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Synthesis of novel 3,4-dihydroquinoxalin-2(1*H*)-one derivatives

Mohammed Shabaan, Azza Taher Taher* and Eman Omar Osman

Department of Organic Chemistry, Faculty of Pharmacy, Cairo University, Cairo, 11562, Egypt

**Corresponding author at: Department of Organic Chemistry, Faculty of Pharmacy, Cairo University, Cairo, 11562, Egypt. Tel.: +2.0101470792; fax: +2.023639307. E-mail address: azzataher2005@yahoo.com (A.T. Taher).*

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ABSTRACT

New derivatives of 3,4-dihydroquinoxaline-2(1*H*)-one were synthesized and characterized. Representative examples were evaluated for their antimicrobial and antifungal activities using Tetracycline and Nystatin as reference compound. One of the tested compounds **10a** was found to exhibit slight activity against *Staphylococcus aureus*. Compounds **10b**, **11b** and **14b** showed slight activity against *Escherichia coli*. Moreover, nineteen compounds were screened for their inhibition effect on CDK5, CK1, and GSK-3β. None of the tested compounds showed an inhibition activity below 10 μM concentration.

1. Introduction

Quinoxaline ring system represents the building block of many biologically active compounds that possess anti-inflammatory [1,2], antibacterial [3-5], antifungal [6], anticancer [7,8], antimalarial [9], CNS depressant [10] and hypoglycemic [11] activities.

Cyclin-dependent kinase 5 (CDK5) is a protein kinase believed to play a critical role in the early development of the central nervous system [12,13]. It is shown to be involved in cellular processes like neuronal differentiation [14], cell adhesion [15], and axonal guidance [16]. Recently, a large body of evidence suggests that deregulation of CDK5 is implicated in the pathology of a number of neurodegenerative disorders. As a consequence, CDK5 inhibitors are of potential therapeutic uses for diseases such as Alzheimer's Disease [17], Parkinson's disease [18], amyotrophic lateral sclerosis [19] and ischemic stroke [20].

From high throughput screening efforts, we identified the structure activity relationship of potent CDK2 and CDK5 inhibitors based on five member ring attached to quinoline-2(1*H*)-one, or 3,4-dihyro-1*H*-quinazoline-2-ones [21,22]. In our efforts to discover more potent inhibitors and an extension of these studies we introduced some modification, firstly the designed compounds included fusion of quinoxaline ring either with six member pyridinone ring compound **4** or five member furan ring compounds **9a-c**. Secondly, quinoxaline-2(1*H*)-ones were linked either to 3-aryl-six member ring compounds **5**, **6a-c** or 3-aryl-five member ring via methylene spacer compounds **10a**, **b** (Scheme 1). In Scheme 2, quinoxaline-2(1*H*)-ones ring is linked directly either to phenylpyrazole compound **12**, or aryloxazine compounds **14a**, **b**. The derivatives prepared in this study were evaluated for their ability to inhibit purified human CDK and the IC_{50} values were reported in Table 1. Unfortunately, none of the tested compounds showed any inhibitory activity. On the other hand, some quinoxaline derivatives were found to have good antibacterial activity against *Staphylococcus aureus* and *Bacillus subtilis* which was more potent than tetracycline. Therefore, it was interesting to test the antibacterial and antifungal activities of new quinoxalines.

2. Experimental

2.1. Chemistry

Melting points were determined on a Griffin apparatus and were uncorrected. IR spectra were determined as KBr discs on Shimadzu IR 435 spectrophotometer and values were represented in cm^{-1}. ^1H NMR was carried out on Varian Gemini 200 MHz and 300 MHz spectrophotometer, Microanalytical center, Cairo University, Egypt, using TMS as an internal standard and chemical shifts were recorded in ppm on δ scale. Mass spectra were run on a Hewlett Packard 5988 spectrometer, Microanalytical center, Cairo University; Elemental analyses were carried out at the Microanalytical center, Cairo University. Progress of the reactions was monitored by thin-layer chromatography (TLC) using TLC sheets pre-coated with UV fluorescent silica gel Merck 60 F_{254} that were visualized using UV lamp.

2.2. Synthesis

2.2.1. Ethyl acetylpyruvate sodium salt 1,3-Acetonylquinoxalin-2(1H)-one 2,3-(2-oxo-4-phenylbut-3-en-1-yl) quinoxalin-2(1H)-ones (3a) and 3-(2-oxo-4-(4-chloro phenyl)-but-3-en-1-yl)quinoxalin-2(1H)-ones (3b)

Ethyl acetylpyruvate sodium salt 1,3-acetonylquinoxalin-2(1*H*)-one 2,3-(2-oxo-4-phenylbut-3-en-1-yl)quinoxalin-2(1*H*)-

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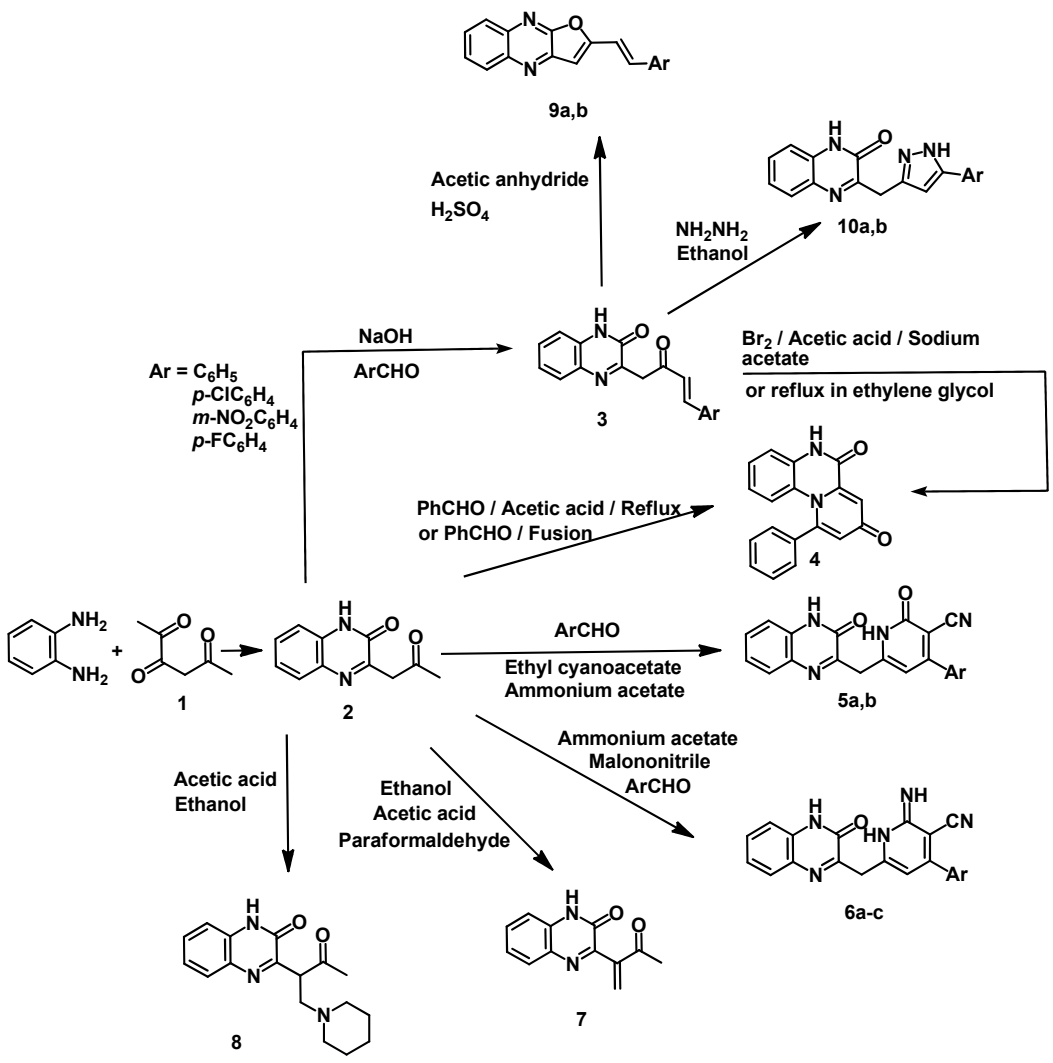


Scheme 1

ones (**3a**), 3-(2-oxo-4-(4-chlorophenyl)-but-3-en-1-yl) quinoxalin-2(1*H*)-ones (**3b**) were prepared as reported [23-25] (Scheme 1).

2.2.2. 3-(2-oxo-4-(3-nitrophenyl)-but-3-en-1-yl)quinoxalin-2(1H)-ones (3c) and 3-(2-oxo-4-(4-flourophenyl)-but-3-en-1-yl)quinoxalin-2(1H)-ones (3d)

To a suspension of 3-acetonylquinoxalin-2(1*H*)-one(2) (0.5 g, 0.0025 mol) in water (10 mL) and 10% sodium hydroxide (2 mL), the appropriate aldehyde (0.0025 mol) was sequentially added with continuous stirring. The formed solid product was filtered, dried and crystallized from acetone (Scheme 1).

3-(2-oxo-4-(3-nitrophenyl)-but-3-en-1-yl)quinoxalin-2(1H)-ones (**3c**) : Yield: 65%. M.p.: 210-212 °C. IR (KBr, cm^{-1}): 3450, 3400 (NH), 1685, 1680 (C=O), 1620(C=N). ^{1}H NMR (DMSO-d_6, δ, ppm): 6.35 (s, 1H, vinylic CH), 7.10-7.78 (m, 10H), 13.88-14.00 (br, 2H, NH, OH, exchanged with D_2O). MS (m/z (%)): 213 (100%), 335 ($M^{+\cdot}$, 26.96%), 336($M+1^{+\cdot}$, 5.83%). anal. Calcd.of $C_{18}H_{13}N_3O_4$ (335): C, 64.48; H, 3.88; N, 12.54. Found: C, 64.71; H, 3.97; N, 12.68%.

3-(2-oxo-4-(4-flourophenyl)-but-3-en-1-yl)quinoxalin-2(1H)-ones (**3d**) : Yield: 72%. M.p.: 213-214 °C. IR (KBr, cm^{-1}): 3400, 3350 (NH), 1690, 1680 (C=O), 1630(C=N). ^{1}H NMR (DMSO-d_6, δ, ppm): 6.33 (s, 1H, vinylic CH), 7.05-7.83 (m, 10H), 12.01, 13.82 (2s, 2H, NH, OH, exchanged with D_2O). Anal. Calcd. for $C_{18}H_{13}FN_2O_2$ (308): C, 70.12; H, 4.23; N, 9.09. Found: C, 70.33; H, 4.22; N, 8.79%.

2.2.3. 10-Phenyl-5H-pyrido[1,2-a]quinoxaline-6,8-dione (4)

Method A: Benzaldehyde (0.53 g, 0.005 mol) was added to a solution of **2** (1.01 g, 0.005 mol) in acetic acid (25 mL). The mixture was heated under reflux for 5 h. The product formed was filtered and crystallized from ethanol (Scheme 1). Yield: 75%. M.p.: >300 °C.

Method B: A mixture of **2** (1.01 g, 0.005 mol) and benzaldehyde (0.53 g, 0.005 mol) was heated at 180-190 °C for 2 h. The solid was then washed with ethanol and crystallized from ethanol. Yield: 70%. M.p.: >300 °C [26].

Method C: To a stirred solution of **3a** (1.45 g, 0.005 mol) and sodium acetate (0.82 g, 0.01 mol) in acetic acid (10 mL), bromine (0.8 g, 0.005 mol) in acetic acid (2.3 mL) was added dropwise. The reaction mixture was heated under reflux for 7 h. Water (20 mL) was then added and the solid product separated was filtered and crystallized from ethanol. Yield: 60%. M.p.: >300 °C.

Method D: A solution of 3a (1.45 g, 0.005 mol) in ethylene glycol (5 mL) was heated under reflux for 6 h. The mixture was cooled to room temperature and then poured onto an ice-water mixture. The product was filtered and crystallized from ethanol. Yield: 70%. M.p.: >300 °C. IR (KBr, cm^{-1}): 3249 (NH), 1660, 1620 (C=O). ^1H NMR ($CHCl_3$/TFA, (5:1), δ, ppm): 6.94-7.58 (m, 11H). MS (m/z (%)): 288 ($M^{+\cdot}$, 100%), 289 ($M+1^{+\cdot}$, 27.01%). Anal. Calcd. for $C_{18}H_{12}N_2O_2$ (288): C, 75.00; H, 4.17; N, 9.72. Found: C, 74.95; H, 3.80; N, 9.92%.

2.2.4. 3[(4-Aryl-5-cyano-6-oxo-1,6-dihydropyridin-2-yl) methyl]quinoxalin-2(1H)-ones (5a,b)

Method A: A mixture of 2 (1.01 g, 0.005 mol), the appropriate aldehyde (0.005 mol), ethyl cyanoacetate (0.56 g, 0.005 mol) and ammonium acetate (3.08 g, 0.04 mol) in absolute ethanol (10 mL) or *n*-butanol (10 mL) was heated under reflux for 6 h. The solvent was evaporated under reduced pressure and the residue was triturated with ethanol, filtered, washed with ethanol and crystallized from acetone (Scheme 1).

Method B: A mixture of the chalcone analogue 3a (1.45 g, 0.005 mol), ethyl cyanoacetate (0.56 g, 0.005 mol) and ammonium acetate (3.08 g, 0.04 mol) in *n*-butanol (10 mL) was heated under reflux for 10 h. The solvent was evaporated under reduced pressure and the residue was triturated with ethanol, filtered, washed with ethanol and crystallized from acetone (Scheme 1).

3[(4-Phenyl-5-cyano-6-oxo-1,6-dihydropyridin-2-yl)methyl] quinoxalin-2(1H)-ones (**5a**): Yield: 68% (Method A), 77% (Method B). M.p.: 276-278 °C. IR (KBr, cm^{-1}): 3450, 3200 (NH), 1690, 1660 (C=O), 2200 (CN). ^1H NMR (DMSO-d_6, δ, ppm): 3.42 (s, 2H, CH_2), 7.18-7.48 (m, 10H, aromatic protons), 12.14 (br, 2H, 2NH, exchanged with D_2O). MS (m/z (%)): 251 (100%), 354 ($M^{+\cdot}$, 0.28%). Anal. Calcd. for $C_{21}H_{14}N_4O_2$ (354): C, 71.18; H, 3.95; N, 15.81. Found: C, 70.80; H, 4.20; N, 15.72%.

3[(4-(3-nitrophenyl)-5-cyano-6-oxo-1,6-dihydropyridin-2-yl)methyl]quinoxalin-2(1H)-ones (**5b**): Yield: 51% (Method A). M.p.: 292-294 °C. IR (KBr, cm^{-1}): 3450, 3350 (NH), 1690, 1670 (C=O), 2200 (CN). ^1H NMR (DMSO-d_6, δ, ppm): 3.31 (s, 2H, CH_2), 7.21-7.42 (m, 9H, aromatic protons), 12.11 (br, 2H, 2NH, exchanged with D_2O). Anal. Calcd. for $C_{21}H_{13}N_5O_4$ (399): C, 63.16; H, 3.25; N, 17.54. Found: C, 63.42; H, 3.57; N, 17.25%.

2.2.5. 3[(4-Aryl-5-cyano-6-amino-1-pyridin-2-yl)methyl] quinoxlin-2(1H)-ones (6a-c)

A mixture of 2 (1.01 g, 0.005 mol), the appropriate aldehyde (0.005 mol), malononitrile (0.33 g, 0.005 mol) and ammonium acetate (3.08 g, 0.04 mol) in absolute ethanol (10 mL) was heated under reflux for 6 h. The solvent was evaporated under reduced pressure and the residue was triturated with ethanol, filtered, washed with ethanol and crystallized from acetone (Scheme 1).

3[(4-phenyl-5-cyano-6-amino-1-pyridin-2-yl)methyl]quinoxlin-2(1H)-one (**6a**): Yield: 55%. M.p.: >300 °C. IR (KBr, cm^{-1}): 3350, 3200 (NH), 1685 (C=O), 2200 (CN). ^1H NMR (DMSO-d_6, δ, ppm): 3.42 (s, 2H, CH_2), 7.15-8.15 (m, 10H, aromatic protons), 7.74, 7.80 and 11.80 (s, 3H, NH exchanged with D_2O). MS (m/z (%)): 83 (100%), 353 ($M^{+\cdot}$, 7.26%). Anal. Calcd. for $C_{21}H_{15}N_5O$ (353): C, 71.38; H, 4.24; N, 19.85. Found: C, 71.10; H, 4.28; N, 19.79%.

3[(4-(4-Chlorophenyl)-5-cyano-6-amino-1-pyridin-2-yl) methyl]quinoxlin-2(1H)-one (**6b**): Yield: 60%. M.p.: >300 °C. IR (KBr, cm^{-1}): 3450, 3400 (NH_2), 1690 (C=O), 2200 (CN). ^1H NMR (DMSO-d_6, δ, ppm): 3.47 (s, 2H, CH_2), 7.06-8.18 (m, 9H, aromatic protons), 11.00, 11.10 and 11.76 (s, 3H, NH exchanged with D_2O). Anal. Calcd. for $C_{21}H_{14}ClN_5O$ (387.5): C, 65.03; H, 3.61; N, 18.06. Found: C, 65.33; H, 3.62; N, 17.99%.

3[(4-(3-nitrophenyl)-5-cyano-6-amino-1-pyridin-2-yl) methyl]quinoxlin-2(1H)-one (**6c**): Yield: 50%. M.p.: 238-240 °C. IR (KBr, cm^{-1}): 3300, 3250 (NH_2), 1690 (C=O), 2200 (CN). ^1H NMR (DMSO-d_6, δ, ppm): 3.90 (s, 2H, CH_2), 7.05-8.63 (m, 9H, aromatic protons), 9.05, 9.15 and 10.95 (s, 3H, NH exchanged with D_2O). Anal. Calcd. for $C_{21}H_{14}N_6O3$ (398): C, 63.31; H, 3.51; N, 21.10. Found: C, 63.57; H, 3.84; N, 20.86%.

2.2.6. 3-(1-methylidene-2-oxopropyl)quinoxalin-2(1H)-one (7)

A solution of 3-acetonylquinoxalin-2(1*H*)-one 2 (1.01 g, 0.005 mol), paraformaldehyde (0.15 g, 0.005 mol) in acetic acid (1 mL) and ethanol (10 mL) was heated under reflux for 3 h, cooled and the separated solid was filtered and crystallized from acetic acid (Scheme 1). Yield: 85%. M.p.: 277-278 °C. IR (KBr, cm^{-1}): 3400 (NH), 1660, 1620 (C=O), 1375 (CH_3 bending). ^1H NMR ($CHCl_3$/TFA, (5:1), δ, ppm): 2.24 (s, 3H, CH_3), 4.98, 5.28 (2s, 2H, CH_2), 7.50-7.97 (m, 4H, aromatic protons). MS (m/z (%)): 53 (100%), 214 ($M^{+\cdot}$, 18%). Anal. Calcd. for $C_{12}H_{10}N_2O_2$ (214): C, 67.28; H, 4.67; N, 13.08. Found: C, 67.40; H, 4.90; N, 13.06%.

2.2.7. 3-[2-Oxo-1-(piperidin-1-ylmethyl)propyl]quinoxalin-2(1H)-one (8)

A solution of 2 (1.01 g, 0.005 mol), paraformaldehyde (0.15 g, 0.005 mol) and piperidine (0.005 mol) in acetic acid (1 mL) and ethanol (10 mL) was heated under reflux for 3 h, cooled and the separated solid was filtered and crystallized from acetic acid (Scheme 1). Yield: 75%. M.p.: >300 °C. IR (KBr, cm^{-1}): 3400 (NH), 1680, 1650 (C=O), 1380 (CH_3 bending). ^1H NMR ($CHCl_3$/TFA, (5:1), δ, ppm): 1.59-1.94 (br., 6H, piperidine), 2.38 (s, 3H, CH_3), 3.03 (br., 4H, piperidine), 3.37 (s, 1H, C*H*-CH_2), 3.90 (d, 2H, CH_2N), 7.47-8.06 (m, 4H, aromatic protons). MS (m/z (%)): 52 (100%), 300 ($M+1^{+\cdot}$, 28.5%). Anal. Calcd. for $C_{17}H_{21}N_3O_2$ (299): C, 68.22; H, 7.02; N, 14.04. Found: C, 68.58; H, 7.34; N, 14.18%.

2.2.8. 2-(2-Arylethenyl)furo[2,3-b]quinoxalines (9a,b)

A mixture of 3a,b (0.003 mol), acetic anhydride (10 mL) and sulphuric acid (few drops) was heated under reflux for 5 h. The reaction mixture was cooled and poured on ice (10 mL) and stirred. The formed product was filtered, dried and crystallized from ethanol (Scheme 1).

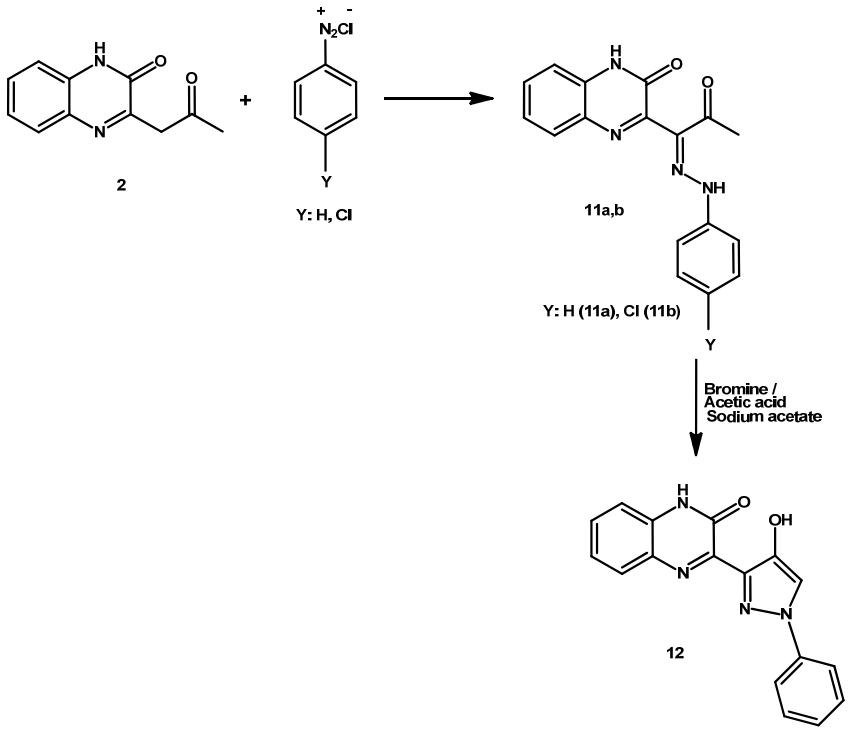
2-(2-phenylethenyl)furo[2,3-b]quinoxalines (**9a**): Yield: 50%. M.p.: >300 °C. IR (KBr, cm^{-1}): 1660 (C=N). MS (m/z (%)): 69 (100%), 272 ($M^{+\cdot}$, 26.69%), 273 ($M+1^{+\cdot}$, 5.13%). Anal. Calcd. for $C_{18}H_{12}N_2O$ (272): C, 79.41; H, 4.41; N, 10.29. Found: C, 79.34; H, 4.45; N, 10.65%.

2-[2-(4- Chlorophenyl)ethenyl]furo[2,3-b]quinoxalines (**9b**): Yield: 55%. M.p.: >300 °C. IR (KBr, cm^{-1}): 1650(C=N). ^1H NMR (DMSO-d_6, δ, ppm): 7.33-7.85 (m, 11H, aromatic protons). MS (m/z (%)): 306 ($M^{+\cdot}$, 100%), 307 ($M+1^{+\cdot}$, 34.08%), 308($M+2^{+\cdot}$, 32.11%). Anal. Calcd. for $C_{18}H_{11}ClN_2O$ (306.5): C, 70.47; H, 3.59; N, 9.14. Found: C, 70.72; H, 3.33; N, 9.46.

2.2.9. 3-[(5-Aryl-1H-pyrazol-3-yl)methyl]quinoxalin-2(1H)-ones (10a,b)

A solution of 3c,d (0.003 mol) and 99% hydrazine hydrate (0.75 g, 0.015 mol) in absolute ethanol (15 mL) containing few drops of glacial acetic acid was heated under reflux for 5 h. The reaction mixture was cooled, and the formed solid was filtered, dried and crystallized from acetone (Scheme 1).

3-{[5-(3-nitrophenyl)-1H-pyrazol-3-yl]methyl}quinoxalin-2(1H)-ones (**10a**): Yield: 50%. M.p.: 232-234 °C. IR (KBr, cm^{-1}): 3300-3250 (NH), 1685 (C=O).



Scheme 2

^{1}H NMR (DMSO-d_6, δ, ppm): 4.27 (s, 2H, CH_2), 6.77-8.60 (m, 9H, aromatic protons), 12.51, 13.06 (2s, 2H, 2NH, exchanged with D_2O). MS (m/z (%)): 347 ($M^{+\cdot}$, 100%), 348 ($M+1^{\cdot+}$, 23.17%). Anal. Calcd. for $C_{18}H_{13}N_5O_3$ (347): C, 62.25; H, 3.74; N, 20.17. Found: C, 62.20; H, 3.85; N, 19.91%.

3-{[5-(4-fluorophenyl)-1H-pyrazol-3-yl]methyl}quinoxalin-2(1H)-ones (10b): Yield: 61%. M.p.: 228-230 °C. IR (KBr, cm^{-1}): 3400 (NH), 1670 (C=O). ^{1}H NMR (DMSO-d_6, δ, ppm): 4.17 (s, 2H, CH_2), 6.51-7.76 (m, 9H, aromatic protons), 12.45, 12.77 (2s, 2H, 2NH, exchanged with D_2O). Anal. Calcd. for $C_{18}H_{13}FN_4O$ (320): C, 67.50; H, 4.06; N, 17.50. Found: C, 67.50; H, 4.11; N, 17.37%.

2.2.10. 3-[1-(2-Arylhydrazinylidene)-2-oxopropyl] quinoxalin-2(1H)-ones (11a,b)

A mixture of 2 (2.02 g, 0.01 mol), sodium hydroxide (1.6 g, 0.04 mol) and water (25 mL) was stirred for 10 min until a clear solution was formed, then chilled at -5 °C. To this solution, an ice cooled solution of aryldiazonium salt [prepared from the appropriate aromatic amine (0.01 mol), concentrated hydrochloric acid (3 mL) and sodium nitrite (0.69 g, 0.01 mol) in water (15 mL)] was added. The reaction mixture was maintained at -5 °C for 30 min then acidified with glacial acetic acid till pH 5-5.5. The formed solid was filtered, washed with water and crystallized from ethyl acetate (Scheme 2).

*3-[1-(2-phenylhydrazinylidene)-2-oxopropyl]quinoxalin-2(1H)-one*s (**11a**): Yield: 60%. M.p.: 140-142 °C. IR (KBr, cm^{-1}): 3500-3450 (NH), 1690, 1675 (C=O), 1370 (CH_3 bending). ^{1}H NMR (DMSO-d_6, δ, ppm): 2.63 (s, 3H, CH_3), 7.02-7.78 (m, 9H, aromatic protons), 11.69, 13.38 (2s, 2H, 2NH exchanged with D_2O). MS (m/z (%)): 77 (100%), 306 ($M^{+\cdot}$, 41.77%), 307 ($M+1^{\cdot+}$, 9.06%). Anal. Calcd. for $C_{17}H_{14}N_4O_2$ (306): C, 66.67; H, 4.58; N, 18.30. Found: C, 66.36; H, 4.30; N, 18.10%.

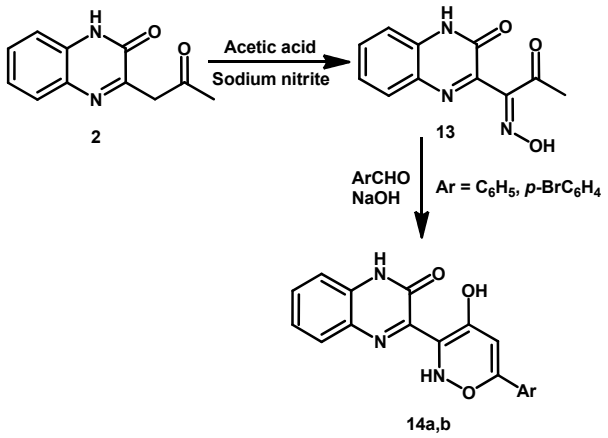
3-[1-(2-(4-Chlorophenyl)hydrazinylidene)-2-oxopropyl] quinoxalin-2(1H)-ones (**11b**): Yield: 80%. M.p.: 224-226 °C. IR (KBr, cm^{-1}): 3450-3400 (NH), 1680, 1660 (C=O), 1360 (CH_3 bending). ^{1}H NMR ($CDCl_3$/TFA, (5:1), δ, ppm): 2.89 (s, 3H, CH_3), 7.55-7.95 (m, 8H, aromatic protons). MS (m/z (%)): 340 ($M^{+\cdot}$, 100%), 341($M+1^{\cdot+}$, 24.1%), 342 ($M+2^{\cdot+}$, 38.4%). Anal. Calcd. for $C_{17}H_{13}ClN_4O_2$ (340.5): C, 59.90; H, 3.82; N, 16.45. Found: C, 60.10; H, 4.05; N, 16.34%.

2.2.11. 3-(4-Hydroxy-1-phenyl-1H-pyrazol-3-yl)quinoxalin-2(1H)-one (12)

To a stirred solution of 11a (1.53 g, 0.005 mol) and sodium acetate (0.82 g, 0.01 mol) in acetic acid (10 mL), bromine (0.8 g, 0.005 mol) in acetic acid (2.3 mL) was added dropwise. The reaction mixture was stirred overnight. Water (20 mL) was then added and the solid product was filtered and crystallized from methanol (Scheme 2). Yield: 55%. M.p.: 244-246 °C. IR (KBr, cm^{-1}): 3500-3350 (NH, OH stretching), 1660 (C=O stretching). ^{1}H NMR (DMSO-d_6, δ, ppm): 7.33-8.27 (m, 10H, aromatic protons), 11.04, 13.27 (2s, 2H, NH, OH, exchanged with D_2O). MS (m/z (%)): 143 (100%), 304 ($M^{+\cdot}$, 15.25%), 305 ($M+1^{\cdot+}$, 9.83%). Anal. Calcd. for $C_{17}H_{12}N_4O_2$ (304): C, 67.10; H, 3.94; N, 18.42. Found: C, 66.76; H, 4.00; N, 18.17%.

2.2.12. 3-(2-Oxo-1-oximinopropyl)quinoxalin-2(1H)-one (13)

A solution of 2 (1.01 g, 0.005 mol) in glacial acetic acid (25 mL) was stirred at room temperature for 5 min; then cooled to -5 °C. To this solution solid sodium nitrite (0.48 g, 0.007 mol) was added portionwise over a period of 30 min. After stirring for an additional 30 min at room temperature, the reaction mixture was diluted with water, and then filtered. The obtained solid was washed with water and crystallized from ethanol (Scheme 3). Yield: 90%. M.p.: 236-238 °C. IR (KBr, cm^{-1}): 3200-3150 (NH, OH stretching), 1690, 1660 (C=O stretching), 1370 (CH_3 bending). ^{1}H NMR (DMSO-d_6, δ, ppm): 2.49 (s, CH_3, 3H), 7.31-7.80 (m, 4H, aromatic protons), 12.61, 12.87 (2s, 2H, NH, OH exchanged with D_2O). MS (m/z (%)): 172 (100%), 231 ($M^{+\cdot}$, 29.28%), 232($M+1^{\cdot+}$, 4.22%). Anal. Cald. for $C_{11}H_9N_3O_3$ (231): C, 57.14; H, 3.89; N 18.18. Found: C, 57.40; H, 3.90; N, 18.06%.



Scheme 3

2.2.13. 3-(6-Aryl-4-hydroxy-2H-1,2-oxazin-3-yl)quinoxalin-2(1H)-ones (14a,b)

To a suspension of 13 (0.46 g, 0.002 mol) in water (10 mL), 10% aqueous sodium hydroxide (2 mL) solution and the appropriate aldehyde (0.002 mol) were sequentially added. The reaction mixture was heated under reflux for 5 h. The formed solid was filtered, washed with ethanol and crystallized from methanol (Scheme 3).

3-(6-phenyl-4-hydroxy-2H-1,2-oxazin-3-yl)quinoxalin-2(1H)-ones (**14a**): Yield: 60%. M.p.: 285-287 °C. IR (KBr, cm^{-1}): 3600-3400 (NH, OH stretching), 1660 (C=O stretching) . ^{1}H NMR (DMSO-d_6, δ, ppm): 6.37 (s, 1H, CH of oxazine), 7.35-7.78 (m, 9H, aromatic protons), 7.91, 10.66, 12.03 (3s, 3H, NH, OH, exchanged with D_2O). MS (m/z (%)): 303 (100%), 319 ($M^{+\cdot}$, 0.4%). Anal. Calcd. for $C_{18}H_{13}N_3O_3$ (319): C, 67.71; H, 4.07; N, 13.16. Found: C, 67.58; H, 3.91; N, 13.33%.

3-(6-(4-bromophenyl)-4-hydroxy-2H-1,2-oxazin-3-yl) quinoxalin-2(1H)-ones (**14b**): Yield: 43%. M.p.: 179-181 °C. IR (KBr, cm^{-1}): 3400-3300 (NH, OH stretching), 1670 (C=O stretching). ^{1}H NMR (DMSO-d_6, δ, ppm): 6.00 (s, 1H, CH of oxazine), 6.88-7.69 (m, 8H, aromatic protons). Anal. Calcd. for $C_{18}H_{12}BrN_3O_3$ (398): C, 54.27; H, 3.01; N, 10.55. Found: C, 54.29; H, 2.92; N, 10.90%.

2.3. Microbiology Test

2.3.1. Test organisms and culture media

Twelve compounds were tested for their antimicrobial activity using: *Staphylococcus aureus* and *Bacillus subtilis* (Gram positive bacteria), *Escherichia coli* and *Pseudomonas aeruginosa* (Gram negative bacteria) and *Candida albicans* (fungus). Culture media: Nutrient broth, Sabouraud's broth and agar.

2.3.2. Method

Agar plate diffusion technique: Agar plates containing 15 mL of agar medium [nutrient agar for bacteria and Sabouraud's broth for fungi] were seeded with 0.2 mL of broth culture of each organism and cultered for 18 h. Sterile filter paper discs (6 mm in diameter) were impregnated each with 10 µL of a 1% solution of the test compound in DMF and allowed to air dry. The discs were then placed onto the surface of agar plates and incubated at 37 °C for 24 h. Control discs impregnated with DMF were used alone to determine the solvent activity. The antibacterial reference tetracycline and the antifungal reference nystatin discs were tested concurrently as standards. The diameter of the inhibition zone around each disc was measured (mm).

2.4. Biological tests

Kinase preparations and assays were performed according to the reported procedures [27-29]. Kinase activities were assayed in Buffer A (10 mM $MgCl_2$, 1 mM EGTA, 1 mM DTT, 25 mM Tris-HCl (pH = 7.5), 50 µg heparin/mL.) or C (60 mM ß-glycerophosphate, 15 mM *p*-nitrophenylphosphate, 25 mM Mops (pH = 7.2), 5 mM EGTA, 15 mM $MgCl_2$, 1 mM DTT, 1 mM sodium vanadate, 1 mM phenylphosphate) at 30 °C, at a final ATP concentration of 15 µM. Blank values were subtracted and activities expressed in % of the maximal activity, i.e. in the absence of inhibitors. Controls were performed with appropriate dilutions of DMSO.

CDK1/cyclin B (M phase starfish oocytes, native), CDK2/cyclin A, CDK2/cyclin E, CDK5/p25 and CDK7/cyclin H (human, recombinant) were prepared as previously described. Their kinase activity was assayed in buffer C, with 1 mg histone H1/mL, in the presence of 15 µM [γ-^{33}P] ATP (3,000 Ci/mmol; 10 mCi/mL) in a final volume of 30 µL. After 30 min incubation at 30 °C, 25 µL aliquots of supernatant were spotted onto 2.5 x 3 cm pieces of Whatman P81 phosphocellulose paper, and, 20 sec later, the filters were washed five times (for at least 5 min each time) in a solution of 10 mL phosphoric acid/liter of water. The wet filters were counted in the presence of 1 mL ACS (Amersham) scintillation fluid (Table 1).

GSK-3α/β (porcine brain, native) was assayed, as described for CDK1 but in Buffer A and using a GSK-3 specific substrate (GS-1: YRRAAVPPSPSLSRHSSPHQSpEDEEE) (pS stands for phosphorylated serine). GS-1 was synthesized by Millegen (Labege, France). CK1 (porcine brain, native) was assayed as described for CDK1 but using the CK1-specific peptide substrate RRKHAAIGpSAYSITA, obtained from Millegen (Labege, France).

3. Results and discussion

3.1. Chemistry

The target compounds were prepared according to Schemes 1-3. Compound **2** can exist in two tautomeric forms: the imine and the enamine which is the more predominant tautomer [30].

In this study, our target was to synthesize novel quinoxalines through the application of aliphatic electrophilic substitution reactions on the methyl and methylene of the side

chain of 3-acetonylquinoxalin-2(1*H*)-one **2**, followed by cyclization reactions.

Table 1. Inhibition effect (μM) on CK1, CDK5 and GSK3β of selected compounds.

Compound	CK1	CDK5	GSK-3β
Compound 3a	> 10	> 10	> 10
Compound 3b	> 10	> 10	> 10
Compound 3c	> 10	> 10	> 10
Compound 5a	> 10	> 10	> 10
Compound 5b	> 10	> 10	> 10
Compound 6a	> 10	> 10	> 10
Compound 6b	> 10	> 10	> 10
Compound 6c	> 10	> 10	> 10
Compound 7	> 10	> 10	> 10
Compound 8	> 10	> 10	> 10
Compound 9a	> 10	> 10	> 10
Compound 9b	> 10	> 10	> 10
Compound 10a	> 10	> 10	> 10
Compound 10b	> 10	> 10	> 10
Compound 11a	> 10	> 10	> 10
Compound 11b	> 10	> 10	> 10
Compound 12	> 10	> 10	> 10
Compound 13	> 10	> 10	> 10
Compound 14a	> 10	> 10	> 10

In Scheme 1, the formation of chalcone product was catalyzed by a basic or acidic catalyst [31]. In alcoholic alkali medium the electrophilic substitution reaction occurred on the terminal methyl group. ^{1}H NMR spectrum of compound **3** revealed the disappearance of the singlet signal at δ 2.17 ppm corresponding to the terminal methyl protons and the presence of a singlet signal at δ 6.38 ppm corresponding to the vinylic protons. On the other hand, reaction in acetic acid afforded compound **4** and its ^{1}H NMR spectrum showed a multiplet signal at δ 6.94-7.58 ppm corresponding to the aromatic protons and the disappearance of both aliphatic protons at δ 2.17 ppm and the vinylic proton at δ 6.01 ppm. Mass spectrum of the product showed a molecular ion peak at m/z 288.

Compound **4** was prepared by two different ways, firstly by refluxing of compound **2** with benzaldehyde in glacial acetic acid or fusing the starting compound **2** with benzaldehyde at 180-190 ^{o}C. Furthermore, refluxing of compound **3**a in bromine/acetic acid afforded compound **4**.

Secondly, Hantzsch reaction was applied on **2** using ethyl cyanoacetate in ethanol or *n*-butanol resulted in the same product **5a** in yields of 68% and 77%, respectively. The reaction mechanism as guided by Katritzky [32] may proceed via the formation of the enaminone intermediate. The second step involved Micheal addition of this intermediate to the α,β-unsaturated ester, initially formed from the reaction of the appropriate aldehyde with ethyl cyanoacetate, followed by cyclization and aromatization to yield the target compounds **5a**,**b**. On the other hand, the reaction of compound **2** with aromatic aldehydes, malononitrile and ammonium acetate in ethanol gave the products **6a**-**c**. Moreover, heating compound **2** with paraformaldehyde in ethanol containing acetic acid yielded product **7** [33]. ^{1}H NMR spectrum of compound **7** showed a singlet signal at δ 2.24 ppm corresponding to the terminal methyl protons and the absence of the vinylic proton. Aminomethylation of compound **2** was carried out using paraformaldehyde and piperidine in ethanol containing few drops of acetic acid gave product **8**. The structure assignment depends on the position at which the electrophilic substitution reaction took place. IR spectra showed bands at 1375 and 1380 cm^{-1}, respectively, corresponding to the bending vibration of the terminal methyl group [34] in addition to bands at 3450 and 3400 cm^{-1}, respectively, due to NH group and bands at 1690-1650 cm^{-1} due to C=O groups. ^{1}H NMR spectrum showed a singlet signal at δ 2.38 ppm corresponding to the terminal methyl group protons and the absence of the vinylic proton. Dehydration of compounds **3a**,**b** in acetic anhydride/sulphuric acid mixture gave the required compounds **9a**,**b** . IR showed the absence of bands corresponding to NH and C=O groups. ^{1}H NMR spectrum revealed the absence of the singlet signal of the vinylic proton and the absence of the exchangeable singlet signals corresponding to NH protons. Refluxing compounds **3a**,**c** with hydrazine hydrate in ethanol containing few drops of glacial acetic acid afforded compounds **10a**,**b** [35]. Mass spectrum of the compound **10a** showed molecular ion peak at m/z 302. ^{1}H NMR spectrum of compound **10b** showed a singlet signal at δ 4.27 ppm corresponding to methylene protons, a multiplet signal at δ 6.77-8.60 ppm corresponding to the aromatic protons and two exchangeable singlet signals at δ 12.51 and 13.06 ppm corresponding to 2 NH protons. Reaction of compound **3a**,**c** with hydrazine hydrate afforded 3-[(5-aryl-1*H*-pyrazol-3-yl)methyl]-quinoxalin-2(1*H*)-ones (**10a**,**b**). The proposed mechanism for this reaction may involve either the intermediate formation of hydrazones or the initial Micheal addition of the hydrazine on the chalcone.

Coupling the alkaline solution of 3-acetonylquinoxalin-2(1*H*)-one (**2**) with freshly prepared diazonium salts at pH= 5.0-5.5 yielded products **11a**,**b**. IR spectrum showed a band at 1380-1370 cm^{-1} due to the bending vibration of the terminal methyl group. ^{1}H NMR spectra of compounds **11a** showed a singlet signal at δ 2.63 ppm corresponding to the terminal methyl group and the absence of the singlet signal corresponding to the vinylic proton.

Reaction of **11a** with bromine in acetic acid containing sodium acetate yielded **12**. Mass spectrum showed a molecular ion peak at m/z 304 and ^{1}H NMR spectrum which revealed a multiplet signal at δ 7.33-8.27 ppm corresponding to the aromatic protons, in addition to two exchangeable singlet signals at δ 11.04 and 13.27 ppm corresponding to NH and OH protons, respectively [36].

Treating a solution of compound **2** in acetic acid with solid sodium nitrite, following the directions of Shawali and Fahmi [37] yielded **13** whose structure depends on the position at which the electrophilic substitution took place. ^{1}H NMR spectrum showed a singlet signal at δ 2.49 ppm corresponding to the methyl protons and the absence of the singlet signal at δ 6.01 ppm due to the vinylic proton. It was concluded that the weak non-bulky electrophile attacked the more activated position 1 at the side chain. Furthermore, reaction of oxime **13** with benzaldehyde in aqueous sodium hydroxide gave product **14**, which showed the expected molecular weight at m/z 319. ^{1}H NMR spectrum showed a singlet signal at δ 6.37 ppm. This suggested an internal Micheal addition of the OH group of the chalcone analogue on the C=C to afford an oxazine ring.

One of the tested compounds **10a** was found to exhibit slight activity against *staphylococcus aureus*. Compounds **10b**, **11b** and **15b** showed slight activity against *Escherichia coli.* Unfortunately, the rest of the tested compounds (**3d**, **4**, **5b**, **6c**, **9a**,**10c**,**12**,**13**,**14a**) showed no inhibition against *Staphylococcus Aureus*, *Bacillus subtitis*, *Escherichia Coli*, *Pseudo aeruginosa* and *Candida albicans.* Nineteen compounds were screened for their inhibition effect on CK1, CDK5, and GSK-3β. None of the tested compounds showed an inhibition activity below 10 μM concentration.

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