compounds **3**, **4$_a$**, **7$_a$**, **8$_a$**, **9$_d$** and **15$_a$** caused 66.67% protection while the other compounds caused 50% protection at the same dose (Table 1). Compounds **5**, **7$_b$**, **7$_c$**, **8$_c$**, **9$_c$**, **12$_b$**, **12$_d$** and **12$_f$** caused 50% protection in a dose of 75 mg/kg body weight while the remaining compounds showed 50% protection at higher doses. The percent of protection per dose as well as the medium effective dose (ED_{50}), which makes 50% protection of animals was calculated using INSTANT 2 program (ICS, Philadelphia, PA, USA), presented in (Table 1). In spite of all compounds exhibited anticonvulsant activities lesser than that of Phenobarbital sodium, it provided a basis for further optimization.

4. Conclusion

The data obtained from biological screening revealed that; all the tested compounds showed anticonvulsant activities lesser than that of Phenobarbital sodium as the reference drug. Their potencies range from 0.33 to 0.11 of that of Phenobarbital. Compounds **9$_c$** and **8$_c$** showed the highest anticonvulsant activities in experimental mice with anticonvulsant potency in comparing to Phenobarbital sodium as the reference drug of 0.33 and 0.32 respectively. Compounds **7$_b$**, **7$_c$** and **12$_f$** exhibited

the same relative potency of 0.30. Compound **14_a** exhibited the lowest relative potency of 0.11. The other compounds showed variable relative potencies of 0.13–0.29. In spite of all compounds exhibited anticonvulsant activities lesser than that of Phenobarbital sodium, it provided a basis for further optimization.

5. Conflict of interest

None.

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