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## Synthesis of quinoxalines and assessment of their inhibitory effects against human non-small-cell lung cancer cells†

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Twenty-six quinoxalin derivatives were synthesized to assess their biological activities against human non-small-cell lung cancer cells (A549 cells). Compound **4b** ( $IC_{50} = 11.98 \pm 2.59 \mu M$ ) and compound **4m** ( $IC_{50} = 9.32 \pm 1.56 \mu M$ ) possess anticancer activity comparable to 5-fluorouracil (clinical anticancer drug) ( $IC_{50} = 4.89 \pm 0.20 \mu M$ ). Western blot tests further confirmed that compound **4m** effectively induced apoptosis of A549 cells through mitochondrial- and caspase-3-dependent pathways. The introduction of bromo groups instead of nitro groups into the quinoxaline skeleton has been shown to provide better inhibition against lung cancer cells in this article. This modification in the molecular structure could enhance the biological activity and effectiveness of quinoxaline derivatives in the design and synthesis of anticancer drugs, making bromo-substituted quinoxalines a promising avenue for further research and development in anticancer therapeutics.

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## 1 Introduction

Quinoxaline, known as benzopyrazine, is naphthalene with some carbon atoms replaced with nitrogen atoms<sup>1</sup> (Fig. 1a). The nitrogen atoms of quinoxalines contribute to the stabilization of radical ion species. Naturally occurring quinoxalines are scarce,<sup>2,3</sup> and their derivatives are mostly derived from synthetic products. Quinoxaline's skeleton is in frame with some natural antibiotics, such as echinomycin,<sup>4,5</sup> quinornycin A,<sup>6</sup> triostins<sup>7</sup> and actinoleutin which show activities against Gram-positive bacteria<sup>8</sup> and tumors.<sup>9</sup> Furthermore, the synthetic quinoxaline derivatives possess antimicrobial,<sup>1,10,11</sup> anti-inflammatory,<sup>12,13</sup> antiviral,<sup>1,14</sup> antioxidant,<sup>13,15</sup> antitumor,<sup>1,9</sup> receptor antagonist,<sup>16</sup> antitubercular,<sup>1,17,18</sup> antifungal,<sup>1,11,15,19-22</sup> antiparasitic<sup>23</sup> and antidiabetic<sup>1,24-28</sup> activities. Besides the pharmacological interest in quinoxalines, they are also employed in material sciences, such as in OLEDs<sup>29</sup> and dye-sensitized solar cells.<sup>30</sup>

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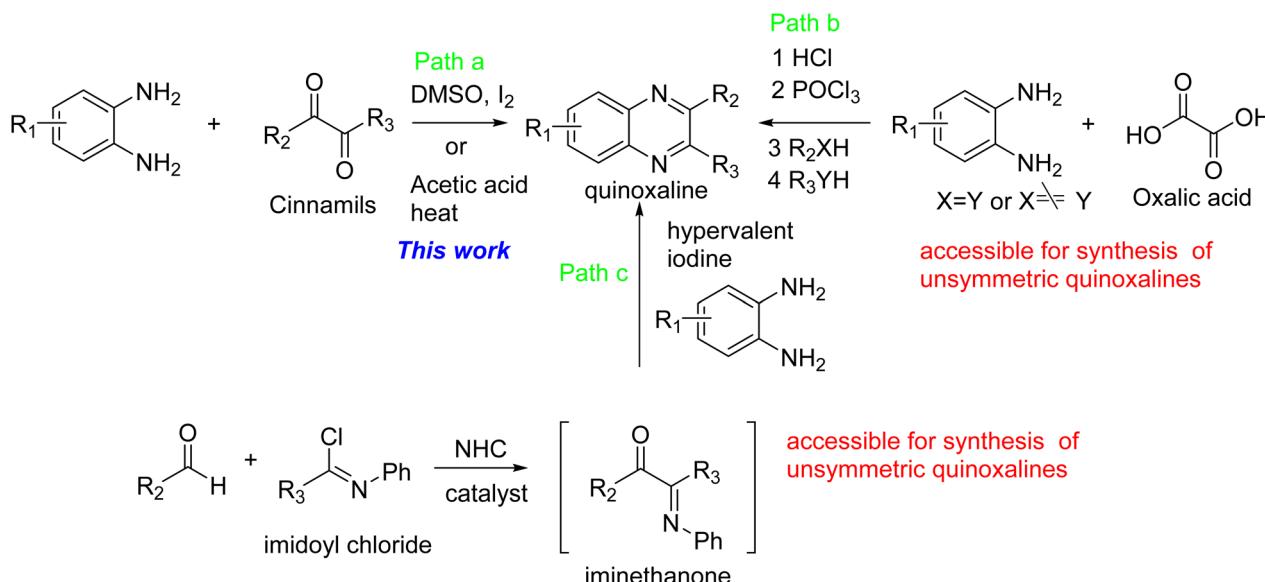
† Electronic supplementary information (ESI) available: Spectroscopic data of  $^1H$  NMR and  $^{13}C$  NMR for new compounds. See DOI: <https://doi.org/10.1039/d4ra04453c>

Quinoxaline urea derivatives were evaluated in the treatment of pancreatic therapy.<sup>31</sup> Therefore, we have reported the synthesis of cinnamils and 2,3-dialkenyl-substituted quinoxalines to evaluate their activities against pancreatic cancer cells.<sup>32</sup> Among them, one of cinnamils exhibited a more potent anticancer effect than that of quinoxalines. However, that series of quinoxalines presented a moderate inhibitory effect. Compared with other quinoxalines, synthesizing 2,3-dialkenyl-substituted quinoxalines is relatively rare.<sup>33,34</sup> They exhibited photophysical properties<sup>34</sup> or as a detector,<sup>35</sup> however, their biological activity is not well studied. A C6-fluoro substituted quinoxaline was reported as a potent inhibitor of JNK stimulatory phosphatase-1 (JSP-1) in an *in vitro* biological assay (Fig. 1c).<sup>36</sup> Due to quinoxalines for their pharmacological interests, it prompted us to introduce an electron-withdrawing group to the C6-position of 2,3-dialkenyl-substituted quinoxalines. Therefore, the bromo or nitro groups were selected to assess their activities against non-small-cell lung cancer cells (Fig. 1b, X = Br, or NO<sub>2</sub>) and pursue hit compounds. According to reports, the NO<sub>2</sub> functional group may be genotoxic, so the genotoxicity of **5a–5m** needs further discussion. However, this article aims to investigate the apoptotic mechanisms of the most effective compound **4m**.



Fig. 1 The skeletons of quinoxaline (a) and C6-substituted alkenyl quinoxalines (b) are reported in this article, and a quinoxaline derivative (c) is reported in the literature with potent biological activity.





Scheme 1 The reported strategies for the synthesis of quinoxalines.

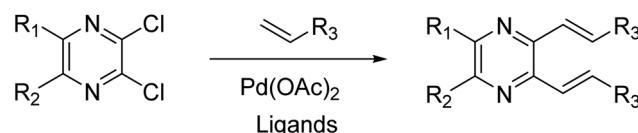


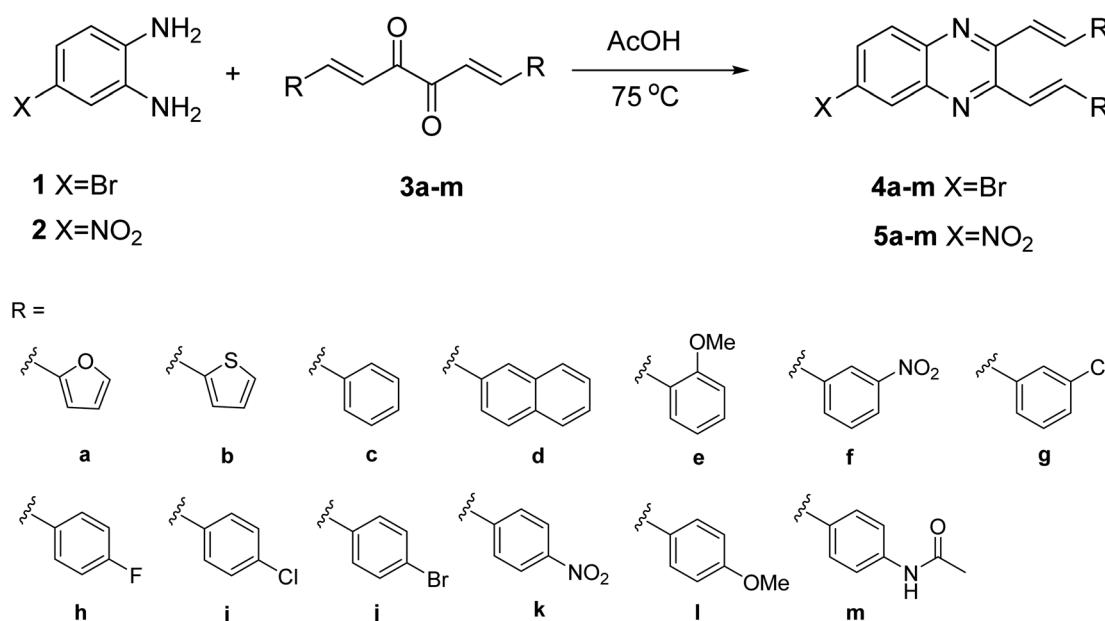
Fig. 2 The synthesis of 2,3-dialkenyl-substituted quinoxalines via the Heck method.

## 2 Results and discussion

### 2.1 Chemistry

There are at least three methods available for the synthesis of quinoxalines. First, the condensation of cinnamils with 1,2-

phenylenediamine derivatives under either the catalytic amount of  $I_2$  in DMSO<sup>37</sup> or reflux acetic acid,<sup>38</sup> which condition is used for the synthesis of target molecules in this article (Path a). Secondly, oxalic acid is condensed with 1,2-phenylenediamine in an acidic condition and then treated with  $POCl_3$  followed by reacting with nucleophiles (Path b).<sup>10</sup> Finally, the key intermediate, iminoethanone, was prepared from aldehyde and imidoyl chloride under the NHC catalyst (path c). The intermediate, iminoethanone, was condensed with 1,2-phenylenediamine catalyzed by hypervalent iodine<sup>39</sup> to obtain quinoxaline. It is noticed that Path b and Path c are more readily available for the synthesis of unsymmetric quinoxalines (Scheme 1).



Scheme 2 Synthesis of quinoxalines 4a-m and 5a-m.



**Table 1** Inhibitory effects of compounds against human non-small-cell lung cancer cells (A549)<sup>a</sup>

Compounds	IC <sub>50</sub> <sup>b</sup> (μM)
<b>4a</b>	54.94 ± 2.13**
<b>4b</b>	11.98 ± 2.59***
<b>4c</b>	>100
<b>4d</b>	>100
<b>4e</b>	35.23 ± 9.29*
<b>4f</b>	>100
<b>4g</b>	42.39 ± 1.40**
<b>4h</b>	>100
<b>4i</b>	>100
<b>4j</b>	>100
<b>4k</b>	>100
<b>4l</b>	38.17 ± 0.48*
<b>4m</b>	9.32 ± 1.56**
<b>5a</b>	>100
<b>5b</b>	>100
<b>5c</b>	>100
<b>5d</b>	>100
<b>5c</b>	>100
<b>5d</b>	>100
<b>5e</b>	>100
<b>5f</b>	>100
<b>5g</b>	75.02 ± 4.50**
<b>5h</b>	>100
<b>5i</b>	>100
<b>5j</b>	>100
<b>5k</b>	>100
<b>5l</b>	>100
<b>5m</b>	>100
5-FU <sup>c</sup>	4.89 ± 0.20**

<sup>a</sup> Results are presented as averages ± SD (n = 3). <sup>b</sup> Concentration necessary for 50% inhibition (IC<sub>50</sub>). <sup>c</sup> 5-Fluorouracil (5-FU) was used as a positive control.

**Table 2** *In vitro* cytotoxic effects of compound **4m** and 5-fluorouracil against Hs68 (human foreskin fibroblast) cells<sup>a</sup>

Compounds	IC <sub>50</sub> <sup>b</sup> (μM)
<b>4m</b>	9.29 ± 1.25
5-FU <sup>c</sup>	3.08 ± 0.40

<sup>a</sup> Results are presented as averages ± SD (n = 3). <sup>b</sup> Concentration necessary for 50% inhibition (IC<sub>50</sub>). <sup>c</sup> 5-Fluorouracil (5-FU) was used as a positive control.

The previous synthesis of 2,3-dialkenyl-substituted quinoxalines employed a Heck method (Fig. 2).<sup>33</sup> We applied the condensation of **1** or **2** with the corresponding **3a–m** in acetic acid under reflux conditions.<sup>32</sup> The resulting solid was filtered by suction and washed with distilled water to obtain **4a–m** and **5a–m** in moderate to high yields without column chromatography (Scheme 2, see experimental section).

## 2.2 Biological evaluation

The cytotoxic effects of all twenty-six synthesized compounds were evaluated by their activity to suppress human non-small cell lung cancer cells (A549 cells). As shown in Table 1,

compound **4m** (IC<sub>50</sub> = 9.32 ± 1.56 μM) exhibited the most potent inhibitory activity against A549 cells. Although compound **4m** showed slightly weaker anticancer activity than 5-fluorouracil (5-FU) against A549 cells. We further compared the cytotoxicity of **4m** and the clinical anticancer drug 5-FU on normal human cells. The cytotoxicity of **4m** (IC<sub>50</sub> = 9.29 ± 1.25 μM) to human foreskin fibroblast (Hs68) cells is approximately 3 times lower than that of 5-FU (IC<sub>50</sub> = 3.08 ± 1.40 μM) (Table 2). Therefore, it shows that **4m** possesses research and development value. In addition, compounds **4a**, **4b**, **4e**, **4g**, **4l**, and **5g** also exhibited cytotoxic activities against A549 cells.

The effect of treating A549 cells with compound **4m** on the expression of apoptosis-related proteins was investigated. Fig. 3a shows that the expression of the pro-apoptotic protein Bax treated with 20 μM of compound **4m** was higher than that treated with 5 or 10 μM of **4m**. In contrast, cells treated with 20 μM of compound **4m** showed lower Bcl-2 (anti-apoptotic protein) expression than those treated with 5 or 10 μM (Fig. 3b). The results show that compound **4m** suppressed the expression of Bcl-2 and increased the expression of Bax.

Besides, caspase 3 activation is a hallmark of apoptosis. We further investigated whether compound **4m** could influence the enzymatic activities of caspase-3. The results show that compound **4m** suppressed pro-caspase 3 and increased the cleaved caspase-3 (Fig. 3c and d). Furthermore, compound **4m** markedly induced apoptosis of A549 cells through caspase-3-dependent pathways.

## 3 Conclusions

The development of quinoxalines as anticancer agents has gained attention in recent years.<sup>40,41</sup> Synthesis of quinoxalines **4a–m** and **5a–m** was facile. Their purifications were simple filtration, then washed with water to obtain the pure target molecules. This series of compounds showed less potency on A549 cells than 5-FU. However, it provides important information that the C6-bromo rather than C6-nitro group is a suitable quinoxaline substitute for designing potential drug candidates. Furthermore, we found compounds **4f**, **4k**, **5f**, and **5k**, which are with two nitro groups, reduced the solubility. Compound **4m** was found to have the highest cytotoxic activity against A549 cells out of all synthesized molecules. The activity of compound **4m** was comparable to that of 5-FU (clinical anticancer drug). Furthermore, compound **4m** markedly induced apoptosis of A549 cells through the mitochondrial- and caspase-3-dependent pathways (Fig. 4). This suggests that the halo substituted at C6-position of 2,3-dialkenyl quinoxalines may be necessary. Thus, compound **4m** is worth further investigation and might be developed as a candidate for treating or preventing human non-small-cell lung cancer.

## 4 Experimental

All chemicals were purchased from either Acros or Alfa from Uniworld in Taiwan and used from received without further purification except otherwise mentioned. The structure's determinations were based on <sup>1</sup>H and <sup>13</sup>C-NMR data recorded



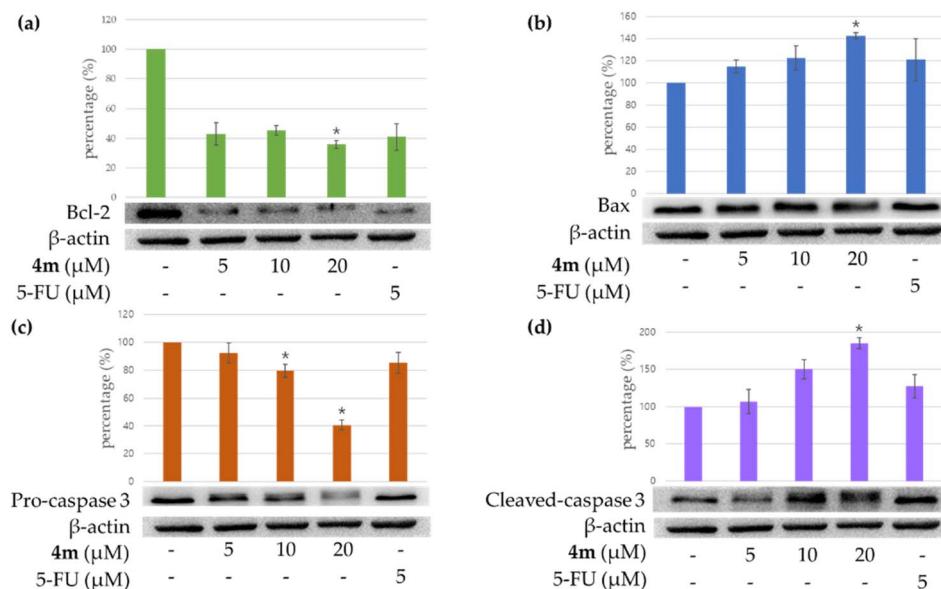


Fig. 3 Western blot analysis for Bcl-2 (a), Bax (b), pro-caspase 3 (c), and cleaved caspase 3 (d) in each group on A549 cells. 4m means compound 4m. 5-Fluorouracil (5-FU) was used as a positive control. Asterisks indicate significant differences ( $*p < 0.05$ ) compared with the control group.

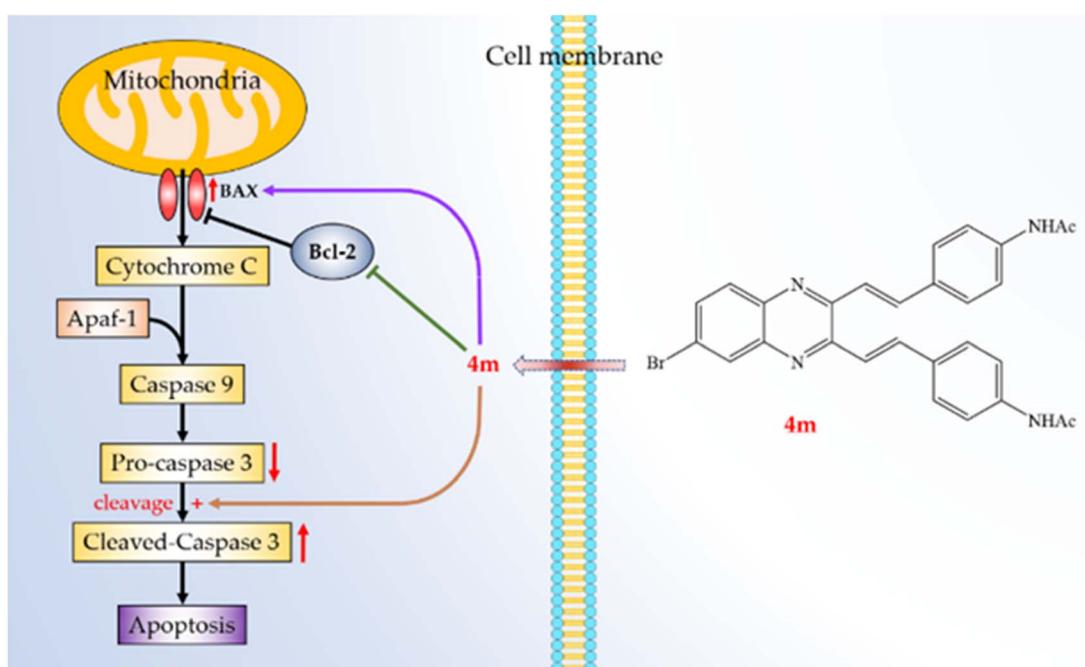


Fig. 4 Schematic diagram for cancer cell apoptosis mechanism of compound 4m in A549 cells.

on a 600 MHz Bruker Ultrashield instrument. The chemical shifts were reported in part per million (ppm) for the residual solvent ( $\text{CDCl}_3$ : 7.26 ppm for  $^1\text{H}$ ; 77.0 ppm for  $^{13}\text{C}$ ). The splitting constant ( $J$ ) was represented in hertz (Hz), and the splitting patterns were designated as s (singlet), br s (broad singlet), d (doublet), dd (doublet of doublets), t (triplet), and m (multiplet). The purity of compounds was performed by high-resolution mass spectrometry (HRMS) using a Bruker Ultra-Flex II instrument (from Bruker Taiwan Co. Ltd) for electrospray ionization (ESI). The reaction processes were monitored by thin

layer chromatography (Analtech Silica gel HLF UV254) and visualized by UV (256 or 365 nm) or stained with  $\text{KMnO}_4$  or *p*-anisaldehyde solution. The melting points were determined using an MP-2D apparatus and were uncorrected.

#### 4.1 General procedure for the synthesis of compounds 4a–m and 5a–m

Compound 1 (4-bromo-1,2-diaminobenzene, 1.0 equivalent) and the corresponding cinnamyl 3a–m (1.2 equivalent) were



dissolved in acetic acid (0.2 M). This resulting mixture was heated at 75 °C for 6 h and then cooled in an ice bath until a precipitate formed. The precipitate was filtrated by suction and washed with H<sub>2</sub>O to obtain the target molecules **4a–m**, respectively. Synthesis of compounds **5a–m** followed the method mentioned above.

## 4.2 Spectra data

### 4.2.1 6-Bromo-2,3-bis[(E)-2-(furan-2-yl)vinyl]quinoxaline (4a).

Use compound **1** (0.093 g, 0.495 mmol) and compound **3a** (0.10 g, 0.413 mmol) to afford compound **4a** (0.144 g, 0.366 mmol). Yield: 89%. Mp 213–214 °C. A brown solid. <sup>1</sup>H NMR (600 MHz, CDCl<sub>3</sub>) δ 8.15 (d, *J* = 1.7 Hz, 1H), 7.83 (d, *J* = 13.4 Hz, 2H), 7.82 (d, *J* = 9.6 Hz, 1H), 7.70 (dd, *J* = 8.9, 1.6 Hz, 1H), 7.55 (d, *J* = 3.2 Hz, 1H), 7.54 (d, *J* = 15.2 Hz, 2H), 7.53 (s, 1H), 6.62 (t, *J* = 4.8 Hz, 2H), 6.51 (d, *J* = 1.4 Hz, 2H). <sup>13</sup>C NMR (150 MHz, CDCl<sub>3</sub>) δ 152.9, 152.8, 149.4, 148.9, 143.8, 143.7, 142.1, 140.3, 132.7, 131.0, 130.0, 125.1, 124.8, 123.1, 119.8, 119.7, 113.1, 112.9, 112.3. HRMS (ESI) calculated for C<sub>20</sub>H<sub>14</sub>BrN<sub>2</sub>O<sub>2</sub> [M + H]<sup>+</sup> 393.0239. Found: 393.0225.

### 4.2.2 6-Bromo-2,3-bis[(E)-2-(thiophen-2-yl)vinyl]quinoxaline (4b).

Use compound **1** (0.082 g, 0.437 mmol) and compound **3b** (0.10 g, 0.364 mmol) to afford compound **4b** (0.118 g, 0.276 mmol). Yield: 76%. Mp 210–211 °C. A yellow solid. <sup>1</sup>H NMR (600 MHz, CDCl<sub>3</sub>) δ 8.17 (d, *J* = 2.1 Hz, 1H), 8.15 (d, *J* = 3.5 Hz, 1H), 8.12 (d, *J* = 3.5 Hz, 1H), 7.84 (d, *J* = 8.8 Hz, 1H), 7.71 (d, *J* = 8.8, 2.1 Hz, 1H), 7.38 (d, *J* = 15.3 Hz, 2H), 7.37 (d, *J* = 15.3 Hz, 2H), 7.32 (t, *J* = 3.4 Hz, 2H), 7.11–7.08 (m, 2H). <sup>13</sup>C NMR (150 MHz, CDCl<sub>3</sub>) δ 149.2, 148.7, 142.1, 141.9, 141.8, 140.3, 132.8, 131.2, 131.0, 130.9, 129.4, 129.3, 128.1, 126.9, 126.8, 123.2, 121.1, 121.0. HRMS (ESI) calculated for C<sub>20</sub>H<sub>14</sub>BrN<sub>2</sub>S<sub>2</sub> [M + H]<sup>+</sup> 424.9782. Found: 424.9798.

### 4.2.3 6-Bromo-2,3-di[(E)-styryl]quinoxaline (4c).

Use compound **1** (0.257 g, 1.373 mmol) and compound **3c** (0.30 g, 1.144 mmol) to afford compound **4c** (0.448 g, 1.084 mmol). Yield: 95%. Mp 190–191 °C. A yellow solid. <sup>1</sup>H NMR (600 MHz, CDCl<sub>3</sub>) δ 8.20 (d, *J* = 2.0 Hz, 1H), 8.00 (d, *J* = 15.5 Hz, 1H), 7.99 (d, *J* = 15.5 Hz, 1H), 7.87 (d, *J* = 8.9 Hz, 1H), 7.72 (d, *J* = 8.9, 2.0 Hz, 1H), 7.67 (d, *J* = 7.7 Hz, 4H), 7.60 (d, *J* = 15.5 Hz, 1H), 7.59 (d, *J* = 15.5 Hz, 1H), 7.44 (d, *J* = 7.4 Hz, 1H), 7.43 (d, *J* = 7.4 Hz, 2H), 7.38 (t, *J* = 7.3 Hz, 2H). <sup>13</sup>C NMR (150 MHz, CDCl<sub>3</sub>) δ 149.7, 149.2, 142.0, 140.3, 138.6, 138.3, 136.3, 136.2, 132.9, 131.1, 130.1, 129.3, 129.2, 128.9, 127.7, 127.6, 123.3, 122.2, 122.1. HRMS (ESI) calculated for C<sub>24</sub>H<sub>18</sub>BrN<sub>2</sub> [M + H]<sup>+</sup> 413.0653. Found: 413.0640.

### 4.2.4 6-Bromo-2,3-bis[(E)-2-(naphthalen-2-yl)vinyl]quinoxaline (4d).

Use Compound **1** (0.031 g, 0.165 mmol) and compound **3d** (0.050 g, 0.138 mmol) to afford compound **4d** (0.065 g, 0.126 mmol). Yield: 91%. Mp 234–235 °C. A yellow solid. <sup>1</sup>H NMR (600 MHz, CDCl<sub>3</sub>) δ 8.25 (d, *J* = 1.5 Hz, 1H), 8.20 (d, *J* = 15.5 Hz, 2H), 8.07 (s, 2H), 7.93–7.85 (m, 9H), 7.78–7.75 (m, 3H), 7.55–7.51 (br m, 4H). <sup>13</sup>C NMR (150 MHz, CDCl<sub>3</sub>) δ 149.8, 149.4, 142.2, 140.4, 138.8, 138.5, 133.9, 133.8, 133.6, 132.9, 131.2, 130.2, 129.1, 129.0, 128.6, 128.5, 128.4, 127.8, 126.8, 126.7, 126.6, 123.7, 123.3, 122.4, 122.3. HRMS (ESI) calculated for C<sub>32</sub>H<sub>22</sub>BrN<sub>2</sub> [M + H]<sup>+</sup> 513.0966. Found: 513.0971.

### 4.2.5 6-Bromo-2,3-bis[(E)-2-methoxystyryl]quinoxaline (4e).

Use compound **1** (0.139 g, 0.744 mmol) and compound **3e** (0.20 g, 0.620 mmol) to afford compound **4e** (0.284 g, 0.601 mmol). Yield: 97%. Mp 182–183 °C. A yellow solid. <sup>1</sup>H NMR (600 MHz, CDCl<sub>3</sub>) δ 8.28 (d, *J* = 15.7 Hz, 1H), 8.27 (d, *J* = 15.7 Hz, 1H), 8.24 (d, *J* = 2.1 Hz, 1H), 7.91 (d, *J* = 8.9 Hz, 1H), 7.75 (d, *J* = 15.7 Hz, 1H), 7.73 (d, *J* = 15.7 Hz, 1H), 7.72 (dd, *J* = 8.7, 2.2 Hz, 1H), 7.70 (d, *J* = 7.6 Hz, 2H), 7.34 (t, *J* = 7.6 Hz, 2H), 7.02 (t, *J* = 7.5 Hz, 2H), 6.97 (d, *J* = 8.3 Hz, 2H). <sup>13</sup>C NMR (150 MHz, CDCl<sub>3</sub>) δ 158.0, 150.5, 150.1, 142.2, 140.3, 133.9, 133.6, 132.5, 131.2, 130.3, 130.2, 130.1, 128.2, 125.6, 125.5, 123.5, 123.3, 122.9, 120.8, 111.1, 55.5. HRMS (ESI) calculated for C<sub>26</sub>H<sub>22</sub>BrN<sub>2</sub>O<sub>2</sub> [M + H]<sup>+</sup> 473.0865. Found: 473.0858.

### 4.2.6 6-Bromo-2,3-bis[(E)-3-nitrostyryl]quinoxaline (4f).

Use compound **1** (0.032 g, 0.170 mmol) and compound **3f** (0.050 g, 0.14 mmol) to afford compound **4f** (0.061 g, 0.121 mmol). Yield: 85%. Mp 290–291 °C. A yellow solid. <sup>1</sup>H and <sup>13</sup>C-NMR spectra were not obtained in perfect forms because of insolubility in CDCl<sub>3</sub>, DMSO-d<sub>6</sub>, CD<sub>3</sub>OD, and acetone-d<sub>6</sub>. HRMS (ESI) calculated for C<sub>24</sub>H<sub>16</sub>BrN<sub>4</sub>O<sub>4</sub> [M + H]<sup>+</sup> 503.0355. Found: 503.0335.

### 4.2.7 6-Bromo-2,3-bis[(E)-3-chlorostyryl]quinoxaline (4g).

Use compound **1** (0.068 g, 0.362 mmol) and compound **3g** (0.10 g, 0.302 mmol) to afford compound **4g** (0.123 g, 0.254 mmol). Yield: 85%. Mp 94–95 °C. A yellow-brown solid. <sup>1</sup>H NMR (600 MHz, CDCl<sub>3</sub>) δ 8.21 (d, *J* = 1.9 Hz, 1H), 7.94 (d, *J* = 15.5 Hz, 2H), 7.88 (d, *J* = 8.9 Hz, 1H), 7.76 (dd, *J* = 8.9, 2.0 Hz, 1H), 7.66 (s, 2H), 7.57 (d, *J* = 3.6 Hz, 1H), 7.55 (d, *J* = 9.2 Hz, 1H), 7.54 (s, 1H), 7.39–7.34 (m, 4H). <sup>13</sup>C NMR (150 MHz, CDCl<sub>3</sub>) δ 149.1, 148.7, 142.2, 140.4, 138.1, 138.0, 137.3, 137.0, 134.9, 133.3, 131.2, 130.2, 130.1, 129.2, 129.1, 127.4, 125.9, 125.8, 123.7, 123.2, 123.1. HRMS (ESI) calculated for C<sub>24</sub>H<sub>16</sub>BrCl<sub>2</sub>N<sub>2</sub> [M + H]<sup>+</sup> 480.9874. Found: 480.9870.

### 4.2.8 6-Bromo-2,3-bis[(E)-4-fluorostyryl]quinoxaline (4h).

Use compound **1** (0.113 g, 0.603 mmol) and compound **3h** (0.150 g, 0.503 mmol) to afford compound **4h** (0.179 g, 0.399 mmol). Yield: 80%. Mp 212–213 °C. A yellow solid. <sup>1</sup>H NMR (600 MHz, CDCl<sub>3</sub>) δ 8.21 (s, 1H), 7.97 (d, *J* = 15.5 Hz, 1H), 7.96 (d, *J* = 15.5 Hz, 1H), 7.88 (d, *J* = 8.9 Hz, 1H), 7.75 (dd, *J* = 8.9, 1.2 Hz, 1H), 7.66 (d, *J* = 7.9 Hz, 2H), 7.65 (d, *J* = 7.9 Hz, 2H), 7.52 (d, *J* = 15.5 Hz, 1H), 7.50 (d, *J* = 15.5 Hz, 1H), 7.13 (t, *J* = 8.3 Hz, 4H). <sup>13</sup>C NMR (150 MHz, CDCl<sub>3</sub>) δ 164.1, 163.3 (<sup>1</sup>J<sub>C–F</sub> = 249.0 Hz), 149.5, 149.0, 142.1, 140.3, 137.4, 137.1, 133.0, 132.6, 132.5, 131.1, 130.1, 129.4 (<sup>3</sup>J<sub>C–F</sub> = 7.5 Hz), 129.3, 123.4, 121.8, 121.7, 116.0 (<sup>2</sup>J<sub>C–F</sub> = 22.5 Hz). HRMS (ESI) calculated for C<sub>24</sub>H<sub>16</sub>BrF<sub>2</sub>N<sub>2</sub> [M + H]<sup>+</sup> 449.0465. Found: 449.0455.

### 4.2.9 6-Bromo-2,3-bis[(E)-4-chlorostyryl]quinoxaline (4i).

Use compound **1** (0.068 g, 0.362 mmol) and compound **3i** (0.10 g, 0.302 mmol) to afford compound **4i** (0.081 g, 0.168 mmol). Yield: 56%. Mp 219–220 °C. A brown solid. <sup>1</sup>H NMR (600 MHz, CDCl<sub>3</sub>) δ 8.22 (s, 1H), 7.96 (d, *J* = 15.5 Hz, 2H), 7.89 (d, *J* = 8.8 Hz, 1H), 7.76 (dd, *J* = 8.8, 1.7 Hz, 1H), 7.61 (d, *J* = 8.3 Hz, 4H), 7.57 (d, *J* = 15.5 Hz, 1H), 7.56 (d, *J* = 15.5 Hz, 1H), 7.41 (d, *J* = 8.1 Hz, 4H). <sup>13</sup>C NMR (150 MHz, CDCl<sub>3</sub>) δ 149.3, 148.9, 142.2, 140.4, 137.4, 137.1, 135.1, 134.8, 134.7, 133.2, 131.1, 130.1,



129.2, 128.8, 128.7, 123.6, 122.5, 122.4. HRMS (ESI) calculated for  $C_{24}H_{16}BrCl_2N_2$  [M + H]<sup>+</sup> 480.9880. Found: 480.9874.

#### 4.2.10 6-Bromo-2,3-bis[(E)-4-bromostyryl]quinoxaline (4j).

Use compound **1** (0.053 g, 0.286 mmol) and compound **3j** (0.10 g, 0.238 mmol) to afford compound **4j** (0.116 g, 0.203 mmol). Yield: 85%. Mp 241–242 °C. A yellow solid. <sup>1</sup>H NMR (600 MHz, CDCl<sub>3</sub>) δ 8.22 (d, *J* = 2.1 Hz, 1H), 7.95 (d, *J* = 15.5 Hz, 1H), 7.94 (d, *J* = 15.5 Hz, 1H), 7.89 (d, *J* = 8.9 Hz, 1H), 7.76 (dd, *J* = 8.9, 2.1 Hz, 1H), 7.59–7.52 (m, 10H). <sup>13</sup>C NMR (150 MHz, CDCl<sub>3</sub>) δ 149.3, 148.9, 142.2, 140.4, 137.5, 137.2, 135.2, 135.1, 133.2, 132.1, 131.9, 131.5, 131.2, 130.4, 130.2, 129.1, 129.0, 123.6, 123.4, 123.3, 122.6, 122.5. HRMS (ESI) calculated for  $C_{24}H_{16}Br_3N_2$  [M + H]<sup>+</sup> 568.8864. Found: 568.8877.

#### 4.2.11 6-Bromo-2,3-bis[(E)-4-nitrostyryl]quinoxaline (4k).

Use compound **1** (0.032 g, 0.170 mmol) and compound **3k** (0.050 g, 0.142 mmol) to afford compound **4k** (0.061 g, 0.121 mmol). Yield: 85%. Mp 307–308 °C. A yellow solid. <sup>1</sup>H NMR (600 MHz, CDCl<sub>3</sub>) δ 8.32–8.28 (m, 5H), 8.11 (d, *J* = 15.2 Hz, 2H), 7.95 (d, *J* = 8.6 Hz, 1H), 7.84 (d, *J* = 8.0 Hz, 5H), 7.75 (d, *J* = 15.2 Hz, 2H). <sup>13</sup>C NMR (150 MHz, CDCl<sub>3</sub>) δ 148.0, 147.9, 147.8, 142.3, 142.2, 140.5, 136.2, 135.9, 133.9, 131.2, 130.2, 128.1, 128.0, 125.7, 125.6, 124.4, 124.2. HRMS (ESI) calculated for  $C_{24}H_{16}BrN_4O_2$  [M + H]<sup>+</sup> 503.0355. Found: 503.0356.

#### 4.2.12 6-Bromo-2,3-bis[(E)-4-methoxystyryl]quinoxaline (4l).

Use compound **1** (0.069 g, 0.372 mmol) and compound **3l** (0.10 g, 0.310 mmol) to afford compound **4l** (0.092 g, 0.195 mmol). Yield: 63%. Mp 190–191 °C. A yellow solid. <sup>1</sup>H NMR (600 MHz, CDCl<sub>3</sub>) δ 8.20 (d, *J* = 2.0 Hz, 1H), 7.97 (d, *J* = 15.5 Hz, 1H), 7.96 (d, *J* = 15.5 Hz, 1H), 7.87 (d, *J* = 8.9 Hz, 1H), 7.71 (dd, *J* = 8.9, 2.0 Hz, 1H), 7.64 (d, *J* = 8.3 Hz, 4H), 7.50 (d, *J* = 15.5 Hz, 1H), 7.49 (d, *J* = 15.5 Hz, 1H), 6.97 (d, *J* = 8.3 Hz, 1H), 3.87 (s, 6H). <sup>13</sup>C NMR (150 MHz, CDCl<sub>3</sub>) δ 160.6, 160.5, 150.0, 149.6, 142.0, 140.2, 138.1, 137.8, 132.5, 131.0, 130.0, 129.2, 129.1, 122.8, 120.0, 119.9, 114.3, 55.4. HRMS (ESI) calculated for  $C_{26}H_{22}BrN_2O_2$  [M + H]<sup>+</sup> 473.0865. Found: 473.0864.

#### 4.2.13 *N,N'*-{[(1*E*,1*E*)-(6-bromoquinoxaline-2,3-diyl)bis(ethene-2,1-diyl)]bis(4,1-phenylene)} diacetamide (4m).

Use compound **1** (0.029 g, 0.159 mmol) and compound **3m** (0.050 g, 0.133 mmol) to afford compound **4m** (0.057 g, 0.108 mmol). Yield: 81%. Mp 239 °C (decomposed). A yellow solid. <sup>1</sup>H NMR (600 MHz, CDCl<sub>3</sub>) δ 10.12 (s, 1H), 10.1 (s, 1H), 8.18 (d, *J* = 1.6 Hz, 1H), 7.95–7.82 (m, 10H), 7.68 (d, *J* = 8.0 Hz, 2H), 7.67 (d, *J* = 8.0 Hz, 2H), 2.07 (s, 6H). <sup>13</sup>C NMR (150 MHz, CDCl<sub>3</sub>) δ 168.6, 149.7, 149.3, 141.5, 140.4, 140.3, 139.7, 137.6, 132.6, 130.8, 130.7, 130.4, 130.3, 128.9, 128.7, 122.3, 120.3, 120.3, 119.0. HRMS (ESI) calculated for  $C_{28}H_{24}BrN_4O_2$  [M + H]<sup>+</sup> 527.1083. Found: 527.1087.

#### 4.2.14 2,3-Bis[(E)-2-(furan-2-yl)vinyl]-6-nitroquinoxaline (5a).

Use compound **2** (0.076 g, 0.495 mmol) and compound **3a** (0.10 g, 0.413 mmol) to afford compound **5a** (0.124 g, 0.345 mmol). Yield: 84%. Mp 218–219 °C. <sup>1</sup>H NMR (600 MHz, CDCl<sub>3</sub>) δ 8.83 (d, *J* = 2.2 Hz, 1H), 8.37 (dd, *J* = 9.1, 2.2 Hz, 1H), 8.03 (d, *J* = 9.1 Hz, 1H), 7.92 (d, *J* = 15.2 Hz, 1H), 7.89 (d, *J* = 15.2 Hz, 1H), 7.56 (s, 1H), 7.55 (s, 1H), 7.52 (d, *J* = 15.2 Hz, 1H), 7.51 (d, *J* = 15.2 Hz, 1H), 6.68 (d, *J* = 3.1 Hz, 1H), 6.67 (d, *J* = 3.1 Hz, 1H), 6.53 (d, *J* = 1.6 Hz, 2H). <sup>13</sup>C NMR (150 MHz, CDCl<sub>3</sub>) δ 152.6, 151.4, 150.8, 147.3, 144.3, 144.1, 144.0, 140.2, 130.0, 126.6,

126.0, 125.1, 122.5, 119.0, 118.8, 114.2, 113.9, 112.5, 112.4. HRMS (ESI) calculated for  $C_{20}H_{14}N_3O_4$  [M + H]<sup>+</sup> 360.0984. Found: 360.0995.

#### 4.2.15 6-Nitro-2,3-bis[(E)-2-(thiophen-2-yl)vinyl]quinoxaline (5b).

Use compound **2** (0.067 g, 0.437 mmol) and compound **3b** (0.10 g, 0.364 mmol) to afford compound **5b** (0.109 g, 0.279 mmol). Yield: 91%. Mp 223–224 °C. <sup>1</sup>H NMR (600 MHz, CDCl<sub>3</sub>) δ 8.86 (d, *J* = 2.4 Hz, 1H), 8.26 (d, *J* = 15.2 Hz, 1H), 8.23 (d, *J* = 15.2 Hz, 1H), 8.06 (d, *J* = 9.1 Hz, 1H), 7.41 (d, *J* = 5.1 Hz, 1H), 7.40 (d, *J* = 5.1 Hz, 1H), 7.38–7.34 (m, 4H), 7.13–7.10 (m, 2H). <sup>13</sup>C NMR (150 MHz, CDCl<sub>3</sub>) δ 151.1, 150.6, 147.3, 144.0, 141.6, 140.2, 133.0, 132.4, 130.3, 130.1, 130.0, 128.3, 128.2, 127.8, 127.5, 125.1, 122.6, 120.3, 120.1. HRMS (ESI) calculated for  $C_{20}H_{14}N_3O_2S_2$  [M + H]<sup>+</sup> 392.0527. Found: 392.0540.

#### 4.2.16 6-Nitro-2,3-di[(E)-styryl]quinoxaline (5c).

Use compound **2** (0.070 g, 0.457 mmol) and compound **3c** (0.10 g, 0.381 mmol) to afford compound **5c** (0.109 g, 0.286 mmol). Yield: 75%. Mp 195–196 °C. A brown solid. <sup>1</sup>H NMR (600 MHz, CDCl<sub>3</sub>) δ 8.91 (d, *J* = 2.5 Hz, 1H), 8.41 (dd, *J* = 9.1, 2.5 Hz, 1H), 8.13 (d, *J* = 15.5 Hz, 1H), 8.11 (d, *J* = 9.1 Hz, 1H), 8.09 (d, *J* = 15.5 Hz, 1H), 7.70 (d, *J* = 7.4 Hz, 1H), 7.62 (d, *J* = 15.5 Hz, 1H), 7.61 (d, *J* = 15.5 Hz, 1H), 7.46 (t, *J* = 7.4 Hz, 4H), 7.42–7.39 (m, 2H). <sup>13</sup>C NMR (150 MHz, CDCl<sub>3</sub>) δ 151.6, 151.1, 147.4, 144.0, 140.5, 140.3, 140.0, 136.0, 135.9, 130.2, 129.9, 129.7, 129.0, 127.9, 127.8, 125.2, 122.7, 121.4, 121.1. HRMS (ESI) calculated for  $C_{24}H_{18}N_3O_2$  [M + H]<sup>+</sup> 380.1399. Found: 380.1405.

#### 4.2.17 2,3-Bis[(E)-2-(naphthalen-2-yl)vinyl]-6-nitroquinoxaline (5d).

Use compound **2** (0.051 g, 0.331 mmol) and compound **3d** (0.10 g, 0.276 mmol) to afford compound **5d** (0.094 g, 0.196 mmol). Yield: 70%. Mp 228–229 °C. An orange solid. <sup>1</sup>H NMR (600 MHz, CDCl<sub>3</sub>) δ 8.93 (d, *J* = 2.3 Hz, 1H), 8.42 (dd, *J* = 9.1, 2.8 Hz, 1H), 8.31 (d, *J* = 15.4 Hz, 1H), 8.28 (d, *J* = 15.4 Hz, 1H), 8.14 (d, *J* = 9.1 Hz, 1H), 8.08 (s, 2H), 7.92–7.86 (m, 9H), 7.78 (d, *J* = 15.4 Hz, 1H), 7.77 (d, *J* = 15.4 Hz, 1H), 7.55–7.53 (m, 5H). <sup>13</sup>C NMR (150 MHz, CDCl<sub>3</sub>) δ 151.7, 151.2, 147.4, 144.1, 140.7, 140.3, 140.0, 134.1, 134.0, 133.5, 133.4, 130.2, 130.0, 129.5, 128.8, 128.7, 128.5, 127.8, 127.1, 127.0, 126.8, 126.7, 125.2, 123.6, 122.7, 121.6, 121.4. HRMS (ESI) calculated for  $C_{32}H_{22}N_3O_2$  [M + H]<sup>+</sup> 480.1712. Found: 480.1719.

#### 4.2.18 2,3-Bis[(E)-2-methoxystyryl]-6-nitroquinoxaline (5e).

Use compound **2** (0.057 g, 0.372 mmol) and compound **3e** (0.10 g, 0.310 mmol) to afford compound **5e** (0.122 g, 0.278 mmol). Yield: 90%. Mp 219–220 °C. An orange solid. <sup>1</sup>H NMR (600 MHz, CDCl<sub>3</sub>) δ 8.95 (s, 1H), 8.40 (dd, *J* = 9.4, 2.5 Hz, 1H), 8.39 (d, *J* = 15.7 Hz, 1H), 8.38 (d, *J* = 15.7 Hz, 1H), 8.14 (d, *J* = 9.1 Hz, 1H), 7.78 (d, *J* = 15.7 Hz, 1H), 7.77 (d, *J* = 15.7 Hz, 1H), 7.71 (d, *J* = 7.5 Hz, 2H), 7.40–7.35 (m, 2H), 7.04 (t, *J* = 7.4 Hz, 2H), 6.99 (d, *J* = 8.3 Hz, 2H), 3.97 (s, 3H), 3.96 (s, 3H). <sup>13</sup>C NMR (150 MHz, CDCl<sub>3</sub>) δ 158.3, 158.2, 152.5, 152.0, 147.2, 135.8, 135.1, 130.9, 130.7, 130.2, 128.6, 128.4, 125.3, 125.2, 125.1, 122.7, 122.5, 122.4, 120.9, 120.8, 119.2, 55.6. HRMS (ESI) calculated for  $C_{26}H_{22}N_3O_4$  [M + H]<sup>+</sup> 440.1610. Found: 440.1594.

#### 4.2.19 6-Nitro-2,3-bis[(E)-3-nitrostyryl]quinoxaline (5f).

Use compound **2** (0.052 g, 0.341 mmol) and compound **3f** (0.10 g, 0.284 mmol) to afford compound **5f** (0.069 g, 0.146 mmol). Yield: 86%. Mp 197–198 °C. A yellow solid. <sup>1</sup>H NMR (600 MHz, CDCl<sub>3</sub>) δ 8.99 (d, *J* = 2.4 Hz, 1H), 8.57 (d, *J* = 2.1 Hz, 2H), 8.51



(dd,  $J = 9.3, 2.7$  Hz, 1H), 8.28–8.24 (m, 3H), 8.21 (dd,  $J = 9.3, 1.8$  Hz, 2H), 8.06–8.01 (m, 2H), 7.76 (d,  $J = 15.6, 3.0$  Hz, 2H), 7.67 (t,  $J = 7.8$  Hz, 2H).  $^{13}\text{C}$  NMR (150 MHz,  $\text{CDCl}_3$ )  $\delta$  150.6, 150.1, 148.9, 148.0, 144.1, 140.6, 138.2, 137.6, 137.5, 133.7, 133.5, 130.6, 130.1, 125.4, 124.2, 124.1, 124.0, 123.8, 123.5, 122.4, 122.2. HRMS (ESI) calculated for  $\text{C}_{24}\text{H}_{16}\text{N}_5\text{O}_6$  [M + H]<sup>+</sup> 470.1101. Found: 470.1094.

#### 4.2.20 2,3-Bis[(E)-3-chlorostyryl]-6-nitroquinoxaline (5g).

Use compound 2 (0.044 g, 0.289 mmol) and compound 3g (0.080 g, 0.242 mmol) to afford compound 5g (0.092 g, 0.205 mmol). Yield: 85%. Mp 200–201 °C. A brown solid.  $^1\text{H}$  NMR (600 MHz,  $\text{CDCl}_3$ )  $\delta$  8.93 (s, 1H), 8.45 (d,  $J = 8.9$  Hz, 1H), 8.15 (d,  $J = 8.9$  Hz, 1H), 8.08 (d,  $J = 15.6$  Hz, 1H), 8.05 (d,  $J = 15.6$  Hz, 1H), 7.69 (s, 2H), 7.62–7.57 (br m, 4H), 7.45–7.38 (br m, 4H).  $^{13}\text{C}$  NMR (150 MHz,  $\text{CDCl}_3$ )  $\delta$  151.1, 150.6, 147.7, 144.1, 140.4, 139.2, 138.6, 137.7, 135.0, 130.4, 129.7, 129.6, 127.7, 127.5, 126.2, 125.3, 123.0, 122.6, 122.4. HRMS (ESI) calculated for  $\text{C}_{24}\text{H}_{16}\text{Cl}_2\text{N}_3\text{O}_2$  [M + H]<sup>+</sup> 448.0620. Found: 448.0521.

#### 4.2.21 2,3-Bis[(E)-4-fluorostyryl]-6-nitroquinoxaline (5h).

Use compound 2 (0.062 g, 0.402 mmol) and compound 3h (0.10 g, 0.335 mmol) to afford compound 5h (0.080 g, 0.195 mmol). Yield: 83%. Mp 258–259 °C. An orange-yellow solid.  $^1\text{H}$  NMR (600 MHz,  $\text{CDCl}_3$ )  $\delta$  8.95 (s, 1H), 8.45 (d,  $J = 9.5$  Hz, 1H), 8.15 (d,  $J = 9.5$  Hz, 1H), 8.13 (d,  $J = 15.5$  Hz, 1H), 8.09 (d,  $J = 15.5$  Hz, 1H), 7.70 (br s, 4H), 7.56 (d,  $J = 15.5$  Hz, 1H), 7.55 (d,  $J = 15.5$  Hz, 1H), 7.16 (d,  $J = 8.3$  Hz, 2H), 7.15 (d,  $J = 8.3$  Hz, 2H).  $^{13}\text{C}$  NMR (150 MHz,  $\text{CDCl}_3$ )  $\delta$  167.0, 163.7 ( $^1\text{J}_{\text{C}-\text{F}} = 249.0$  Hz), 163.6 ( $^1\text{J}_{\text{C}-\text{F}} = 249.0$  Hz), 151.5, 151.0, 147.5, 144.0, 140.3, 139.4, 138.7, 132.2, 130.2, 130.0, 129.7 ( $^2\text{J}_{\text{C}-\text{F}} = 24.0$  Hz), 129.6 ( $^3\text{J}_{\text{C}-\text{F}} = 7.5$  Hz), 125.2, 122.8, 121.1 ( $^2\text{J}_{\text{C}-\text{F}} = 28.5$  Hz), 116.2 ( $^4\text{J}_{\text{C}-\text{F}} = 4.5$  Hz), 116.0 ( $^4\text{J}_{\text{C}-\text{F}} = 4.5$  Hz). HRMS (ESI) calculated for  $\text{C}_{24}\text{H}_{16}\text{F}_2\text{N}_3\text{O}_2$  [M + H]<sup>+</sup> 416.1222. Found: 416.1215.

#### 4.2.22 2,3-Bis[(E)-4-chlorostyryl]-6-nitroquinoxaline (5i).

Use compound 2 (0.031 g, 0.199 mmol) and compound 3h (0.055 g, 0.166 mmol) to afford compound 5h (0.062 g, 0.138 mmol). Yield: 89%. Mp 221–222 °C. A yellow-brown solid.  $^1\text{H}$  NMR (600 MHz,  $\text{CDCl}_3$ )  $\delta$  8.93 (s, 1H), 8.44 (d,  $J = 7.4$  Hz, 1H), 8.14 (d,  $J = 8.8$  Hz, 1H), 8.11 (d,  $J = 15.8$  Hz, 1H), 8.06 (d,  $J = 15.8$  Hz, 1H), 7.64 (d,  $J = 6.8$  Hz, 1H), 7.59 (d,  $J = 15.8$  Hz, 1H), 7.58 (d,  $J = 15.8$  Hz, 1H), 7.43 (d,  $J = 7.4$  Hz, 4H).  $^{13}\text{C}$  NMR (150 MHz,  $\text{CDCl}_3$ )  $\delta$  151.3, 150.8, 147.6, 144.1, 140.4, 139.3, 138.6, 135.7, 135.6, 134.4, 130.3, 129.3, 129.1, 129.0, 125.3, 122.9, 121.9, 121.7. HRMS (ESI) calculated for  $\text{C}_{24}\text{H}_{16}\text{Cl}_2\text{N}_3\text{O}_2$  [M + H]<sup>+</sup> 448.0620. Found: 448.0617.

#### 4.2.23 2,3-Bis[(E)-4-bromostyryl]-6-nitroquinoxaline (5j).

Use compound 2 (0.022 g, 0.143 mmol) and compound 3j (0.050 g, 0.119 mmol) to afford compound 5h (0.059 g, 0.111 mmol). Yield: 93%. Mp 247–248 °C. A yellow-brown solid.  $^1\text{H}$  NMR (600 MHz,  $\text{CDCl}_3$ )  $\delta$  8.93 (d,  $J = 2.3$  Hz, 1H), 8.44 (dt,  $J = 9.2, 1.2$  Hz), 8.14 (d,  $J = 9.2$  Hz, 1H), 8.08 (d,  $J = 15.4$  Hz, 1H), 8.04 (d,  $J = 15.4$  Hz, 1H), 7.62–7.55 (m, 11H).  $^{13}\text{C}$  NMR (150 MHz,  $\text{CDCl}_3$ )  $\delta$  150.9, 150.4, 147.2, 143.7, 140.0, 138.9, 138.3, 134.5, 134.4, 131.9, 131.8, 129.9, 128.9, 128.8, 124.9, 123.7, 123.5, 122.6, 121.5, 121.3. HRMS (ESI) calculated for  $\text{C}_{24}\text{H}_{16}\text{Br}_2\text{N}_3\text{O}_2$  [M + H]<sup>+</sup> 535.9609. Found: 535.9607.

#### 4.2.24 6-Nitro-2,3-bis[(E)-4-nitrostyryl]quinoxaline (5k).

Use compound 2 (0.052 g, 0.341 mmol) and compound 3k

(0.10 g, 0.284 mmol) to afford compound 5k (0.108 g, 0.231 mmol). Yield: 81%. Mp 297–298 °C. A yellow solid.  $^1\text{H}$  NMR (600 MHz,  $\text{CDCl}_3$ )  $\delta$  9.00 (s, 1H), 8.53 (d,  $J = 9.1$  Hz, 1H), 8.34 (d,  $J = 8.5$  Hz, 4H), 8.25–8.20 (m, 3H), 7.87 (d,  $J = 8.2$  Hz, 4H), 7.78 (d,  $J = 16.2$  Hz, 1H), 7.76 (d,  $J = 16.2$  Hz, 1H). No satisfaction of the  $^{13}\text{C}$  NMR spectrum was obtained due to solubility. HRMS (FAB) calculated for  $\text{C}_{24}\text{H}_{16}\text{N}_5\text{O}_6$  [M + H]<sup>+</sup> 470.1101. Found: 470.1101.

#### 4.2.25 2,3-Bis[(E)-4-methoxystyryl]-6-nitroquinoxaline (5l).

Use compound 2 (0.057 g, 0.372 mmol) and compound 3l (0.10 g, 0.335 mmol) to afford compound 5l (0.069 g, 0.184 mmol). Yield: 89%. Mp 144–145 °C. A dark-orange solid.  $^1\text{H}$  NMR (600 MHz,  $\text{CDCl}_3$ )  $\delta$  8.85 (d,  $J = 2.4$  Hz, 1H), 8.36 (dd,  $J = 9.1, 2.4$  Hz, 1H), 8.05 (d,  $J = 15.2$  Hz, 1H), 8.04 (d,  $J = 9.1$  Hz, 1H), 8.02 (d,  $J = 15.2$  Hz, 1H), 7.63 (d,  $J = 8.5$  Hz, 4H), 7.45 (d,  $J = 15.4$  Hz, 1H), 7.44 (d,  $J = 15.4$  Hz, 1H), 6.97 (d,  $J = 8.5$  Hz, 4H).  $^{13}\text{C}$  NMR (150 MHz,  $\text{CDCl}_3$ )  $\delta$  161.0, 160.9, 151.9, 151.4, 147.1, 144.0, 140.1, 140.0, 139.3, 129.9, 129.5, 129.4, 128.9, 128.8, 125.0, 122.3, 119.1, 118.9, 114.4, 114.3, 55.4. HRMS (ESI) calculated for  $\text{C}_{26}\text{H}_{22}\text{N}_3\text{O}_4$  [M + H]<sup>+</sup> 440.1610. Found: 440.1613.

#### 4.2.26 *N,N'*-[(E,E)-(6-nitroquinoxaline-2,3-diyl)bis(ethene-2,1-diyl)bis(4,1-phenylene)]diacetamide (5m).

Use compound 2 (0.024 g, 0.159 mmol) and compound 3m (0.050 g, 0.133 mmol) to afford compound 5m (0.060 g, 0.121 mmol). Yield: 91%. Mp 325–326 °C. An orange solid.  $^1\text{H}$  NMR (600 MHz,  $\text{CDCl}_3$ )  $\delta$  10.15 (s, 1H), 10.14 (s, 1H), 8.66 (d,  $J = 2.1$  Hz, 1H), 8.34 (d,  $J = 9.1$  Hz, 1H), 8.09 (d,  $J = 9.1$  Hz, 1H), 7.99 (d,  $J = 15.6$  Hz, 1H), 7.95 (d,  $J = 15.6$  Hz, 1H), 7.85–7.81 (m, 6H), 7.67 (d,  $J = 8.0$  Hz, 4H).  $^{13}\text{C}$  NMR (150 MHz,  $\text{CDCl}_3$ )  $\delta$  168.7, 151.7, 151.1, 146.8, 143.6, 140.9, 140.7, 139.6, 139.5, 138.9, 130.6, 130.5, 130.1, 129.3, 129.2, 124.4, 122.6, 119.9, 119.7, 119.0, 24.2. HRMS (FAB) calculated for  $\text{C}_{28}\text{H}_{24}\text{N}_5\text{O}_4$  [M + H]<sup>+</sup> 494.1828. Found: 494.1824.

### 4.3 Cell culture

Prof. Y. Su of National Yang-Ming Chiao Tung University, Taipei, Taiwan kindly provided human non-small lung cancer cells (A549 cells). The cells were stored in liquid nitrogen at –196 °C. After the cells were thawed, the cells were incubated at 37 °C in 5%  $\text{CO}_2$  and cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS), 100 U per mL penicillin, 100  $\mu\text{g}$  per mL streptomycin, 2  $\mu\text{M}$  L-glutamine, and 1 mM sodium pyruvate.<sup>42,43</sup>

### 4.4 *In vitro* cytotoxicity assay

The cell viability was evaluated with MTT assay, as previously reported to further assess the cytotoxicity.<sup>32,43</sup> In brief, the cells were plated in 96-well culture plates at a concentration of  $3 \times 10^3$  cells in 200  $\mu\text{L}$  per well. After 24 hours, cells were treated with different concentrations (6.25, 12.5, 25, 50, and 100  $\mu\text{M}$ ) of all twenty-six synthesized compounds, and 5-fluorouracil (5-FU) was used as a positive control. After 72 hours, the attached cells were added with MTT reagent at 0.5 mg  $\text{mL}^{-1}$  in 100  $\mu\text{L}$  per well and incubated at 37 °C for 3 h. Then, the MTT reagent was removed, 100  $\mu\text{L}$  of DMSO was added per well to dissolve the formazan metabolite, and the amount of formazan was measured by the absorbance at 570 nm, using an ELISA plate



reader, TECAN Spark (Tecan Group Ltd) ( $\mu$  Quant). The optical density recorded previously in the control group (untreated cells) was considered to be 100% cell viability.

#### 4.5 Western blot

Western blot analysis was performed according to the method previously described.<sup>32,43</sup> Briefly, the cells were seeded to 6-well culture plates. After reaching 85–90% confluence, cells were treated with compound **4m** (5, 10, and 20  $\mu$ M) and 5-FU (5  $\mu$ M). The cells were incubated for 48 h after treatment. Afterward, the cells were collected and lysed using radioimmunoprecipitation assay (RIPA) buffer. Lysates of total protein were separated by 12.5% sodium dodecyl sulfate-polyacrylamide gels and transferred to polyvinylidene difluoride (PVDF) membranes. Then, the membranes were blocked with 2% bovine serum albumin (BSA) solution and incubated with anti-Bax, anti-Bcl-2 (Cell Signaling Inc., Danvers, MA, USA), anti-caspase-3, and anti- $\beta$ -actin (GeneTex Inc., Irvine, CA, USA) primary antibodies at 4 °C overnight. Each membrane was washed with tris-buffered saline containing 0.1% Tween 20 (TBST) three times and incubated with horseradish peroxidase (HRP)-conjugated secondary antibodies at room temperature for 2 h. Finally, the membranes were developed with an enhanced chemiluminescence (ECL) detection kit and visualized by ImageQuant LAS 4000 Mini biomolecular imager (GE Healthcare, MA, USA). ImageJ software quantified the band densities (BioTechniques, NY, USA).

## Data availability

The data supporting this article have been included as part of the ESI.† No code was produced as part of this review.

## Author contributions

Jia-Hua Liang performed the bioassay and analyzed the data and manuscript writing. Shu-Tse Cho conducted the isolation and structure elucidation of the constituents. Jih-Jung Chen and Tzeng-Lien Shih planned, designed, and organized this study's research and the manuscript's preparation. All authors read and approved the final version of the manuscript.

## Conflicts of interest

The authors declare no conflicts of financial interest.

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