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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Synthesis of new 2(1H)-quinoxalinone derivatives for antimicrobial and antiinflammatory evaluation

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Abstract New 2(1H)-quinoxalinones and their hexahydro derivatives were prepared for investigating their antimicrobial and antiinflammatory activities. The study showed that thiosemicarbazide **6a** derived from the hexahydro-2(1H)-quinoxalinone series has nearly the same antibacterial activity as the reference drug ciprofloxacin. Moreover, the 2(1H)-quinoxalinones bearing N-phenyltriazole (**9a**) or 4-chlorophenyl-2,3-dihydrothiazole (**13b**) moieties were the most active ones after 4 h with use of the rat hind paw edema method, whereas their antiinflammatory and ulcerogenic activities were comparable with the selective COX-2 inhibitor celecoxib.

Keywords Antiinflammatory activity · Antimicrobial activity · 2(1H)-Quinoxalinones · Synthesis

Introduction

Among the various classes of heterocyclic compounds, quinoxaline forms an essential component of those that are biologically active. Morbidity and mortality due to enteric bacterial infections have caused important health problems worldwide, mainly in developing countries (Devasia *et al.*, 2006; Qadri *et al.*, 2005). Toxicity and resistance to the drugs also have played an important role in

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treatment failure (Nolan *et al.*, 1979). Consequently, there is an urgent need to screen new compounds for the development of new antibacterial agents.

The study of quinoxaline derivatives has generated much interest in recent years because of their antimalarial (Vicente *et al.*, 2008), antidepressant (Trivedi and Bruns, 1988), anticancer (Daina *et al.*, 2008; Moarbess *et al.*, 2008; Ortega *et al.*, 2002), and antiviral (El Ashry *et al.*, 1999) activities. In addition, other compounds bearing quinoxaline moiety have shown antibacterial (Carta *et al.*, 2003; Khan, 2008; Khan *et al.*, 2007; Refaat *et al.*, 2004), antifungal (Badran *et al.*, 2003; Carta *et al.*, 2001), and antiinflammatory (Burguete *et al.*, 2007; Smits *et al.*, 2008) activities.

We report simple and efficient methods for preparation of 2(1H)-quinoxalinone derivatives bearing different chemical entities that promise superior antimicrobial and antiinflammatory activities.

Materials and methods

Chemistry

Melting points, determined with a Gallenkamp melting point apparatus (London, UK), are uncorrected. Infrared (IR) spectra (KBr, cm^{-1}) were recorded on a Bruker Vector, 22FT-IR (Bavaria, Germany) or Testscan Shimadzu FT 8000 spectrometer (Tokyo, Japan), and ^{1}H-NMR spectra were recorded on Varian Mercury-300 (300 MHz) (Palo Alto, CA, USA) and Varian Gemini 200 MHz spectrometers (Foster City, CA, USA) using dimethyl sulfoxide (DMSO)-d_6 or $CDCl_3$ as a solvent and tetramethylsilane (TMS) as an internal standard (Chemical shift in δ, ppm). Electron impact mass spectra were determined using a GC/MS Mat 112 S at 70 eV spectrometer. Elemental analyses were determined using the Heraeus (Hanau, Germany) and Vario EL-III (Elemntar) CHNS analyzer (Hanau, Germany) at the National Research Center and Microanalytical Center, Faculty of Science, University of Cairo, Egypt.

All the results of the elemental analyses were in an acceptable error range. Thin-layer chromatography (TLC) was performed on silica gel G for TLC (Merck), and spots were visualized by iodine vapors or by irradiation with ultraviolet light (UV; 254 nm). All chemicals were purchased from Sigma Chemical (St. Louis, MO, USA). Intermediate 3-(ethoxycarbonylmethyl)-2(1H)-quinoxalinone was prepared according to the reported procedure (Kurasawa *et al.*, 1995). The animal experiments were performed in accordance with international guidelines.

*3-Methyl-4a,5,6,7,8,8a-hexahydro-2(1H)-quinoxolinone (**1**)*

A mixture of 1,2-cyclohexanediamine (35 mmol), ethanol (30 mL), and glacial acetic acid (1 mL) was heated up to 80°C. Ethyl pyurvate (40 mmol) was added to the reaction mixture with intensive stirring for 12 h at room temperature. The separated product was filtered, dried, and crystallized from dimethylformamide (DMF)/H_2O. Yield: 73%; m.p.: 187°C; spectroscopic analysis: ^{1}H-NMR (300 MHz, DMSO-d_6): δ = 1.133–1.866 (m, 8H, cyclohexane-H), 2.02 (s, 3H, CH_3), 2.134–

2.996 (m, 2H, cyclohexane-H), 8.24 (s, 1H, NH) ppm. Anal. calcd. for $C_9H_{14}N_2O$: C, 65.03; H, 8.49; N, 16.85%; found: C, 64.83; H, 8.45;N, 16.56%.

*General procedure for preparation of compounds **2a–e***

A mixture of the appropriate aromatic amine (5 mmol) in HCl (1.5 mL, 37%) and water (4 mL) was cooled to 0°C, then diazotized using cooled solution of $NaNO_2$ (5 mmol) in water (2 mL). The mixture was stirred for 20 min at 0°C, then added to the stirred solution prepared from compound **1** (5 mmol) dissolved in aqueous acetic acid (10 mL) containing CH_3COONa (1 g) at 0°C. The mixture was set aside at 0° to 5°C for 24 h, then diluted with water. The separated product was filtered, washed with water, and crystallized from ethanol.

*3-[(2-Phenylhydrazono)methyl]-4a,5,6,7,8,8a-hexahydro-2(1H)-quinoxalinone (**2a**)* Yield: 70%; m.p.:215°C; spectroscopic analysis: ^{1}H-NMR (300 MHz, $CDCl_3$): $\delta = 1.421–2.389$ (m, 8H, cyclohexane-H), 3.249–3.385 (m, 2H, cyclohexane-H), 6.250 (br s, 1H, NH), 6.971–7.522 (m, 5H, ArH), 7.547 (s, 1H, CH=N), 14.400 (s, 1H, NH) ppm. Anal. calcd. for $C_{15}H_{18}N_4O$: C, 66.64; H, 6.71; N, 20.73%; found: C, 66.84; H, 6.57; N, 20.59%.

*3-[(2-(4-Bromophenyl)hydrazono)methyl]-4a,5,6,7,8,8a-hexahydro-2(1H)-quinoxalinone (**2b**)* Yield: 86%; m.p.: 236°C; spectroscopic analysis: IR (KBr): $\nu = 3188$ (NH), 3078 (CH, aromatic), 2936 (CH, aliphatic), 1689 (C=O) cm^{-1}; ^{1}H-NMR (300 MHz, $CDCl_3$): $\delta = 1.269–2.401$ (m,8H,cyclohexane-H), 3.276–3.444 (m,2H, cyclohexane-H), 6.196 (br s, 1H, NH), 7.106–7.692 (m, 5H, ArH+ CH=N), 14.311 (br s, 1H, NH) ppm. Anal. calcd. for $C_{15}H_{17}BrN_4O$: C, 51.59; H, 4.91; N, 16.04%; found: C, 51.13; H, 5.04; N, 15.52%.

*3-[(2-(4-Chlorophenyl)hydrazono)methyl]-4a,5,6,7,8,8a-hexahydro-2(1H)-quinoxalinone (**2c**)* Yield: 82%; m.p.: 254–255°C; spectroscopic analysis: ^{1}H-NMR (300 MHz, $CDCl_3$): $\delta = 1.269–2.434$ (m, 8H, cyclohexane-H), 3.194–3.304 (m, 2H, cyclohexane-H), 5.857–5.918 (d, 1H, NH), 7.174–7.556 (m, 5H, ArH+ CH=N), 14.306 (br s, 1H, NH) ppm. Anal. calcd. for $C_{15}H_{17}ClN_4O$:C, 59.11; H, 5.62; N, 18.38%; found: C, 59.17; H, 5.76; N, 17.84%.

*3-[(2-(4-Flurophenyl)hydrazono)methyl]-4a,5,6,7,8,8a-hexahydro-2(1H)-quinoxalinone (**2d**)* Yield: 84%; m.p.: 241–242°C; spectroscopic analysis: IR (KBr): $\nu = 3188$ (NH), 3078 (CH, aromatic), 2936 (CH, aliphatic), 1689 (C=O) cm^{-1}; ^{1}H-NMR (300 MHz, $CDCl_3$): $\delta = 1.267–2.373$ (m, 8H, cyclohexane-H), 3.264–3.387 (m, 2H, cyclohexane-H), 6.058 (br s, 1H, NH), 6.983–7.272 (m, 4H, ArH), 7.526 (s, 1H, CH=N), 14.150 (br s, 1H, NH) ppm. Anal. calcd. for $C_{15}H_{17}FN_4O$:C, 62.49; H, 5.94; N, 19.43%; found: C, 62.65; H, 6.12; N, 19.36%.

*3-[(2-(4-Methylphenyl)hydrazono)methyl]-4a,5,6,7,8,8a-hexahydro-2(1H)-quinoxalinone (**2e**)* Yield: 90%; m.p.: 243–245°C; spectroscopic analysis: IR (KBr):

v = 3188 (NH), 3079 (CH, aromatic), 2935(CH, aliphatic), 1687 (C=O)cm^{-1}. MS: *m/z* (rel. int.) = 284 (M^+, 76.0), 255 (17.8), 202 (31.8), 186 (30.0), 159 (14.2), 132 (18.6), 106 (69.7), 81 (100.0). Anal. calcd. for $C_{16}H_{20}N_4O$: C, 67.58; H, 7.09; N, 19.70%; found: C, 67.79; H, 7.13; N, 19.58%.

*3-(Ethoxycarbonylmethyl)-4a,5,6,7,8,8a-hexahydro-2(1H)-quinoxalinone (**3**)* A mixture of 1,2-cyclohexanediamine (35 mmol), ethanol (35 mL), and glacial acetic acid (1 mL) was heated up to 80°C. Diethyl oxaloacetate sodium (35 mmol) was then added to the reaction mixture with intensive stirring for 12 h at room temperature. The separated product was filtered, dried, and crystallized from ethanol. Yield: 73%; m.p.: 182–183°C; spectroscopic analysis: MS: *m/z* (rel. int.) = 238 (M^+, 48.63), 193 (45.25), 192 (32.19), 166 (100.0), 123 (38.59), 81 (43.22), 68 (54.07). Anal. calcd. for $C_{12}H_{18}N_2O_3$:C, 60.49; H, 7.61; N, 11.76%; found: C, 60.43; H, 7.11; N, 11.81%.

*3-(Hydrazinocarbonylmethyl)-4a,5,6,7,8,8a-hexahydro-2(1H)-quinoxalinone (**4**)* A mixture of the ester **3** (20 mmol) and hydrazine hydrate (40 mmol) in ethanol (30 mL) was refluxed for 2 h. The obtained product was collected, dried, and crystallized from DMF. Yield: 70%; m.p.: 260–261°C; spectroscopic analysis: ^{1}H-NMR (200 MHz, DMSO-d_6): δ = 1.207–2.964 (m, 10H, cyclohexane-H), 3.538–3.947 (m, 2H, CH_2CO), 5.635 (s, 2H, NH_2, exch.), 7.532 (d, 1H, NH, exch.), 7.776–7.819 (d, 1H, NH, exch.) ppm. Anal. calcd. for $C_{10}H_{16}N_4O_2$:C, 53.56; H, 7.19; N, 24.98%; found: C, 53.46; H, 7.24; N, 24.79%.

*3-[N-(Phthalimido)aminocarbonylmethyl]-4a,5,6,7,8, 8a-hexahydro-2(1H)-quinoxalinone (**5**)* A mixture of the hydrazide **4** (5 mmol), and phthalic anhydride (5 mmol) in glacial acetic acid (20 mL) was heated at reflux for 2 h. After cooling, the separated product was filtered and crystallized from DMF/H_2O. Yield: 79%; m.p.: 290–291°C; spectroscopic analysis: IR (KBr): v = 3376, 3190 (NH), 3064 (CH, aromatic), 2946 (CH, aliphatic), 1764, 1699, 1648 (C=O) cm^{-1}. MS: *m/z* (rel. int.) = 354 (M^+, 10.9), 243 (55.3), 207 (99.6), 160 (24.2), 111 (100.0), 56 (90.7). Anal. calcd. for $C_{18}H_{18}N_4O_4$:C, 61.01; H, 5.12; N, 15.81%; found: C, 60.88; H, 5.09; N, 15.77%.

*General procedure for preparation of compounds **6a–c***

A mixture of hydrazide **4** (5 mmol) and the appropriate isothiocyante (5 mmol) in ethanol (20 mL) was heated at reflux for 2 h. After cooling, the separated product was filtered and crystallized from dioxane/H_2O.

*N^1-[(2-Oxo-1,2,4a,5,6,7,8,8a-octahydroquinoxalin-3-yl)methylcarbonyl]-N^4-phenylthiosemicarbazide (**6a**)* Yield: 80%; m.p.: 200–201°C; spectroscopic analysis: IR (KBr): v = 3216 (NH), 2933 (CH, aliphatic), 1646 (C=O) cm^{-1}. ^{1}H-NMR (300 MHz, DMSO-d_6): δ = 1.134–2.140 (m, 10H, cyclohexane-H), 3.803–3.864 (m, 1H, CH_2CO), 4.267 (m, 1H, CH_2CO), 7.036–7.412 (m, 5H, ArH), 7.525–7.552 (d, 1H, NH, exch.), 8.159 (br s, 1H, NH, exch.), 9.491 (s, 1H, NH, exch.), 12.240 (br s,

1H, NH, exch.) ppm. Anal. calcd. for $C_{17}H_{21}N_5O_2S$: C, 56.80; H, 5.89; N, 19.48%; found: C, 56.50; H, 5.88; N, 19.20%.

*N^1-[(2-Oxo-1,2,4a,5,6,7,8,8a-octahydroquinoxalin-3-yl)methylcarbonyl]-N^4-ethyl thiosemicarbazide (**6b**)* Yield: 60%; m.p.: 244–245°C; spectroscopic analysis: ^{1}H-NMR (300 MHz, DMSO-d_6): $\delta = 0.969$–2.011 (m, 13H, cyclohexane-H+ CH_3), 3.245–3.237 (br s, 2H, CH_2), 3.677–4.125 (m, 2H, CH_2), 7.123–7.148 (d, 1H, NH, exch.), 7.389 (s, 1H, NH, exch.), 8.098 (br s, 1H, NH, exch.), 12.208 (br s, 1H, NH, exch.) ppm. Anal. calcd. for $C_{13}H_{21}N_5O_2S$: C, 50.14; H, 6.80; N, 22.49%; found: C, 49.76; H, 6.76; N, 22.27%.

*N^1-[(2-Oxo-1,2,4a,5,6,7,8,8a-octahydroquinoxalin-3-yl)-methyl-carbonyl]-N^4-allyl thiosemicarbazide (**6c**)* Yield: 65%; m.p.: 224–225°C; spectroscopic analysis: ^{1}H-NMR (300 MHz, DMSO-d_6): $\delta = 1.031$–2.15 (m, 10H, cyclohexane-H), 3.389–3.955 (m,4H, two CH_2), 4.961–5.097 (m,2H, $=CH_2$), 5.697–5.824 (m,1H, =CH), 7.224–7.242 (d, 1H, NH, exch.), 7.534 (br s, 1H, NH, exch.), 8.050 (br s, 1H, NH, exch.), 12.20 (br s, 1H, NH, exch.) ppm. Anal. calcd. for $C_{14}H_{21}N_5O_2S$: C, 51.99; H, 6.54; N, 21.65%; found: C, 51.56; H, 6.80; N, 21.10%.

*3-(Hydrazinocarbonylmethyl)-2(1H)-quinoxalinone (**7**)* It was prepared and crystallized as previously mentioned under intermediate **4**, but the 3-(ethoxycarbonylmethyl)-2(1H)-quinoxalinone intermediate was used instead of the ester **3**. Yield: 72%; m.p.: 255–256°C; spectroscopic analysis: ^{1}H-NMR (300 MHz, DMSO-d_6): $\delta = 3.618$, 5.586 (two s, 2H, CH_2), 4.278 (br s, 2H, NH_2, exch.), 6.865–7.734 (m, 4H, ArH), 9.121 (s, 1H, NH, exch.), 11.372 (br s, 1H, NH, exch.) ppm. Anal. calcd. for $C_{10}H_{10}N_4O_2$: C, 55.04; H, 4.62; N, 25.68%; found: C, 55.32; H, 4.41; N, 25.66%.

*General procedure for preparation of compounds **8a–c***

These compounds were prepared and crystallized as previously mentioned for compounds **6a–c**, but hydrazide **7** was used instead of hydrazide **4**.

*N^1-[(2-Oxo-1,2-dihydroquinoxalin-3-yl)methylcarbonyl]-N^4-phenyl-thiosemicarbazide (**8a**)* Yield: 82%; m.p.: 224–225°C; spectroscopic analysis: ^{1}H-NMR (200 MHz, DMSO-d_6): $\delta = 2.741$–2.897 (two s, 1H, CH_2), 3.805 (s, 1H, CH_2), 6.547–7.758 (m, 9H, ArH), 9.877–9.934 (d, 1H, NH, exch.), 10.485 (s, 1H, NH, exch.), 11.596 (s, 1H, NH, exch.),12.660 (br s, 1H, NH, exch.) ppm. Anal. calcd. for $C_{17}H_{15}N_5O_2S$: C, 57.78; H, 4.28; N, 19.82%; found: C, 57.45; H, 4.35; N, 20.11%.

*N^1-[(2-Oxo-1,2-dihydroquinoxalin-3-yl)methylcarbonyl]-N^4-ethyl thiosemicarbazide (**8b**)* Yield: 63%; m.p.: 235–236°C; spectroscopic analysis: ^{1}H-NMR (300 MHz, DMSO-d_6): $\delta = 1.029$–1.151 (m, 3H, CH_3), 3.429–3.527 (m, 2H, CH_2), 3.710, 5.688 (two s, 2H, CH_2CO), 6.946–7.776 (m, 4H, ArH), 7.90 (br s, 1H, NH, exch.), 9.70 (s, 1H, NH, exch.), 10.151 (s, 1H, NH, exch.), 11.556 (d, 1H, NH, exch.) ppm. Anal. calcd. for $C_{13}H_{15}N_5O_2S$: C, 51.13; H, 4.95; N, 22.94%; found: C, 51.30; H, 4.96; N, 23.32%.

*N^1-[(2-Oxo-1,2-dihydroquinoxalin-3-yl)methylcarbonyl]-N^4-allyl-thiosemicarbazide (**8c**)* Yield: 68%; m.p.: 240–241°C; spectroscopic analysis: IR (KBr): $\nu = 3452$, 3271(NH), 3096 (CH, aromatic), 2926 (CH, aliphatic), 1685, 1632 (C=O) cm^{-1}. MS: *m/z* (rel. int.) = 317 (M^+, 0.18), 299 (2.90), 218 (15.08), 187 (73.47), 159 (30.19), 131 (44.88), 41 (99.10), 39 (100.00). Anal. calcd. for $C_{14}H_{15}N_5O_2S$: C, 52.98; H, 4.76; N, 22.07%; found: C, 52.71; H, 5.00; N, 22.35%.

*General procedure for preparation of compounds **9a–c***

Compounds **9a–c** (5 mmol) were dissolved in NaOH (2N, 20 mL), then heated under reflux for 2 h. The solution was cooled, filtered, and then acidified with HCl (2 N). The separated solid was filtered and crystallized from DMF/ethanol.

*3-[(5-Mercapto-4-phenyl-4H-1,2,4-triazol-3-yl)methyl]-2(1H)-quinoxalinone (**9a**)* Yield: 81%; m.p.: 369–370°C; spectroscopic analysis: ^{1}H-NMR (200 MHz, DMSO-d_6): $\delta = 4.107$ (s, 1H, CH_2), 5.453 (s, 1H, CH_2), 7.022–7.730 (m, 9H, ArH), 10.058 (s, 1H, NH, exch.), 14.60 (br s, 1H, SH, exch.) ppm. Anal. calcd. for $C_{17}H_{13}N_5OS$: C, 60.88; H, 3.91; N, 20.88%; found: C, 60.83; H, 3.95; N, 20.62%.

*3-[(5-Mercapto-4-ethyl-4H-1,2,4-triazol-3-yl)methyl]-2(1H)-quinoxalinone (**9b**)* Yield: 70%; m.p.: 345–346°C; spectroscopic analysis: ^{1}H-NMR (300 MHz, DMSO-d_6): $\delta = 1.189$–1.236 (t, 3H, CH_3), 3.987–4.104 (q, 2H, CH_2), 4.275, 5.982 (two s, 2H, CH_2), 6.964–7.698 (m, 4H, ArH), 10.088 (s, 1H, NH, exch.), 13.90 (br s, 1H, SH, exch.) ppm. Anal. calcd. for $C_{13}H_{13}N_5OS$: C, 54.34; H, 4.56; N, 24.37%; found: C, 53.98; H, 4.31; N, 24.29%.

*3-[(5-Mercapto-4-allyl-4H-1,2,4-triazol-3-yl)methyl]-2(1H)-quinoxalinone (**9c**)* Yield: 72%; m.p.: 324–326°C; spectroscopic analysis: IR (KBr): $\nu = 3441$(NH), 3088, 3040 (CH, aromatic), 2992, 2923 (CH, aliphatic), 1676 (C=O)cm^{-1}. MS: *m/z* (rel. int.) = 299 (M^+, 84.41), 284 (22.99), 185 (45.63), 171 (12.26), 157 (20.25), 90 (24.80), 41 (100.00). Anal. calcd. for $C_{14}H_{13}N_5OS$: C, 56.17; H, 4.38; N, 23.40%; found: C, 56.39; H, 4.50; N, 23.24%.

*3-[(5-Mercapto-1,3,4-oxadiazol-2-yl)methyl]-2(1H)-quinoxalinone (**10**)* The acid hydrazide **7** (5 mmol) was stirred in ethanol (30 mL) containing potassium hydroxide (5 mmol) for 1 h until a clear solution was obtained. Carbon disulfide (12.5 mmol) was added dropwise to the stirred reaction mixture, and then it was heated under reflux for 6 h. The reaction mixture was concentrated, cooled, and acidified with diluted HCl. The separated product was filtered, washed with water, and crystallized from dioxane. Yield: 90%; m.p.: 280–281°C; spectroscopic analysis: IR (KBr): $\nu = 3284$ (NH), 3042, 3010 (CH, aromatic), 2964 (CH, aliphatic), 1681 (C=O)cm^{-1}. MS: *m/z* (rel. int.) = 260 (M^+, 100.0), 187 (68.9), 171 (30.0), 131 (41.6), 90 (25.4), 63 (31.5). Anal. calcd. for $C_{11}H_8N_4O_2S$: C, 50.76; H, 3.10; N, 21.53%; found: C, 50.62; H, 3.39; N, 21.30%.

*Potassium,3-[2-(2-oxo-1,2-dihydroquinoxalin-3-yl)acetyl]dithiocarbazate (**11**)* Carbon disulfide (15 mmol) was added dropwise to an ice-cooled solution of ethanol (20 mL) containing KOH (10 mmol) and hydrazide **7** (10 mmol). The mixture was stirred for 14 h, and then dry diethyl ether (10 mL) was added. The separated solid was filtered and then washed twice with diethyl ether (20 mL). The obtained product was used in the next reaction without further purification.

*3-[(4-Amino-5-mercapto-4H-1,2,4-triazol-3-yl)methyl]-2(1H)-quinoxalinone (**12**)* A mixture of intermediate **11** (5 mmol) and hydrazine hydrate (98%; 10 mmol) in ethanol (20 mL) was refluxed for 4 h. The reaction mixture was diluted with cold water, then neutralized by portionwise addition of concentrated HCl. The formed precipitate was filtered, washed with water, and crystallized from DMF. Yield: 83%; m.p.: 301–302°C; spectroscopic analysis: IR (KBr): $v = 3480$, 3324 (NH, NH_2), 3106, 3049 (CH, aromatic), 2949 (CH, aliphatic), 1663 (C=O) cm^{-1}. MS: *m/z* (rel. int.) = 274 (M^+, 100.0), 228 (14.8), 186 (18.5), 157 (22.5), 131 (27.1), 90 (24.0), 60 (32.5). Anal. calcd. for $C_{11}H_{10}N_6OS$: C, 48.17; H, 3.67; N, 30.64%; found: C, 47.96; H, 3.81; N, 30.68%.

*General procedure for preparation of compounds **13a–c***

To a solution of hydrazide **7** (10 mmol) in dioxane (30 mL), carbon disulfide (15 mmol) and KOH (10 mmol) in water (10 mL) were added. The reaction mixture was heated in a boiling water bath for 1 h, then left to cool to 20°C. The appropriate phenacyl bromide (10 mmol) was added to the reaction mixture and then stirred at room temperature for 12 h. The solid formed at acidification with HCl was filtered and crystallized from DMF/ethanol.

*3-[N-(4-Phenyl-2-thioxo-2,3-dihydrothiazol-3-yl)aminocarbonyl methyl]-2(1H)-quinoxalinone (**13a**)* Yield: 83%; m.p.: 238–239°C; spectroscopic analysis: IR (KBr): $v = 3340$, 3171 (NH), 3018 (CH, aromatic), 2959 (CH, aliphatic), 1681,1630 (C=O) cm^{-1}. ^{1}H-NMR (300 MHz, DMSO-d_6): $\delta = 5.077$–5.105 (d, 2H, CH_2), 6.995–8.080 (m, 10H, ArH+ C_5-thiazole H), 10.346 (s, 1H, NH), 11.675 (s, 1H, NH) ppm. Anal. calcd. for $C_{19}H_{14}N_4O_2S_2$:C, 57.85; H, 3.58; N, 14.20%; found: C, 57.76; H, 3.38; N, 13.90%.

*3-[N-(4-Chlorophenyl-2-thioxo-2,3-dihydrothiazol-3-yl)aminocarbonylmethyl]-2(1H)-quinoxalinone (**13b**)* Yield: 85%; m.p.: 243–244°C; spectroscopic analysis: ^{1}H-NMR (300 MHz, DMSO-d_6): $\delta = 5.054$–5.095 (d, 2H, CH_2), 6.979–8.094 (m, 9H, ArH+ C_5-thiazole H), 10.343 (s, 1H, NH), 11.713 (s, 1H, NH) ppm. Anal. calcd. for $C_{19}H_{13}ClN_4O_2S_2$: C, 53.20; H, 3.05; N, 13.06%; found: C, 53.22; H, 3.31; N, 12.77%.

*3-[N-(4-Methylphenyl-2-thioxo-2,3-dihydrothiazol-3-yl)aminocarbonylmethyl]-2(1H)-quinoxalinone (**13c**)* Yield: 80%; m.p.: 246–247°C; spectroscopic analysis: ^{1}H-NMR (300 MHz, DMSO-d_6): $\delta = 2.389$ (s, 3H, CH_3), 5.039–5.073 (d, 2H, CH_2),

7.005–7.977 (m, 9H, ArH+ C_5-thiazole H), 10.357 (s, 1H, NH), 11.682 (s, 1H, NH) ppm. Anal. calcd. for $C_{20}H_{16}N_4O_2S_2$: C, 58.80; H, 3.95; N, 13.72%; found: C, 58.55; H, 4.15; N, 13.43%.

Biological activities

Antimicrobial activity

Methodology: the cup-plate technique (Reeves and White, 1983) The chosen compounds (**2a–e, 5, 6a,c, 8a,c, 9a,c, 10, 12**, and **13a,b**) were dissolved in DMF at a concentration of 10 mg/mL. The antibacterial ciprofloxacin and the antifungal nystatin were used as references drugs at the same dose level, while DMF was used as a negative control.

Nutrient agar (Oxoid, England) was inoculated with one bacterial strain. The bacterial strains used were Gram-positive bacteria (*Staphylococcus aureus* and *Bacillus subtilis*) and Gram-negative bacteria (*Escherichia coli*). Saburaud's dextrose agar (Oxoid, England) was seeded with *Candida albicans* as the representative of fungi. After solidification, cups were made by cork borer, then filled with 50 μL from compound solution. Each compound was assessed in triplicate. The plates were incubated overnight at 37°C, and then the inhibition zones were measured in millimeters. The results of the antimicrobial activity for the new compounds were recorded in Table 1. Bacterial and fungal strains were isolated and identified by the Department of Microbiology, Faculty of Pharmacy, Zagazig University, Zagazig Egypt.

Statistical analysis

Data were analyzed using computer program SPSS (SPSS Inc., Chicago, IL, USA). The differences in mean values were determined by analysis of variance (one-way ANOVA) followed by least significant difference (LSD).

Pharmacology

Antiinflammatory activity The rat hind paw edema method (Winter *et al.*, 1962) was applied to determine the antiinflammatory activity of the test compounds (**9a–b, 10, 12**, and **13a–c**) using celecoxib as a standard. Mature albino rats of both sexes weighing 150–200 g were used. The animals were divided into nine equal groups (each of five). The first group was left as a control group while the second group was injected (intraperitoneal; i.p.) with celecoxib at a dose of 0.9 mg/100 g body weight.

The test compounds were injected i.p. in the remaining groups at the same dose level. After 1 h, edema in the right hind paw was induced by injecting 0.1 mL of 10% carrageenin. The thickness of the paw was measured using a skin caliber 1, 2, 3, and 4 h after the carrageenin injection to determine the antiinflammatory activity of the test compounds (Table 2).

Table 1 Antimicrobial activity evaluation for compounds **2a–e, 5, 6a,c, 8a,c, 9a,c, 10, 12**, and **13a,b** expressed by diameter of the inhibition zone[#]

Compound	Diameter of inhibition zone (mm)			
	Gram-positive		Gram-negative	Fungi
	Staphylococcus aureus	*Bacillus subtilis*	*Escherichia Coli*	*Candida albicans*
2a	6 ± 0.2*	8 ± 0.2*	4 ± 0.4*	–
2b	13 ± 0.3*	11 ± 0.5*	8 ± 0.3*	–
2c	16 ± 0.3	15 ± 0.3*	6 ± 0.01*	–
2d	16 ± 0.12	17 ± 0.3*	7 ± 0.2*	–
2e	6 ± 0.2*	7 ± 0.01*	5 ± 0.3*	–
5	–	–	6 ± 0.2*	–
6a	16 ± 0.3	18 ± 0.3	12 ± 0.5	–
6c	12 ± 0.7*	11 ± 0.1*	9 ± 0.1*	–
8a	10 ± 0.11*	9 ± 0.3*	8 ± 0.2*	–
8c	9 ± 0.4*	8 ± 0.4*	7 ± 0.2*	–
9a	–	–	–	–
9c	–	–	–	–
10	11 ± 0.18*	10 ± 0.3*	–	–
12	–	–	–	–
13a	–	–	–	–
13b	–	–	–	–
Ciprofloxacin	17 ± 0.8	19 ± 0.5	12 ± 0.3	–
Nystatin	–	–	–	20 ± 0.5

[#] Values were expressed as mean ± standard deviation

* $p < 0.001$ versus ciprofloxacin

Table 2 Antiinflammatory activity evaluation of compounds **9a–b, 10, 12, 13a–c**, and celecoxib at a dose of 0.9 mg/100 g body weight on the inflamed rat paw (mean ± SE; $n = 5$)[#]

Compound	Initial thickness (zero time)	Thickness of rat paw (mm) after			
		1 h	2 h	3 h	4 h
Control	0.22 ± 0.025	0.77 ± 0.02^{a}	0.88 ± 0.03^{a}	0.88 ± 0.03^{a}	0.88 ± 0.03^{a}
Celecoxib	0.22 ± 0.025	0.45 ± 0.06^{d}	0.42 ± 0.04^{d}	0.45 ± 0.04^{c}	$0.50 \pm 0.05^{c,d}$
9a	0.22 ± 0.025	$0.55 \pm 0.02^{b,c,d}$	$0.55 \pm 0.02^{b,c,d}$	0.52 ± 0.02^{b}	$0.47 \pm 0.02^{c,d}$
9b	0.22 ± 0.025	$0.50 \pm 0.04^{c,d}$	$0.50 \pm 0.04^{c,d}$	0.52 ± 0.04^{b}	0.67 ± 0.04^{b}
10	0.22 ± 0.025	$0.57 \pm 0.04^{b,c,d}$	$0.52 \pm 0.02^{c,d}$	0.60 ± 0.04^{b}	$0.52 \pm 0.02^{c,d}$
12	0.22 ± 0.025	$0.67 \pm 0.02^{a,b,c}$	$0.62 \pm 0.02^{b,c}$	0.60 ± 0.04^{b}	$0.62 \pm 0.04^{b,c}$
13a	0.22 ± 0.025	$0.57 \pm 0.08^{b,c,d}$	$0.55 \pm 0.07^{b,c,d}$	0.55 ± 0.06^{b}	$0.52 \pm 0.05^{c,d}$
13b	0.22 ± 0.025	$0.55 \pm 0.08^{b,c,d}$	$0.50 \pm 0.06^{c,d}$	0.47 ± 0.03^{c}	0.45 ± 0.04^{d}
13c	0.22 ± 0.025	$0.70 \pm 0.07^{a,b}$	0.67 ± 0.04^{b}	0.60 ± 0.08^{b}	$0.55 \pm 0.06^{b,c,d}$

[#] Means in the same column with different letters were significantly different at p value of 0.05 or less according to one-way analysis of variance (ANOVA)

Table 3 Ulcerogenic activity of compounds **9a–b, 10, 12, 13a–c**, celecoxib, and indomethacin (mean ± SE; $n = 5$)

Compound	Mean ulcer score	Incidence of gastric ulceration (%)	Ulcer index
Control	0.0	0.0	0.0
Celecoxib	0.0	0.0	0.0
Indomethacin	5 ± 0.035	100	500
9a	0.0	0.0	0.0
9b	0.0	0.0	0.0
10	3 ± 0.07	80	240
12	0.0	0.0	0.0
13a	3 ± 0.08	80	240
13b	0.0	0.0	0.0
13c	0.0	0.0	0.0

Statistical analysis Data were analyzed using the computer program SPSS. The differences in mean values were determined by one-way ANOVA followed by Duncan's multiple rank tests (means with different letters were significantly different).

Ulcerogenic activity The titled compounds were tested for their ulcerogenic activity using celecoxib and indomethacin as references drugs. Male albino rats weighing 150–200 g were fasted for 12 h before drug administration. Water was given ad libitum. The animals were divided into 10 equal groups of 5 each. The first group received 1% gum acacia (suspending vehicle) orally once a day and was left as a control while the second group received celecoxib at a dose of 0.9 mg/100 g/day orally using a metallic stomach tube. The third group received indomethacin at the same dose level. The remaining groups received the test compounds at a dose of 0.9 mg/100 g/day. The drugs were administered orally once a day for three successive days.

The animals were killed by an overdosage of ether 6 h after the last dose. The stomach was removed, opened along the greater curvature, and examined for ulceration. The number and severity of the discrete areas of damage in the glandular mucosa were scored (Table 3). The ulcer score was calculated using the 1–5 scoring system devised by Wilhelmi and Menasse-Gdynia (1972).

Stomach ulceration was expressed in terms of the ulcer index (UI) as follows:

$$\mathrm{UI} = \text{mean ulcer score of animals similarly treated} \times \% \text{ of ulcerated animals in the group.}$$

Results and discussion

Chemistry

In this work, the novel 2(1H)-quinoxalinone derivatives **1–13** were prepared as illustrated in Schemes 1 and 2. The preparation of 3-methyl-4a, 5, 6, 7, 8, 8a-

hexahydro-2(1H)-quinoxolinone(**1**) was through cyclocondensation of equimolar amounts of 1,2-cyclohexanediamine and ethyl pyurvate in hot ethanolic solution containing a catalytic amount of glacial acetic acid. Diazotization of the aromatic amines was realized using sodium nitrite in the presence of hydrochloric acid at 0°C, and the resulting diazonium salts were subsequently coupled at 0°C with an equimolar amount of compound **1** in acetic acid, which was already buffered by sodium acetate solution to provide the novel hydrazones **2a–e**.

A good yield of the new ester **3** was obtained (73%) through the cyclocondensation reaction of 1,2-cyclohexanediamine with diethyl oxaloacetate in ethanol containing drops of acetic acid at 80°C followed by intensive stirring for 12 h at room temperature. The new hydrazide **4** belonging to hexahydro-2(1H)-quinoxalinone was obtained through condensation of the ester **3** with hydrazine hydrate by heating the reactants in ethanol at reflux for 2 h. Similarly, hydrazide **7** was prepared from its corresponding 3-(ethoxycarbonylmethyl)-2(1H)-quinoxalinone (Kurasawa *et al.*, 1995). Hydrazide **4** was allowed to react with phthalic anhydride in glacial acetic acid by heating the reactants at reflux for 2 h to afford the new phthalimido derivative **5** in a 79% yield (Scheme 1).

In the current work, the novel thiosemicarbazide derivatives **6a–c** and **8a–c** were obtained in 60% to 82% yields through the reaction of their respective acid hydrazides **4** and **7** with the appropriate isothiocyante by heating the reactants at reflux in ethanol for 2 h. Furthermore, the thiosemicarbazides **8a–c** were cyclized in hot NaOH solution as a base catalyzed reaction to obtain the novel 5-mercapto-4-substituted-4H-1,2,4-triazole derivatives **9a–c**. Unfortunately, the thiosemicarbazides **6a–c** derived from hexahydro-2(1H)-quinoxalinone failed to undergo cyclization using different reaction conditions.

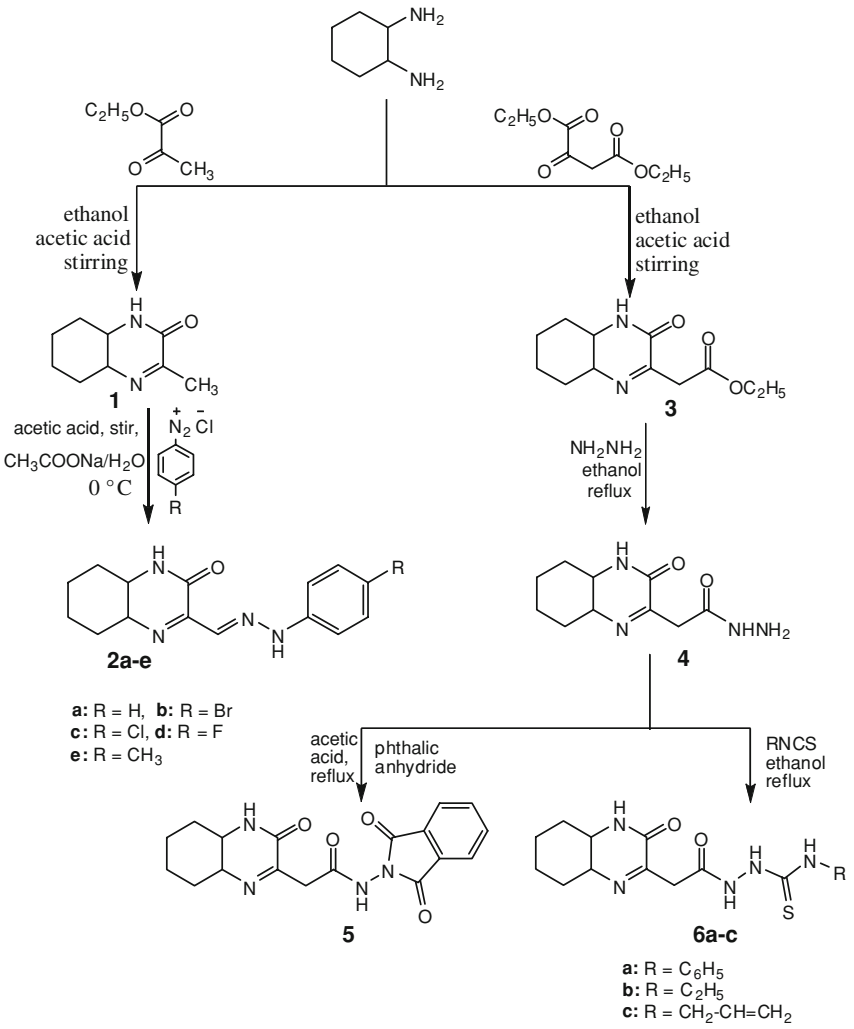
Moreover, the novel oxadiazole **10** was synthezied in a 90% yield through cyclization of the acid hydrazide **7** using potassium hydroxide and carbon disulfide in hot ethanolic solution. The intermediate potassium, 3-(2-[2-oxo-1,2-dihydroquinoxalin-3-yl]acetyl)dithiocarbazate (**11**), was prepared from hydrazide **7** and carbon disulfide in ethanolic potassium hydroxide solution with the aim to be cyclized to the novel triazole **12** under the effect of hydrazine hydrate. Moreover, the intermediate **11** can be formed by heating the former reactants for 1 h in aqueous dioxane instead of ethanol for in situ cyclization to the novel 3,4-dihdrothiazole derivatives **13a–c** at the use of phenacyl bromide derivatives (Scheme 2).

The new compounds were characterized by the use of thin-layer chromatography and melting point techniques. Moreover, the structures of the novel compounds were determined using elemental analyses, IR, ^{1}H NMR, and mass spectroscopy.

Biological activities

Antimicrobial activity

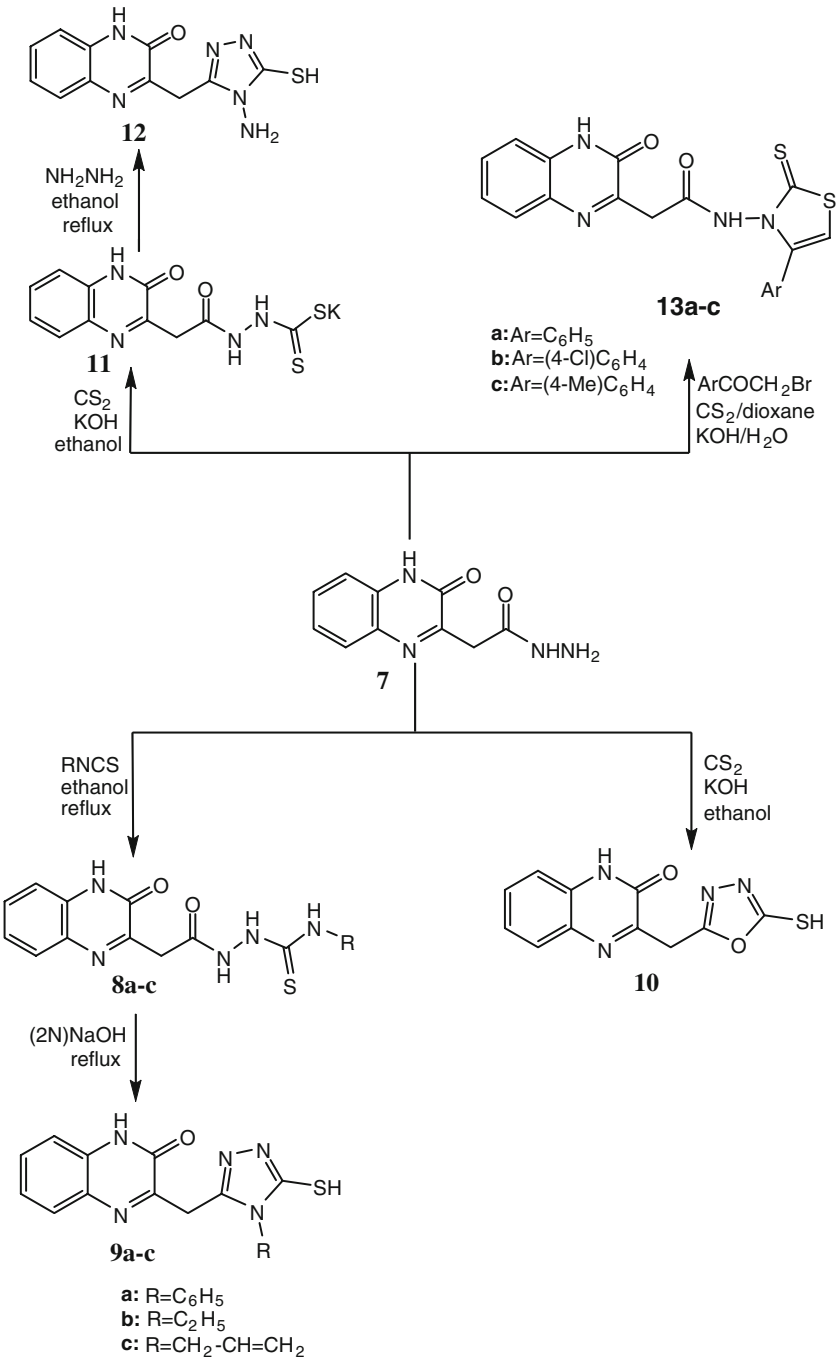
The preliminary antimicrobial activity for most 2(1H)-quinoxalinone derivatives (**2a–e, 5, 6a,c, 8a,c, 9a,c, 10, 12**, and **13a,b**) was carried out using the cup-plate technique (Reeves and White, 1983). Compounds **9a,c, 12**, and **13a,b** were devoid of both antibacterial and antifungal activities (Table 1). Among the hydrazone



Scheme 1 Synthesis of compounds **2a–e, 5**, and **6a–c**

derivatives **2a–e**, it was found that compounds **2c** and **2d** showed the highest activity against *Staphylococcus aureus*, which was comparable to the reference drug ciprofloxacin.

It was noted that the thiosemicarbazides belonging to hexahydro-2(1H)-quinoxalinones **6a,c** exhibited a higher antibacterial activity than their corresponding unsaturated ones **8a,c**. Moreover, it was observed that thiosemicarbazide **6a** bearing a phenyl moiety had nearly the same activity as the reference drug ciprofloxacin against both Gram-positive and -negative bacteria. Unfortunately, cyclization of the unsaturated thiosemicarbazides **8a,c** to their corresponding triazole derivatives **9a,c** led to loss of antibacterial activity.



Scheme 2 Synthesis of compounds **8a–c, 9a–c, 10, 12**, and **13a–c**

The oxadiazole derivative **10** showed moderate activity against Gram-positive bacteria only. None of the tested compounds showed any antifungal activity.

Pharmacology

The novel 2(1H)-quinoxalinones bearing five-membered heterocyclic rings, namely, N-substituted triazoles (**9a,b**), oxadiazole (**10**), aminotriazole (**12**), and 2,3-dihydrothiazoles (**13a–c**), which nearly fulfill the pattern of COX-2 inhibition, were selected for evaluation of their antiinflammatory activities by the rat hind paw edema method (Winter *et al.*, 1962) using the selective COX-2 inhibitor celecoxib as a reference drug. In addition, the ulcerogenic activities (Wilhelmi and Menasse-Gdynia, 1972) for such 2(1H)-quinoxalinones were determined using celecoxib and indomethacin as references drugs.

Antiinflammatory activity Table 2 showed that i.p. injection of the tested compounds at a dose of 0.9 mg/100 g into mature male albino rats with inflammation of the paw induced by carrageenin (Winter *et al.*, 1962) caused a decrease in rat paw thickness starting after 1 h and reaching its maximum effect after 4 h.

It was noted that the 2(1H)-quinoxalinones bearing N-phenyltriazole (**9a**) and 4-chlorophenyl-2,3-dihydrothiazole (**13b**) were the most active ones after 4 h with use of the rat hind paw edema method and that their antiinflammatory activities were comparable with the selective COX-2 inhibitor celecoxib (Fig. 1).

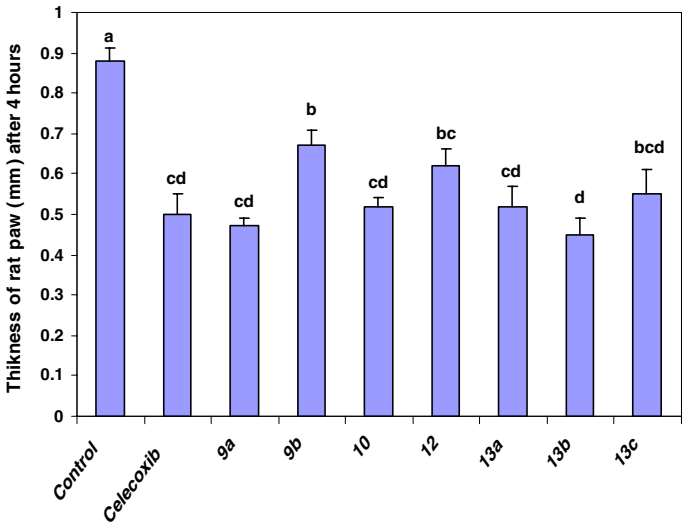


Fig. 1 Antiinflammatory activity of compounds **9a–b, 10, 12, 13a–c** and celecoxib at a dose of 0.9 mg/100 g body weight of rats on the inflamed rat paw (mean ± SE; $n = 5$) after 4 h. Means with different letters were significantly different at a p value of 0.05 or less according to one-way analysis of variance (ANOVA)

Ulcerogenic activity The results presented in Table 3 showed that compounds **10** and **13a** evoked a significant increase in mean ulcer score, incidence of gastric ulceration, and calculated ulcer index, while other tested compounds (**9a, 9b, 12, 13b**, and **13c**) elicited no ulcerogenic activity.

It was observed that 2(1H)-quinoxalinones bearing N-phenyltriazole (**9a**) or 4-chlorophenyl-2,3-dihydrothiazole (**13b**) moieties showed a pronounced antiinflammatory activity without causing ulcerogenic activity like the selective COX-2 inhibitor celecoxib and thus such compounds can be considered as promising antiinflammatory agents that need further future optimization.

Conclusion

New 2(1H)-quinoxalinones and their hexahydro derivatives were prepared for evaluation of their antimicrobial and antiinflammatory activities. It was found that the thiosemicarbazide (**6a**) derived from hexahydro-2(1H)-quinoxalinone exhibited a high in vitro antibacterial activity. Moreover, the N-phenyltriazole (**9a**) and 4-chlorophenyl-2,3-dihydrothiazole (**13b**) derived from 2(1H)-quinoxalinones exhibited high antiinflammatory activity after 4 h upon use of the rat hind paw edema method and also the same ulcerogenic index as the selective COX-2 inhibitor celecoxib.

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