Synthesis and *In Vitro* Antitumor Evaluation of Some New Pyrimido[4,5-*b*] quinoxaline 5,10-Dioxide Derivatives

Mohamed Waly,[a]* Sameh Elgogary,[a,b] Ahmed Lashien,[a] and Ahmad Farag[c]

[a]Department of Chemistry, Faculty of Science, Damietta University, Egypt
[b]Department of Chemistry, Faculty of Science (Jazan), Jazan University, Saudi Arabia
[c]Department of Chemistry, Faculty of Science, Cairo University, Egypt
*E-mail: mohamedwaly7@yahoo.com

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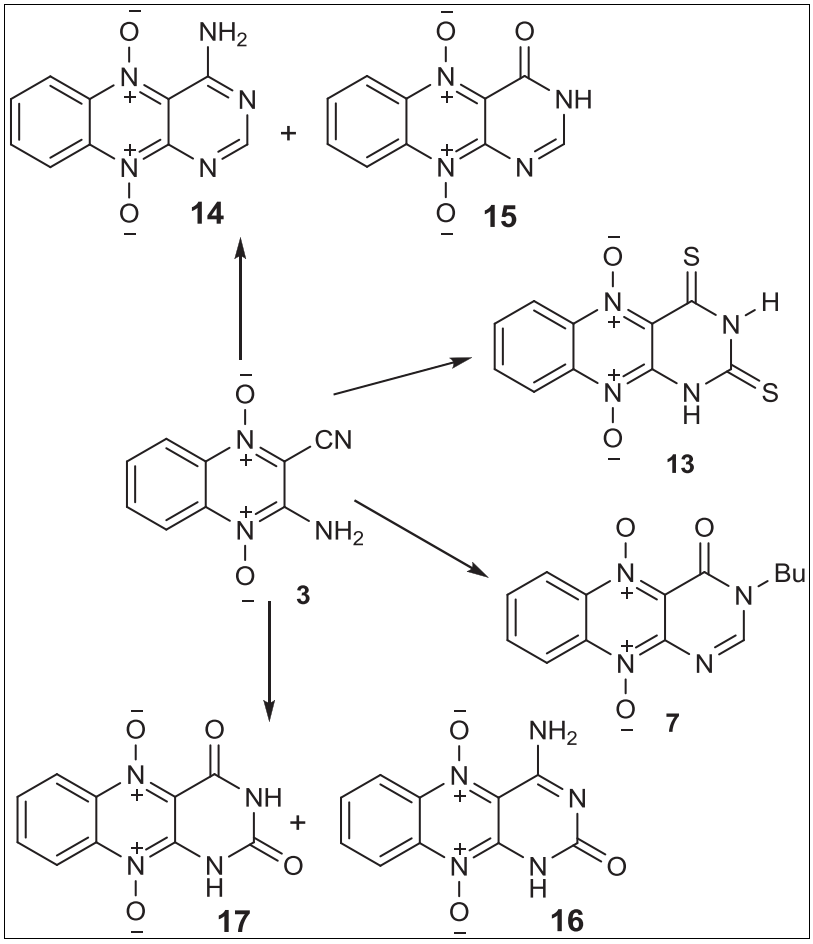


A new series of tricyclic pyrimidoquinoxaline derivatives were synthesized and evaluated as antitumor assays and compared with standard drug 5-fluorouracil. These new pyrimidoquinoxaline derivatives were synthesized by the reaction with *o*-aminonitrilequinoxaline derivative **3** with various reagents. One from which, the condensation of *o*-aminonitrile with potassium cyanate in acetic acid was stated as a new procedure for building the pyrimidine ring incorporate to quinoxaline moiety. Further condensation of aminonitrile **3** with formamide or Vilsmeier reaction followed by transamination or carbon disulphide was applied as procedures for the pyrimidine ring syntheses. Compound **15** achieved significant *in vitro* antitumor activity, and compounds **9** and **14** have high activities.

INTRODUCTION

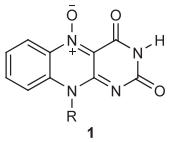
Quinoxaline derivatives play an important role in heterocyclic chemistry owing to their industrial drugs and their wide range of biological activities [1–3]. Notable among these are antioxidant, anti-inflammatory, anticancer, and antihistamic activities.

Among the wide variety of quinoxaline derivatives that have been explored for their pharmaceutical importance are pyridoquinoxaline, which is used as anticonvulsant

and imidazoquinoxaline as receptor antagonist [4,5]. Pyrazoloquinoxaline showed a relatively high antibacterial wherein minimum inhibitory concentration value was 25 μg/mL activity against Bacillus lichen formic and "Cellulomonas" SP [6]. Quinoxaline-1,4-dioxides were used for treatment of tuberculosis [7]. Pyrimido[1,2-*a*] quinoxaline-6-oxide was used as inhibitor of *trypanosome cruzi*, and pyrimido[4,5-*b*]quinoxaline was used as antihypertensive agent and blood platelet anti-aggregating agents [8]. Also, some quinoxaline derivatives have been reported to possess cytotoxic effects on human cancer cell lines [9,10].

Thus, compounds comprising flavin-*N*-oxides **1** were designed aiming to become stronger binding affinity and more hydrogen bonds to the binding pocket of the protein tyrosine kinase due to the polar part of the N-oxide moiety. Recently, it has been used for treatment of solid tumors, nonsolid tumor masses, leukemia, and non-small cell lung cancer involving *in situ* activator mixed with the flavin-*N*-oxide for a period of time, resulting in damage to the DNA in the cancer cells without substantial damage to that of normal cell [12]. Some quinoxalines are classified as kinase inhibitors bind to the inactivated DFG-out conformation. They are competitive with adenosine triphosphate binding and selectively bind to the Met1160, one of the crucial residues of the hinge linkage [13–18].

Motivated by the aforementioned findings and in continuation of our previous studies directed towards design and synthesis of biologically active molecules [11], the main goal of the present study is the development of potential inhibitors by synthesis of newly pyrimido[4,5-*b*]quinoxalinedithione 5,10-dioxide derivatives **2** and their analogs as antitumor agents. Target structures:



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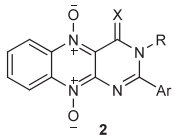

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RESULTS AND DISCUSSION

On the basis of the formerly mentioned facts, it was contemplated to prepare the starting material, 3-amino-2-quinoxalinecarbonitrile-1,4-dioxide **3** [13], that was used as a precursor for the synthesis of pyrimidoquinoxaline-5,10-dioxide derivatives by several procedures.

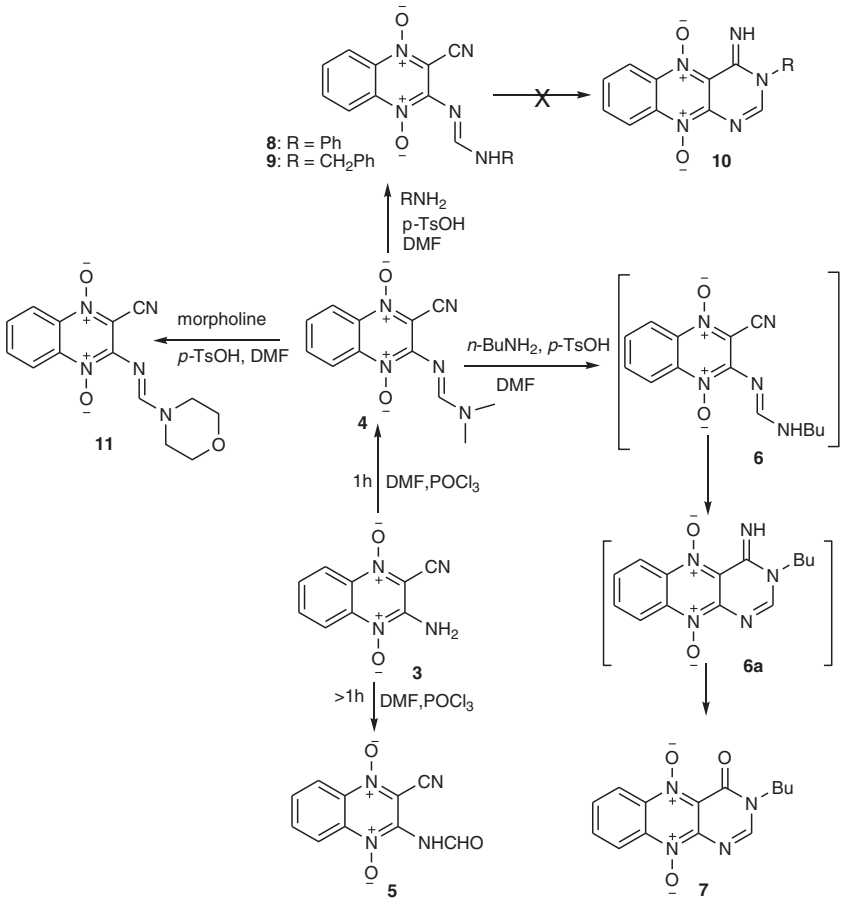
The first method involves the reaction of the *o*-aminonitrile derivative **3** with Vilsmeier reagent (*N*,*N*-dimethylformamide and $POCl_3$) to form the amidine derivative **4** (Scheme 1). The formation of amidine derivative was produced during the first hour of the reaction while extending the reaction time more than 1 h provided the 2-cyano-3-formamidoquinoxaline 1,4-dioxide **5**. The formation of the formyl derivative **5** was attributed to the on time *in situ* protonation of amidine derivative **4** with the HCl forming quaternary ammonium salt, which on workup was hydrolyzed to produce the formyl derivative **5**. The structure of compound **4** was established on the basis of its IR spectrum that exhibits bands characteristic for the stretching vibrations of nitrile group at (2229.31 cm^{-1}). Its ^1H NMR spectrum assured the presence of $N(Me)_2$ protons at δ 3.13 and 3.21 ppm in addition to a sharp singlet signal at δ 10.00 ppm assigned to vinyl protons. The IR data for compound **5** approved the presence of cyano group absorption band at 2229.31 cm^{-1} and amide carbonyl absorption (NCHO) at 1654.62 cm^{-1}. The ^1H NMR indicated the presence of the NH proton at δ 7.36 ppm (exchangeable with D_2O) and formyl proton (CHO) at 7.49 ppm.

One-pot reaction was performed by the transamination [14] of the amidine derivative **4** to afford the corresponding pyrimidoquinoxaline derivatives as in Scheme 1. Aliphatic *n*-butyl amine (*n*-$BuNH_2$) was transaminated to afford the corresponding pyrimidoquinoxaline derivative **7** through formation of the amidine intermediate **6**. Although the aromatic amine ($PhNH_2$) and alkyl amine ($PhCH_2NH_2$) were transaminated to produce the corresponding amidines **8** and **9**, respectively, the amidines **8** and **9** were unable to consequently cyclocondensed to provide the pyrimidoquinoxaline derivatives **10**. The amidine derivative **4** afford the morpholino amidine **11** using the same reaction condition (Scheme 1).

In compound **7**; the *n*-butyl protons appeared at δ 1.38, 1.68, 2.56, and 3.08 ppm in the ^1H NMR spectrum, whereas its IR spectrum showed the presence of carbonyl absorption at 1695.12 cm^{-1}. The presence of the carbonyl group was confirmed by ^{13}C NMR spectrum for this compound, which reflected the presence of a carbonyl signal at δ168.33 ppm. IR spectra of compounds **8** and **9** showed nitrile absorption band at 2229.31 cm^{-1}, whereas their ^1H NMR spectra reflected the presence of an amidine NH proton at δ10.38 and 9.98 ppm, respectively. ^1H NMR spectrum of compound **11** showed morpholine protons as a multiplete at δ 3.12–3.29. IR data revealed a nitrile group absorption band at 2229.31 cm^{-1}. The N3 alkyl substitution of the pyrimidoquinoxaline ring system was accomplished, whereas the corresponding N3 aryl substituted derivatives were failed to be achieved.

One-step synthesis of the 2,4-(1*H*,3*H*)-pyrimido[4,5-*b*] quinoxaline-dithione-5,10-dioxide 13 was achieved by the reaction of the aminonitrile **3** with carbon disulphide (Scheme 2). Apparently, one-step conversion of the aminonitrile **3** to pyrimidinedithiones **13** should involve initial formation of the intermediate dithiocarbamate salt **12** followed by cyclization to thiazine **12a**, which sequentially go through ring-opening and ring-closure (initiated by pyridine that acts as the requisite base) leading speedily and irreversibly to afford the pyrimidoquinoxaline

Scheme 1. Vilsmeier reaction for compound **3** and transamination.



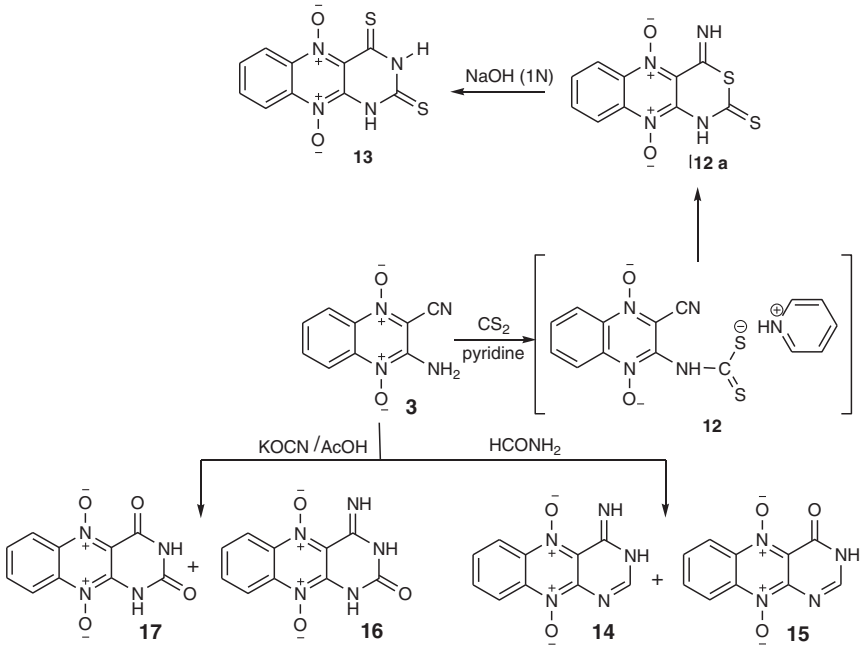
derivative **13** [15]. Chemical structure of compound **13** was elucidated from its spectral data; the IR spectrum showed the disappearance of CN group vibration band. ^{1}H NMR spectra of compound **13** showed the presence of two exchangeable protons at δ 9.34 and 11.38 ppm due to N1 and N3 protons, respectively.

Refluxing of aminonitrile **3** with formamide for 10 h afforded a mixture of 4-aminopyrimido[4,5-*b*]quinoxaline-5,10-dioxide **14** and 4-oxo-3,4- dihydropyrimido [4,5-*b*]quinoxaline-5,10-dioxide **15** (Scheme 4). The pyrimidoquinoxaline-5,10-dioxide **15** was produced as a result of acidic hydrolysis of the imino group during the reaction workup. The IR spectrum of compound **14** revealed the presence of NH group absorption bands at 3435.22 and 3411.46 cm^{-1}, whereas the IR spectrum of compound **15** showed one NH broad band for NH group at 3432.67 and CO group at 1690.30 cm^{-1}, respectively. The ^{1}H NMR spectra of compounds **14** and **15** declared the presence of pyrimidine ring C(2)-H proton at δ 7.93 and 8.62 ppm, respectively, and compound **14** showed two signals for NH groups proton as a broad signal at δ 7.40 ppm, whereas the ^{1}H NMR spectra of compound **15** showed one signal for NH proton at δ 8.72 ppm.

The reaction of compound **3** with potassium cyanate in glacial acetic acid gave a mixture of 4-amino-2-oxo-1,2-dihydropyrimido[4,5-*b*]quinoxaline-5,10-dioxide (**16**) and 2,4-dioxo-1,2,3,4-tetrahydropyrimido[4,5-*b*]quinoxaline-5,10-dioxide (**17**) as depicted in Scheme 2. Compound **16** revealed two NH proton at δ 7.41 and NH amide proton at δ 8.58 ppm, whereas compound **17** gave an imide NH proton signal at δ 9.77 ppm in their ^{1}H NMR spectra. The ^{13}C NMR spectra for compound **16** showed one CO signal at δ 168.35, whereas compound **17** indicated two carbonyl signals at δ 167.67 and 162.61 ppm. This method provided oxo substituents at positions 2 and 4 in pyrimidoquinoxaline ring system.

Alternative synthesis for the pyrimidoquinoxaline ring system with aromatic substituent in position 2 was achieved as in Scheme 3. Hydrolysis of the aminonitrile derivative **3** with H_2SO_4 (98%) gave the amide derivative **18**, which undergoes intermolecular cyclization with the aromatic aldehydes in the presence of iodine [16] to afford the corresponding 2-aryl pyrimidoquinoxaline derivatives **19a–c** (Scheme 3). Compound **18** showed amide carbonyl absorption band at 1672.95 cm^{-1} and revealed the disappearance of the nitrile group absorption in its IR spectrum,

Scheme 2. Reaction of aminonitrile **3** with CS_2, $HCONH_2$, and KOCN.



whereas the ^{1}H NMR spectrum showed two proton signals assigned for NH_2 and $CONH_2$ groups at δ 7.69 and 9.75 ppm, respectively. Compounds **19a–c** showed in each case a carbonyl absorption band in their IR spectra and aromatic protons in their ^{1}H NMR spectra.

In addition, an alternative one-pot synthesis of the pyrimidoquinoxaline ring system **19a** was synthesized by refluxing the aminonitrile derivative **3** with excess of benzoyl chloride that proceed in promoted three reaction steps as illustrated in Scheme 3. Benzoylation of aminonitrile **3** with benzoyl chloride to give the intermediate **20**, that followed by *in situ* intramolecular cyclization in the presence of excess of benzoyl chloride to afford the non-isolated oxazinoquinoxaline intermediate **21** (Scheme 3). Subsequently, the intermediate **21** undergoes intramolecular rearrangement [17] to afford the target compound **19a** (Scheme 3).

***In vitro* antitumor activity.** The newly synthesized pyrimidoquinoxaline-5,10-dioxide derivatives **7**, **9**, **14–17**, and **19a–c** were examined *in vitro* for cytotoxic effect on Ehrlich ascites cells (EAC) viability, using standard anticancer drug 5-fluorouracil [18]. The number of the viable cells values for the active compounds are summarized in Table 1. The biological data designated that compounds **9**, **14**, and **15** showed high activity, whereas the other compounds retained moderate activities. Structure activity relationship indicated that the unsubstituted pyrimidoquinoxaline derivative **15** achieved the most

Scheme 3. Formation of 2-aromatic substituted pyrimidoquinoxaline.

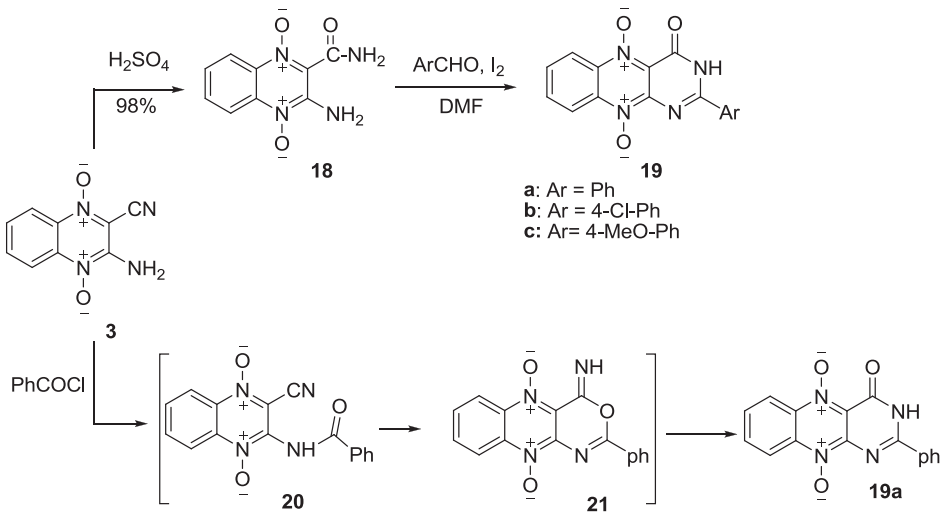


Table 1

Viability of Earlich ascites carcinoma cells.

Compound no.	Number of viable cell/mL ($\times 10^6$) (M ± SD)
Control*	44.5 ± 0.25
DMSO	30.3 ± 2.5
7	18.8 ± 0.32
9	13.6 ± 0.21
14	13 ± 0.25
15	9.2 ± 0.15
16	20 ± 0.36
17	17 ± 0.30
19a	32 ± 2.6
19b	29 ± 2.55
19c	22 ± 0.44
5-FU	8.2 ± 0.11

*Control: saline solution.

in vitro antitumor activity in comparison with the other tested analogs that held bulky substitution at N3 position.

EXPERIMENTAL

Melting points were obtained on digital Gallen Kamp melting point apparatus. The IR spectra were recorded on a Jasco 4100 FTIR spectrophotometer in KBr discs (v max in cm^{-1}). ^1H NMR and ^{13}C NMR spectra ($CDCl_3$) and (DMSO-d_6) were obtained using Pucker 600 MH_Z spectrometer, and chemical shift values were expressed in δ values (ppm) relative to that of the solvent. Coupling constants are given in Hz. All NH and OH protons were exchangeable with D_2O. The mass spectra were recorded on a Shimodzu GCMS-QP 1000 EX mass spectrometer at 70 eV. Elemental analyses were recorded on a PERKIN-ELMER 2400 C, H, N elemental analyzer, Cairo University. Reaction progress was monitored by analytical thin layer chromatography (TLC) on precoated glass plate (silica gel $60F_{254}$-plate-Merck), and the products were visualized by UV light.

N′-(2-cyano-1,4-dioxo-quinoxalin-3-yl)-N,N-dimethylformamidine (4). To a cold solution DMF (20 mL), phosphorus oxychloride (5 mL) was added dropwise with stirring for 15 min. The mixture was allowed to reach room temperature and then was added dropwise to a cold solution of compound **3** (2 g, 10 mmol) in DMF (10 mL). The mixture was stirred for 1 h and then poured on a crushed iced and neutralized with NH_4OH. The solid material was collected by filtration, washed with water, dried, and recrystallized from EtOH to give the titled compound **4**, mp: 210–212°C, (2 g, 94% yield); IR (KBr, cm^{-1}); 2229.31 (CN); ^1H NMR (DMSO-d_6) δ 3.13 (3H, s, N-C*H*$_3$), 3.21 (3H, s, N-C*H*$_3$), 7.79 (1H, t, C(7)-H), 7.98 (1H, t, C(6)-H), 8.29–8.33 (2H, m, C(8)-H and C(5)-H) and 10.00 (1H, s, NC*H*N); ^{13}C NMR (DMSO-d_6) δ 31.26, 34.37, 112.08, 117.78, 119.19, 120.21, 129.66, 133.37, 134.87, 145.61 and 158.38; MS (*m*/*z*): 258.09 (M^+, 0.96%); *Anal*. Calcd for $C_{12}H_{11}N_5O_2$ (257.25): C, 56.03; H, 4.31; N, 27.22; found: C, 55.66; H, 4.70; N, 27.53.

2-Cyano-3-formamidoquinoxaline 1,4-dioxide (5). The same procedure was followed as for synthesis of compound **4**, and the reaction mixture was left at room temperature for 4 h. Compound **5** was recrystallized from ethanol, mp: 246–248°C, yield 70%; IR (KBr, cm^{-1}) 2229.31 (CN), 1654.62 (CO); ^1H NMR (DMSO-d_6); δ 7.36 (1H, s, NH, D_2O exchangeable), 7.46 {1H, t, C(7)-H}, 7.49 (1H, s, C*H*O), 7.63{1H, t, C(6)-H}, 7.48 {1H, t, C(5)-H}, 8.11 {1H, m, C(8)-H}; ^{13}C NMR (DMSO-d_6); δ 117.82, 126.27, 127.17, 129.19, 130.40, 132.20, 135.09, 143.95, 145.11, 155.09; MS (*m*/*z*): 231.95 (M+, 0.25%); *Anal*. Calcd for $C_{10}H_6N_4O_3$ (230.18): C, 52.18; H, 2.63; N, 24.34; found: C, 52.43; H, 3.00; N, 24.12.

General procedure: transamination reaction. An equimolar mixture of the amidine derivative **4** and amine (2 mmol) in DMF (10 mL) was refluxed for 8 h in the presence of *p*-toluenesulphonic acid (0.34 g, 2 mmol). The mixture was then poured onto cold water, and the aqueous layer was extracted with $CHCl_3$ (3 × 100 mL). The collected $CHCl_3$ layers were dried over anhydrous Na_2SO_4 and evaporated *in vacuo* to leave a brown solid residue. The solid was triturated with cold ether and then filtered off and recrystallized from DMF to give compounds **7**, **8**, **9**, and **11**.

3-Butyl-4-oxo-(3*H*)-pyrimido[4,5-*b*]quinoxaline 4,10-dioxide (7). Obtained as pale brown product, (55% yield) and mp 128–130°C. IR (KBr, cm^{-1}); 1695.12 (CO). ^1H NMR (DMSO-d_6); δ 1.38 (3H, t, CH_3), 1.68 (2H, q, C*H*$_2$CH$_3$), 2.56 (2H, q, C*H*$_2$CH$_2$CH$_3$), 3.08 (2H, t, NC*H*$_2$CH$_2$), 7.37–7.95 (4H, m, aromatic protons) and 9.47 {1H, s, C(2)-H}. ^{13}C NMR (DMSO-d_6); δ 14.28, 20.20, 30.98, 55.23, 125.45, 126.23, 132.69, 133.77, 134.95, 135.47, 152.98, 154.84, 160.79 and 168.33. MS (*m*/*z*): 287.10 (M+, 2.24%); *Anal*. Calcd. for $C_{14}H_{14}N_4O_3$(286.29): C, 58.73; H, 4.93; N, 19.57; found: C, 58.44; H, 5.23; N, 19.33.

***N′*-(2-Cyano-1,4-dioxo-quinoxalin-3-yl)-*N*-phenylformamidine (8)**. Compound **8** was produced in 49% yield, mp 170–172°C (ethanol); IR (KBr, cm^{-1}); 3413.39 (NH), 2229.31 (CN); ^1H NMR (DMSO-d_6); δ 7.26 (1H, s, NC*H*), 7.49, 7.72, 8.37 (4H, m, aromatic protons); MS (*m*/*z*): 306.10 (M+, 0.07%).

***N*- Benzyl-*N′*-(2-cyano-1,4-dioxo-quinoxalin-3-yl) formamidine (9)**. Compound **9** was produced in 56% yield, mp: 182–184°C; IR (KBr, cm^{-1}) 3417.21 (NH), 2229.31 (CN); ^1H NMR (DMSO-d_6); δ 4.11 (2H, d, PhC*H*$_2$), 7.36 (1H, s, NC*H*), 8.06, 8.14, 8.23, 8.30 (4H, m, aromatic) and 9.98 (1H, s, NH, exchangeable. with D_2O); MS (*m*/*z*): 320.11 (M^+ 0.042%); *Anal*. Calcd for $C_{17}H_{13}N_5O_2$ (319.32): C, 63.94; H, 4.10; N, 21.93; found C, 64.21; H, 4.60; N, 21.63.

2-(Morpholinomethylideneamino)-quinoxalin-3-carbonitrile-1,4-dioxide (11). Compound **11** was produced by purification using column chromatography (2% ethanol/dichloromethane) in 77% yield, mp: 200°C; IR (KBr, cm^{-1}) 3413.39 (NH), 2229.31 (CN); ^1H NMR (DMSO-d_6) δ 3.12–3.29 (8H, m, morpholine protons), 7.40 (1H, s, NC*H*), 7.72–8.32 (4H,m, aromatic protons), and 10.07 (1H, s, NH, exchangeable. with D_2O); MS (*m*/*z*): 300.10 (M^+ 0.91%); *Anal*. Calcd. for $C_{14}H_{13}N_5O_3$ (299.28): C, 56.18; H, 4.38; N, 23.40; found C, 56.33; H, 4.58; N, 23.70.

2,4(1*H*,3*H*)-Dithiopyrimido(4,5-*b*)quinoxxaline-5,10-dioxide (13). A solution of 3-amino-2-quinoxaline carbonitrile-1,4-dioxide **3**, (1.0 g, 5 mmol) in pyridine (10 mL) and carbon disulphide (10 mL) was refluxed for 24 h; the solvents was removed by vacuum distillation. The remaining residue was stirred with NaOH (50 mL, IN) and was filtered. The filtrate was acidified with glacial AcOH to and recrystallized from DMF to afford the product **13** as brown needles (0.44 g, 30%), mp >300°C; IR (KBr, cm^{-1}) 3408.57 (NH, br.), 1698.98 (CS), 1635.34 (CS); ^1H NMR (DMSO-d_6) 7.39–8.13 (4H, m, aromatic protons), 9.34 {1H, N(1)-H, exchangeable. with D_2O} and 11.38 {1H, N(3)-H, exchangeable. with D_2O}; MS (*m*/

z): 279.01 (M^+ 0.76%); *Anal*. Calcd for $C_{10}H_6N_4O_2S_2$ (278.31): C, 43.16; H, 2.17; N, 20.13; found C, 43.25; H, 2.44; N, 19.83.

Reaction of the aminonitrile 3 with formamide. A mixture of the aminonitrile **3** (2.0 g, 10 mmol) and formamide (20 mL) was refluxed for 10 h. The reaction mixture was allowed to cool and then pouring onto ice/water medium. The solid product was filtered off and was purified by column chromatography using an eluent solution of dichlomethane : ethanol : ammonia, 300:8:1 to separate the mixture of compounds **14** and **15**.

4-Aminopyrimido[4,5-*b*]quinoxaline-5,10-dioxide (14). Compound **14** was obtained (0.8 g, 35% yield), mp: >300°C; IR (KBr, cm^{-1}) 3435.22 and 3411.46 (NH_2, d); ^1H NMR (DMSO-d_6) δ 7.40 (2H, br., NH_2, exchangeable), 7.90–8.52 {5H, m, aromatic protons and C(2)-H}; MS (*m*/*z*): 230.15 (M^+ 0.70%); *Anal*. Calcd for $C_{10}H_7N_5O_2$ (229.19): C, 52.40; H, 3.08; N, 30.56; found C, 52.44; H, 3.54; N, 31.11.

4-Oxo-pyrimido[4,5-*b*]quinoxaline-5,10-dioxide (15). Compound **15**: (1.0 g, 43% yield), mp >300°C; IR (KBr, cm^{-1}) 3432.67 (NH), 1690.30 (CO), ^1H NMR (DMSO-d_6); δ 7.95–8.25 (4H, m, aromatic protons), 8.62 {1H, s, C(2)-H} and 8.72 (1H, br., NH, exchangeable). MS (*m*/*z*): 231.05 (M^+ 4.04%), *Anal*. Calcd for $C_{10}H_6N_4O_3$ (230.18): C, 52.18; H, 2.63; N, 24.34; found C, 52.88; H, 2.33; N, 24.77.

Reaction of the aminonitrile 3 with potassium cyanate. A mixture of compound **3** (2 g, 10 mmol) and potassium cyanate (0.082 g, 10 mmol) was refluxed in glacial AcOH (20 mL) for 6 h. The reaction mixture was allowed to cool, poured onto ice/water, and the solid product was collected by filtration. The solid was subject to column chromatography and was eluted by a mixture of dichlomethane : ethanol : ammonia, 300:8:1. Two products were separated, **16** and **17**.

4-Aminopyrimido[4,5-*b*]quinoxaline-2(1*H*)-one-5,10-dioxide (16). Compound **16**: (0.98 g, 40% yield) mp: 280–282°C; IR (KBr, cm^{-1}) 3441.35 and 3395.07 (NH_2, d), 1699.05 (CO), ^1H NMR (DMSO-d_6); δ 7.41 (2H, br., 2NH, exchangeable), and 7.67–8.28 (4H, m, aromatic), 8.58 (1H, s, exchangeable, NH); ^{13}C NMR (DMSO-d_6) δ 119.88, 125.48, 125.57, 128.85, 129.87, 130.80, 132.91, 152.99, 153.45, 168.35; MS (*m*/*z*): 245.02 (M^+ 0.04%); *Anal*. Calcd for $C_{10}H_7N_5O_3$ (245.19): C, 48.98; H, 2.88; N, 28.56; found C, 48.94; H, 3.45; N, 29.12.

Pyrimido[4,5-*b*]quinoxaline-2,4(1*H*, 3*H*)-dione-5,10-dioxide (17). mp 240–242°C, (0.8 g, 33% yield); IR (KBr, cm^{-1}) 3343.96 (NH), 3255.25 (NH); ^1H NMR (DMSO-d_6) δ 7.67–8.64 {5H, m, aromatic protons and N(1)-H, exchangeable} and 9.77 {1H, s, N(3)-H, exchangeable}; ^{13}C NMR (DMSO-d_6) δ 120.75, 124.75, 127.83, 128.17, 134.18, 135.58, 144.29, 145.70, 162.61 and 167.67; MS (*m*/*z*): 247.21 (M^+ 0.05%); *Anal*. Calcd for $C_{10}H_6N_4O_4$ (246.18): C, 48.79; H, 2.46; N, 22.76; found C, 48.52; H, 2.33; N, 22.52.

2-Amino-3-carbamoylquinoxaline 1,4-dioxide (18). A mixture of compound **3** (2.0 g, 10 mmol) and H_2SO_4, 98% (5 mL), was heated in a water bath at 60°C for 6 h and then poured onto ice/water and neutralized with Na_2CO_3 solution. The solid product was filtered off, washed with water, and recrystallized with EtOH to give pure compound **18**, mp: 238–240°C, (94% yield); IR (KBr, cm^{-1}) 3390.24, 3328.53 (NH_2, d), 3120.26 (NH, br.), and 1672.95 (CO); ^1H NMR (DMSO-d_6); δ 7.36 {2H, br., C(3)NH_2, exchangeable}, 7.46, 7.63, 7.78, 8.11(4H, m, aromatic protons) and 8.33 (1H, br., CONH_2, exchangeable); ^{13}C NMR (DMSO-d_6) δ 117.82, 118.80, 126.27, 127.17, 129.19, 130.40, 145.11, 155.09 and 163.34. MS (*m*/*z*): 221.05 (M^+ 3.69%); *Anal*. Calcd for $C_9H_8N_4O_3$ (220.18): C, 49.09; H, 3.66; N, 25.45; found C, 49.25; H, 3.86; N, 25.22.

4-Oxo-2-phenyl-3,4-dihydropyrimido[4,5-*b*]quinoxaline 5,10-dioxide (19a). Method A: To a mixture of 2-amino-3-carbamoylquinoxaline 1,4-dioxide (**18**) (0.05 g, 22 mmol) and benzaldehyde (0.233 g, 22 mmol) in DMF (5 mL), molecular iodine (0.057 g, 22 mmol) was added. Then, the mixture was refluxed and the progress of the reaction was monitored by TLC using a mixture of dichlomethane : ethanol : ammonia (300:8:1) as eluent. After the reaction was completed, the mixture was allowed to cool to room temperature, and a solution of sodium thiosulphate (5%) was added, and the formed solid was filtered off, washed with water, and dried. The crud product was recrystallized from ethanol to give product **19a**, mp: 320–322°C, 0.3 g, 45% yield; IR (KBr, cm^{-1}) 3445.23 (NH), 1688.12 (CO); ^1H NMR ($CDCl_3$) δ 7.27–8.44 (9H, m, aromatic protons), 10.47 (1H, br., NH, exchangeable); MS (*m*/*z*): 307.10 (M^+ 0.04%); *Anal*. Calcd for $C_{16}H_{10}N_4O_3$ (306.28): C, 62.74; H, 3.29; N, 18.29; found C, 62.61; H, 3.55; N, 18.43.

Method B. A mixture of the aminonitrile **3** (2 g, 10 mmol) and excess of benzyl chloride (10 mL) was refluxed for 24 h. After cooling, the precipitated solid was filtered off, washed with diethyl ether, and then with water, dried and recrystallized from ethanol to give compound **19a**, 2.0 g, 66% yield.

2-(4-Chlorophenyl)-4-oxo-3,4-dihydropyrimido[4,5-*b*] quinoxaline-5,10-dioxide (19b). The same procedure was followed as that used for the synthesis of compound **19a** (method A) to give product **19b**, mp: 310–312°C, 53% yield; IR (KBr, cm^{-1}) 3533.24 (NH), 1697.84 (CO); ^1H NMR ($CDCl_3$); δ 7.87–8.88 (8H, m, aromatic protons), 11.30 (1H, br., NH, exchangeable); MS (*m*/*z*): 341.01 (M^+ 0.05%); *Anal*. Calcd for $C_{16}H_9ClN_4O_3$ (340.72): C, 56.40; H, 2.66; N, 16.44; found C, 56.25; H, 3.03; N, 16.77.

2-(4-Methoxyphenyl)-4-oxo-3,4-dihydropyrimido[4,5-*b*] quinoxaline-5,10-dioxide (19c). The same procedure was followed as that used for the synthesis of compound **19a** (method A) to give product **19c**, mp: 344–346°C, 60% yield; IR (KBr, cm^{-1}) 3323.44 (NH), 1678.55 (CO); ^1H NMR (DMSO-d_6); δ 3.74 (3H, s, OCH_3), 7.64–8.39 (8H, m, aromatic protons), 9.33 (1H, br., NH, exchangeable); ^{13}C NMR (DMSO-d_6); δ 55.75, 114.43, 114.59, 125.48, 125.571, 128.04, 130.46, 130.99, 133.05, 133.41, 134.26, 135.49, 152.22, 153.00, 160.05, 163.34, 168.35; MS (*m*/*z*): 337.03 (M^+ 4.37%); *Anal*. Calcd for $C_{17}H_{12}N_4O_4$ (336.30): C, 60.71; H, 3.60; N, 16.66; found C, 61.11; H, 3.43; N, 16.99.

VIABILITY TEST

A viable EAC cell counting was carried out by trypan blue exclusion using the method of MacLimans *et al*. [18]. Trypan blue (1 g, Sigma, USA) was dissolved in distilled water (100 mL). This solution was diluted with distilled water to give ultimately 0.16% solution when mixed with cells. A volume of trypan blue (0.2 mL of 0.32%) was mixed with EAC cells (0.2 mL), incubated for 10 min at 37°C, and then the number of viable tumor cells (unstained) was counted within 5 min after incubation using a hemocytometer with a microscopic

magnification of ×100. The number of viable tumor cells/mL was then calculated.

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