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## Synthesis and in vitro anticancer evaluation of 1,4-phenylene-bis-pyrimidine-2-amine derivatives

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**Abstract:** A series of 1,4-phenylene-bis-chalcones **3a–3h** were synthesized by the reaction of terephthalaldehyde with substituted arylketones in this study. The novel 1,4-phenylene-bis-pyrimidine-2-amine derivatives **5a–5h** were obtained by the addition of guanidine hydrochloride to 1,4-phenylene-bis-chalcone **3a–3h** in ethanolic KOH under reflux conditions. The structure of the compounds was explained by means of IR, <sup>1</sup>H NMR, <sup>13</sup>C NMR, and elemental analyses. The anticancer activities of **3a–3h** and **5a–5h** were investigated against rat brain tumor cells and human uterus carcinoma in vitro. Activity tests were performed as dose-dependent assays at eight concentrations. The positive control was 5-fluorouracil (5-FU). Compounds **3c** and **3d** were examined and they showed high activities as compared to 5-FU against C6 (rat brain tumor) and HeLa (human uterus carcinoma) cells. The anticancer activity of **5h** was better than that of 5-FU at high concentrations cell-selectively against C6 cells.

**Key words:** 1,4-Phenylene-bis-chalcone, 1,4-phenylene-bis-pyrimidine, HeLa, C6, anticancer activity, 5-fluorouracil

### 1. Introduction

Cancer is a very dangerous disease and it is the second most common cause of death after heart disease in the world.<sup>1</sup> Numerous anticancer agents that can be used for cancer treatment have been developed, but most of them have high toxicity rates.<sup>2</sup> Therefore, the need to discover some new anticancer agents that are very efficacious in the treatment of cancer, but at the same time have very minimal toxicity rates, is one of the main objectives of organic and medicinal chemistry.<sup>3</sup> Pyrimidines attract great attention on account of their wide range of biological and pharmaceutical properties, such as anticancer,<sup>4–7</sup> antibacterial,<sup>8,9</sup> antiinflammatory,<sup>10–13</sup> antiviral,<sup>14</sup> antituberculosis,<sup>15,16</sup> antihypertensive,<sup>17,18</sup> and anticonvulsant<sup>19</sup> properties. For this reason, the design and synthesis of pyrimidine derivatives as potential cancer agents have been extensively studied<sup>20</sup> and hundreds of pyrimidine derivatives have been synthesized and evaluated for their anticancer activity.<sup>21–23</sup> Moreover, various drugs containing a pyrimidine nucleus like 5-fluorouracil (5-FU), tegafur, and thioguanine were prepared and used as anticancer agents.<sup>24,25</sup> Chalcones are known to show different biological activities, such as antioxidant,<sup>26</sup> antiinflammatory,<sup>27</sup> antimalarial,<sup>28</sup> antileishmanial,<sup>29</sup> anticancer,<sup>30</sup> and antitumor<sup>31</sup> activities. Besides their biological activities, chalcones are very useful in starting materials for the preparation of bioactive heterocycles such as pyridine, pyrimidine, pyrazoline, and isoxazoline derivatives.<sup>32–35</sup>

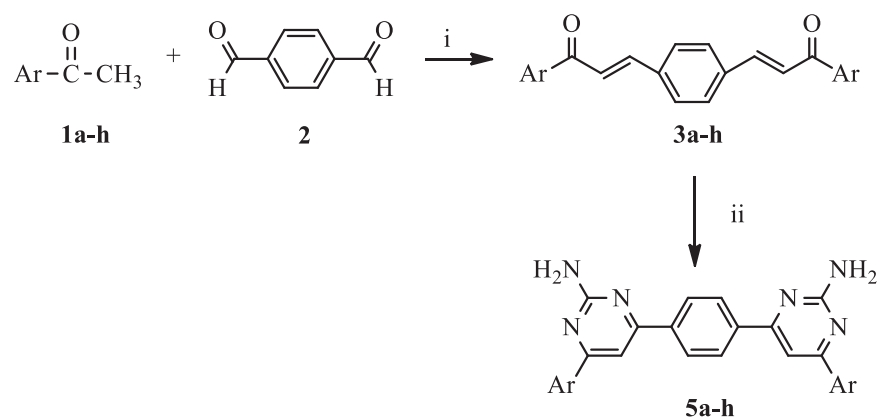
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As a follow-up to these results, we aim at the synthesis of novel 1,4-phenylene-bis-pyrimidine-2-amine derivatives from 1,4-phenylene-bis-chalcones, and we also investigate their anticancer activities against the C6 and HeLa cell lines.

## 2. Results and discussion

### 2.1. Chemistry

The synthetic approach to the desired compounds is given in the Scheme. The starting materials, 1,4-phenylene-bis-chalcones (**3a–3h**), were prepared by Claisen–Schmidt condensation of terephthalaldehyde (**2**) and related arylketones (**1a–h**) in the presence of NaOH in EtOH (Scheme; Table 1). 1,4-Phenylene-bis-chalcones are known as **3a**,<sup>36</sup> **3b**,<sup>37</sup> **3c**,<sup>38</sup> and **3e–3h**.<sup>39</sup>



**i)** NaOH, EtOH, 3h, room temp.

**ii)** 1.  $\text{C}(\text{NH}_2)_3\text{Cl}$  (**4**), KOH, EtOH, 2h, 2.  $\text{H}_2\text{O}_2$ , 4h, reflux.

**Ar:** 1-Naphthyl, 3-Thienyl, 2-Thienyl, 2-Furyl, Ph, 4- $\text{CH}_3\text{Ph}$ , 4- $\text{CH}_3\text{OPh}$ , 4- $\text{ClPh}$ .

**Scheme.** The synthetic approach to the desired compounds.

Synthesized 1,4-phenylene-bis-chalcones were submitted to reaction with guanidine hydrochloride (**4**) to get 1,4-phenylene-bis-pyrimidine-2-amine derivatives. The 1,4-phenylene-bis-pyrimidine-2-amine derivatives (**5a–5h**) were synthesized by literature procedures.<sup>40</sup> Reaction of 1,4-phenylene-bis-chalcones (**3a–3h**) (1 equiv.) with guanidine hydrochloride (**4**) (8 equiv.) and KOH (2 equiv., 2.5 M) was started in dry ethanol under reflux conditions. After 2 h of reaction, the mixture was added to  $\text{H}_2\text{O}_2$  (35%, 20 equiv.) dropwise for 2 h. The mixture was then cooled to r.t. and transferred to HCl/ice. The precipitated solid products were filtered off, washed with methanol several times, and dried to yield the 1,4-phenylene-bis-pyrimidine-2-amine derivatives (**5a–5h**) (Scheme; Table 1).

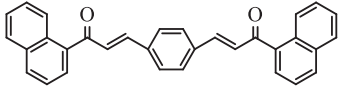
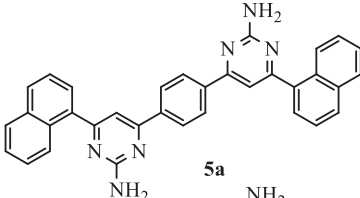
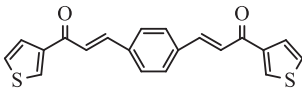
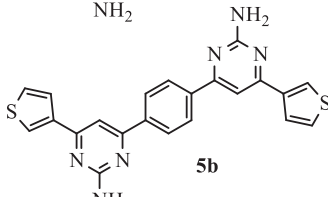
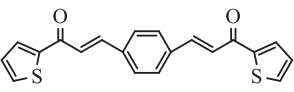
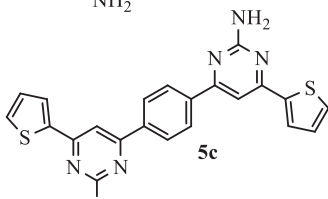
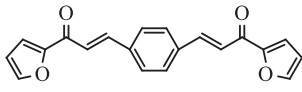
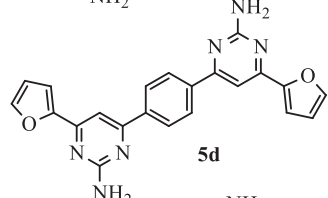
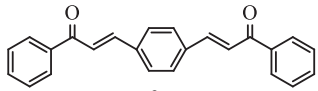
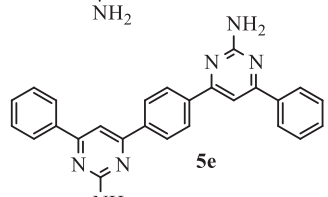
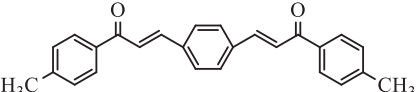
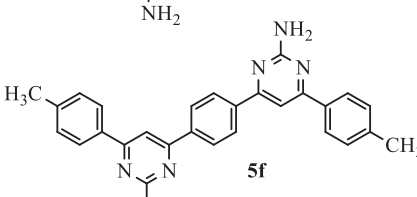
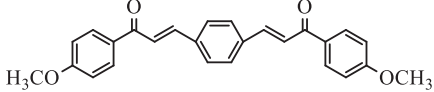
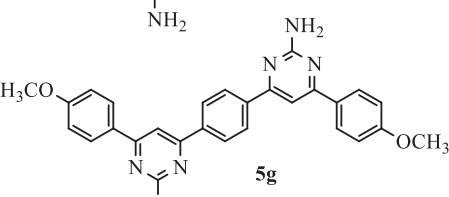
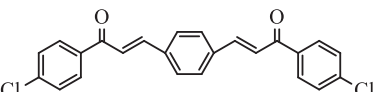
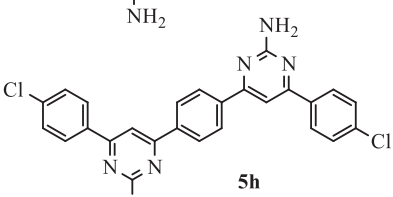
The structures of **5a–5h** were explained by spectral data (IR and NMR) and elemental analysis. All spectral data are in good agreement with the expected structures.

### 2.2. Anticancer activity results against C6 and HeLa cells

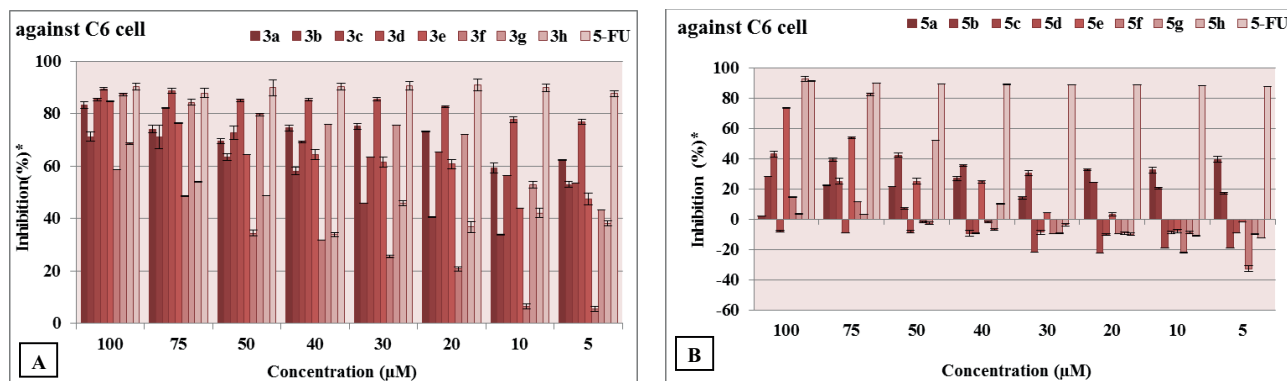
Anticancer activities against C6 and HeLa cells of **3a–3h** and **5a–5h** were investigated. The results were compared with 5-FU, which was used as a positive control.

The anticancer activities of **3a–3h** were shown to increase dose-dependently against C6 cells (Figure 1A). Most of compounds **3a–3h** exhibited significant activity compared to the 5-FU as the reference drug.

Table 1. Synthesized compounds.

Entry	Reagent	Product	Yield (%)
1	 <b>3a</b>	 <b>5a</b>	84
2	 <b>3b</b>	 <b>5b</b>	78
3	 <b>3c</b>	 <b>5c</b>	83
4	 <b>3d</b>	 <b>5d</b>	73
5	 <b>3e</b>	 <b>5e</b>	90
6	 <b>3f</b>	 <b>5f</b>	75
7	 <b>3g</b>	 <b>5g</b>	82
8	 <b>3h</b>	 <b>5h</b>	86

Compounds **3c**, **3d**, and **3g** showed almost the same activity as 5-FU, particularly at 75–100  $\mu\text{M}$  concentrations. While compounds **3a** and **3e** showed considerable activity, compounds **3b**, **3f**, and **3h** exhibited moderate anticancer activities when compared to 5-FU. Inhibitory effects of the compounds on HeLa cells at 100  $\mu\text{M}$  revealed the following potency order: 5-FU > **3d** ~ **3g** ~ **3c** > **3e** ~ **3a** > **3b** > **3h** > **3f**.



**Figure 1.** The anticancer activities of **3a–3h** (A) and **5a–5h** (B) against C6 cells. \*Each substance was tested twice in triplicate against the cell line. Data show the average of two individual experiments ( $P < 0.01$ ).

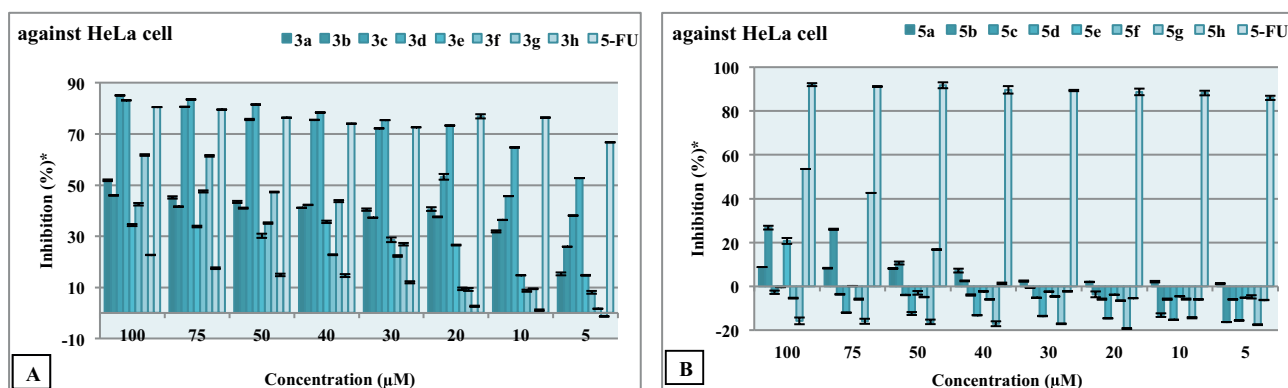
The anticancer activities of **5a–5h** were also determined by the increase in dose-dependent activity against C6 cells (Figure 1B). The anticancer activity of **5h** was better than that of 5-FU at high concentrations. Compound **5e** exhibited a moderate anticancer effect, while compounds **5a–5d** and **5f** and **5g** did not show any significant activity when compared to 5-FU. Inhibitory effects of the compounds on C6 cells at 100  $\mu\text{M}$  revealed the following potency order: **5h** > 5-FU > **5e** > **5c** > **5b** > **5f** > **5g** > **5a** > **5d**.

Anticancer activities of **3a–3h** and **5a–5h** were also determined against HeLa cells. The anticancer activities of **3a–3h** and **5a–5h** were shown to increase dose-dependently against HeLa cells (Figures 2A and 2B, respectively). The anticancer activity of compounds **3c** and **3d** was better than that of 5-FU at high concentrations (30–100  $\mu\text{M}$ ). Moreover, the others (except **3h**) showed moderate activity when compared to 5-FU. Inhibitory effects of the compounds against HeLa cells at 100  $\mu\text{M}$  revealed the following potency order: **3c** > **3d** > 5-FU > **3g** > **3a** > **3b** > **3f** > **3e** > **3h** (Figure 2A). The anticancer activities of compounds **5a–5h** are given in Figure 2B. Compound **5h** exhibited moderate activity, whereas the others did not show any significant activity when compared to 5-FU (Figure 2B). Inhibitory effects of the compounds on HeLa cells at 100  $\mu\text{M}$  revealed the following potency order: 5-FU > **5h** > **5b** > **5e** > **5a** > **5d** > **5c** > **5f** > **5g**.

When comparing 1,4-phenylene-bis-chalcones **3a–3h** with 1,4-phenylene-bis-pyrimidines **5a–5h**, it was seen that 1,4-phenylene-bis-chalcones **3a–3h** were more effective against both cell lines (C6 and HeLa) than 1,4-phenylene-bis-pyrimidines **5a–5h**.

Among all tested compounds, the most active compounds against both cell lines were compounds **3c** and **3d**, followed by compounds **3e** and **3h** against C6 and **5h** against C6 (at 75–100  $\mu\text{M}$  concentrations), when compared with 5-FU. This result indicated that the thiophene and furan ring and the *p*-chloro substituent enhanced anticancer activity.

Additionally,  $\text{IC}_{50}$  values were calculated by using ED50 Plus v1.0 and are given in Table 2.



**Figure 2.** The anticancer activities of **3a–3h** (A) and **5a–5h** (B) against HeLa cells. \*Each substance was tested twice in triplicate against the cell line. Data show the average of two individual experiments ( $P < 0.01$ ).

**Table 2.** The  $IC_{50}$  values of **3a–3h** and **5a–5h** against C6 and HeLa cells.

Sample codes	IC <sub>50</sub>	
	HeLa	C6
<b>3a</b>	< 5	< 5
<b>3b</b>	< 5	< 5
<b>3c</b>	< 5	< 5
<b>3d</b>	< 5	< 5
<b>3e</b>	< 5	< 5
<b>3f</b>	39.48	41.86
<b>3g</b>	38.65	< 5
<b>3h</b>	44.71	31.43
<b>5a</b>	34.39	55.31
<b>5b</b>	60.66	< 5
<b>5c</b>	> 100	73.75
<b>5d</b>	> 100	> 100
<b>5e</b>	89.18	58.38
<b>5f</b>	< 5	71.39
<b>5g</b>	> 100	82.09
<b>5h</b>	62.62	57.64
<b>5-FU</b>	16.32	5.8

### 3. Experimental

#### 3.1. General

IR spectra (KBr disks) were measured on a JASCO FT/IR-430 spectrometer.  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectra were recorded on a Bruker Avance DPX-400 instrument. As internal standards TMS ( $\delta$  0.00) was used for  $^1\text{H}$  NMR and  $\text{CDCl}_3$  ( $\delta$  77.0) for  $^{13}\text{C}$  NMR spectroscopy;  $J$  values are given in Hz. The multiplicities of the signals in the  $^1\text{H}$  NMR spectra were abbreviated as s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), br (broad), and combinations thereof. Melting points were measured on an Electrothermal 9100 apparatus. Elemental analyses were obtained from a LECO CHNS 932 Elemental Analyzer.

**3.1.1. General procedure for the synthesis of bis-pyrimidine derivatives (5a–5h)**

A solution of bis-chalcone (3a–3h) (1 mmol), guanidine hydrochloride (4) (8 mmol), and KOH (2 mmol, 2.5 M) in ethanol was refluxed for 2 h. Continuing the reflux operation, to the reaction medium H<sub>2</sub>O<sub>2</sub> (20 mmol, 35%) was added dropwise within 2 h. After reflux, the reaction mixture was cooled to r.t. and the product was precipitated by the addition of dilute HCl. The precipitate was filtered and washed with methanol and dried.

**6,6'-(1,4-Phenylene)bis(4-(naphthalen-1-yl)pyrimidin-2-amine) (5a):** Orange solid (433 mg, 84% yield); mp 290–292 °C. IR (KCl, cm<sup>-1</sup>): 3471, 3288, 3143, 1627, 1567, 1538, 1454, 1400, 1353, 1220, 831, 808, 777. <sup>1</sup>H NMR (400 MHz, DMSO, ppm): δ 8.33 (s, 4H), 8.27–8.24 (m, 2H), 8.07–8.02 (m, 4H), 7.75 (d, *J* = 6.8 Hz 2H), 7.64 (t, *J* = 7.6 Hz 2H), 7.60–7.54 (m, 4H), 7.49 (s, 2H), 6.95 (s, 4H). <sup>13</sup>C NMR (100 MHz, DMSO, ppm): δ 168.5 (2C), 164.2 (2C), 164.0 (2C), 139.4 (2C), 137.3 (2C), 133.8 (2C), 130.6 (2C), 129.8 (2C), 128.7 (2C), 127.7 (4C), 127.5 (2C), 127.0 (2C), 126.5 (2C), 125.9 (2C), 125.8 (2C), 107.3 (2C). Anal. calc. for C<sub>34</sub>H<sub>24</sub>N<sub>6</sub>: C, 79.05; H, 4.68; N, 16.27. Found: C, 78.88; H, 4.49; N, 16.21.

**6,6'-(1,4-Phenylene)bis(4-(thiophen-3-yl)pyrimidin-2-amine) (5b):** Orange solid (334 mg, 78% yield); mp 296–297 °C. IR (KCl, cm<sup>-1</sup>): 3316, 3195, 1631, 1573, 1513, 1454, 1411, 1334, 1228, 829, 784, 742. <sup>1</sup>H NMR (400 MHz, DMSO, ppm): δ 8.41 (s, 2H), 8.33 (s, 4H), 7.87 (d, *J* = 4.0 Hz 2H), 7.71 (s, 2H), 7.67 (s, 2H), 6.67 (s, 4H). <sup>13</sup>C NMR (100 MHz, DMSO, ppm): δ 164.4 (2C), 164.3(2C), 164.1 (2C), 141.3 (2C), 139.4 (2C), 127.5 (6C), 127.3 (2C), 126.9 (2C), 102.7 (2C). Anal. calc. for C<sub>22</sub>H<sub>16</sub>N<sub>6</sub>S<sub>2</sub>: C, 61.66; H, 3.76; N, 19.61. Found: C, 61.54; H, 3.61; N, 19.41.

**6,6'-(1,4-Phenylene)bis(4-(thiophen-2-yl)pyrimidin-2-amine) (5c):** Orange solid (355 mg, 83% yield); mp 324–325 °C. IR (KCl, cm<sup>-1</sup>): 3326, 3201, 1639, 1565, 1509, 1444, 1415, 1365, 1243, 1216, 813, 771, 715. <sup>1</sup>H NMR (400 MHz, DMSO, ppm): δ 8.35 (s, 4H), 8.17 (d, *J* = 4.0 Hz 2H), 7.80 (s, 2H), 7.76 (d, *J* = 5.2 Hz 2H), 7.26–7.24 (m, 2H), 6.81 (s, 4H). <sup>13</sup>C NMR (100 MHz, DMSO, ppm): δ 164.8 (2C), 164.7 (2C), 161.2 (2C), 144.1 (2C), 139.9 (2C), 131.0 (2C), 129.4 (2C), 129.1 (2C), 128.1 (4C), 101.5 (2C). Anal. calc. for C<sub>22</sub>H<sub>16</sub>N<sub>6</sub>S<sub>2</sub>: C, 61.66; H, 3.76; N, 19.61. Found: C, 61.58; H, 3.56; N, 19.49.

**6,6'-(1,4-Phenylene)bis(4-(furan-2-yl)pyrimidin-2-amine) (5d):** Orange solid (289 mg, 73% yield); mp 292–296 °C. IR (KCl, cm<sup>-1</sup>): 3486, 3309, 3183, 1600, 1562, 1486, 1448, 1349, 1236, 1018, 991, 954, 885, 819, 779, 757, 595. <sup>1</sup>H NMR (400 MHz, DMSO, ppm): δ 8.30 (s, 4H), 7.94 (s, 2H), 7.56 (s, 2H), 7.33 (d, *J* = 3.2 Hz 2H), 6.87 (s, 4H), 6.74–6.72 (dd, *J* = 3.2, 1.6 Hz 2H). <sup>13</sup>C NMR (100 MHz, DMSO, ppm): δ 164.3 (4C), 157.1 (2C), 152.4 (2C), 145.8 (2C), 139.3 (2C), 127.5 (4C), 112.9 (2C), 112.2 (2C), 100.5 (2C). Anal. calc. for C<sub>22</sub>H<sub>16</sub>N<sub>6</sub>O<sub>2</sub>: C, 66.66; H, 4.07; N, 21.20. Found: C, 66.54; H, 4.00; N, 21.12.

**6,6'-(1,4-Phenylene)bis(4-phenylpyrimidin-2-amine) (5e):** Orange solid (375 mg, 90% yield); mp 294–298 °C. IR (KCl, cm<sup>-1</sup>): 3330, 3203, 1644, 1565, 1496, 1455, 1359, 1216, 827, 969, 694, 649. <sup>1</sup>H NMR (400 MHz, DMSO, ppm): δ 8.39 (s, 4H), 8.27–8.24 (m, 4H), 7.82 (s, 2H), 7.55–7.54 (m, 6H), 6.82 (s, 4H). <sup>13</sup>C NMR (100 MHz, DMSO, ppm): δ 165.5 (2C), 164.6 (2C), 164.4 (2C), 139.5 (2C), 137.6 (2C), 131.0 (2C), 129.1 (4C), 127.7 (4C), 127.4 (4C), 102.6 (2C). Anal. calc. for C<sub>26</sub>H<sub>20</sub>N<sub>6</sub>: C, 74.98; H, 4.84; N, 20.18. Found: C, 74.87; H, 4.79; N, 20.14.

**6,6'-(1,4-Phenylene)bis(4-(p-tolyl)pyrimidin-2-amine) (5f):** Orange solid (333 mg, 75% yield); mp 311–312 °C. IR (KCl, cm<sup>-1</sup>): 3484, 3313, 3193, 1625, 1573, 1533, 1508, 1448, 1361, 1220, 1148, 811, 790, 773. <sup>1</sup>H-NMR (400 MHz, DMSO, ppm): δ 8.38 (s, 4H), 8.17 (d, *J* = 8.0 Hz 4H), 7.79 (s, 2H), 7.35 (d, *J* = 8.0

Hz 4H), 6.80 (s, 4H), 2.39 (s, 6H).  $^{13}\text{C}$  NMR (100 MHz, DMSO, ppm):  $\delta$  165.3 (2C), 164.4 (4C), 140.7 (2C), 139.5 (2C), 134.9 (2C), 129.6 (4C), 127.6 (4C), 127.4 (4C), 102.1 (2C), 21.4 (2C). Anal. calc. for  $\text{C}_{28}\text{H}_{24}\text{N}_6$ : C, 75.65; H, 5.44; N, 18.91. Found: C, 75.58; H, 5.39; N, 18.82.

**6,6'-(1,4-Phenylene)bis(4-(4-methoxyphenyl)pyrimidin-2-amine) (5g)**: Orange solid (390 mg, 82% yield); mp 320–323 °C. IR (KCl,  $\text{cm}^{-1}$ ): 3451, 3309, 3193, 1606, 1573, 1535, 1509, 1438, 1361, 1238, 1176, 1027, 823, 580.  $^1\text{H}$ -NMR (400 MHz, DMSO, ppm):  $\delta$  8.37 (s, 4H), 8.25 (d,  $J = 8.4$  Hz 4H), 7.77 (s, 2H), 7.09 (d,  $J = 8.8$  Hz 4H), 6.76 (s, 4H), 3.85 (s, 6H).  $^{13}\text{C}$  NMR (100 MHz, DMSO, ppm):  $\delta$  165.0 (2C), 164.4 (2C), 164.2 (2C), 161.7 (2C), 139.6 (2C), 130.0 (2C), 129.0 (4C), 127.5 (4C), 114.4 (4C), 101.7 (2C), 55.7 (2C). Anal. calc. for  $\text{C}_{28}\text{H}_{24}\text{N}_6\text{O}_2$ : C, 70.57; H, 5.08; N, 17.64. Found: C, 70.54; H, 4.99; N, 17.59.

**6,6'-(1,4-Phenylene)bis(4-(4-chlorophenyl)pyrimidin-2-amine) (5h)**: Orange solid (416 mg, 86% yield); mp 308–310 °C. IR (KCl,  $\text{cm}^{-1}$ ): 3490, 3332, 3201, 1631, 1567, 1492, 1359, 1218, 1091, 1012, 809, 485.  $^1\text{H}$  NMR (400 MHz, DMSO, ppm):  $\delta$  8.39 (s, 4H), 8.30 (d,  $J = 8.4$  Hz 4H), 7.86 (s, 2H), 7.62 (d,  $J = 8.4$  Hz 4H), 6.89 (s, 4H).  $^{13}\text{C}$ -NMR (100 MHz, DMSO, ppm):  $\delta$  164.8 (2C), 164.4 (2C), 164.2 (2C), 139.5 (2C), 136.5 (2C), 135.7 (2C), 129.3 (4C), 129.1 (4C), 127.7 (4C), 102.4 (2C). Anal. calc. for  $\text{C}_{26}\text{H}_{18}\text{Cl}_2\text{N}_6$ : C, 64.34; H, 3.74; N, 17.31. Found: C, 64.29; H, 3.67; N, 17.26.

## 3.2. Biological part

### 3.2.1. Preparation of stock solution

The compounds and 5-FU were solved by sterile dimethyl sulfoxide (DMSO) and were diluted with Dulbecco's modified Eagle's medium. The final concentration of DMSO was kept below 0.1% in all tests.

### 3.2.2. Cell culture and cell proliferation assay

The anticancer activity tests and cell culture studies were performed according to the literature.<sup>41,42</sup> HeLa and C6 cells were used for the anticancer tests. The experiments were carried out at eight concentrations (5, 10, 20, 30, 40, 50, 75, and 100  $\mu\text{M}$ ).

### 3.2.3. Statistical analysis

The results are means  $\pm$  SDs of nine values. Differences between groups were determined by the ANOVA method ( $P < 0.01$ ). Statistical analysis was performed with SPSS 13.5.

## 4. Conclusion

A series of 1,4-phenylene-bis-chalcone derivatives (**3a–3h**) and new 1,4-phenylene-bis-pyrimidine derivatives (**5a–5h**) were designed, synthesized, and evaluated for their anticancer activity against the C6 (rat brain tumor) and HeLa (human uterus carcinoma) cell lines. Among all the compounds that were tested, compounds **3c** and **3d** were found to be the most promising agents due to their significant antiproliferative effects against both cell lines. In addition, compounds **3e** and **3h** showed very high activity against C6. The pyrimidine series **5a–5h** (except **5h**) demonstrated very low activity against both cell lines, while compound **5h** exhibited very high activity against C6 (at 75–100  $\mu\text{M}$  concentrations) when compared to 5-FU. These results are encouraging, but further studies are required to evaluate the mechanism of action for the anticancer activity of compounds **3c**, **3d**, **3e**, **3h**, and **5h**.



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## References

1. Siegel, R. L.; Miller, K. D.; Jemal, A. *Ca. Cancer. J. Clin.* **2015**, *65*, 5-29.
2. Meenalosini, S.; Janet, J.; Kannan, E. *Am. J. Applied. Sci.* **2012**, *9*, 1020-1029.
3. Alfonse, M.; Aref, M. M.; Salem, A. B. M. *International Journal of Information Engineering and Electronic Business* **2014**, *6*, 55-63.
4. Cordeu, L.; Cubedo, E.; Bandrés, E.; Rebollo, A.; Sáenz, X.; Chozas, H.; Domínguez, M. V.; Echeverría, M.; Mendivil, B.; Sanmartin, C. et al. *Bioorg. Med. Chem.* **2007**, *15*, 1659-1669.
5. Mohammed, A. M.; Abdel, G. E.; Alsharai, M. A. *Am. J. Biochem. Biotech.* **2011**, *7*, 43-54.
6. Abdel Mohsen, H. T.; Ragab, F. A. F.; Ramla, M. M.; El Diwani, H. I. *Eur. J. Med. Chem.* **2010**, *45*, 2336-2344.
7. Xie, F.; Zhao, H. B.; Zhao, L.; Luo, L.; Hu, Y. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 275-278.
8. Deshmukh, M. B.; Salunkhe, S. M.; Patil, D. R.; Anbhule, P. V. *J. Med. Chem.* **2009**, *44*, 2651-2654.
9. Chandrashekar, S.; Nagarajan, S. *Il Farmaco* **2005**, *60*, 279-282.
10. Sondhi, S. M.; Singh, N.; Johar, M.; Kumar, A. *Bioorg. Med. Chem.* **2005**, *13*, 6158-6166.
11. Amin, K. M.; Hanna, M. M.; Abo-Youssef, H. E.; George, R. F. *Eur. J. Med. Chem.* **2009**, *30*, 1-13.
12. Kota, R. K.; Kompelly, K. K.; Surampudi, R.; Kulkarni, R. *J. Chem. Pharm. Res.* **2011**, *3*, 848-853.
13. Gupta, J. K.; Sharma, P. K.; Dudhe, R.; Chaudhary, A. *Acta. Pol. Pharm.* **2011**, *68*, 785-793.
14. Huckova, D.; Holy, A.; Masojdkova, M.; Andrei, G.; Snoeck, R.; De Clercq, E.; Balzarini, J. *Bioorg. Med. Chem.* **2004**, *12*, 3197-3202.
15. Chitre, T. S.; Kathiravan, M. K.; Chothe, A. S.; Rakholiya, V. K.; Asgaonkar, K. D.; Shital, M. M. *J. Pharm. Res.* **2011**, *4*, 1882-1883.
16. Mohamed, Y. A.; Mohamed, S. F.; Abdullah, M. M. *J. Chem. Sci.* **2012**, *124*, 693-702.
17. Amin, K. M.; Awadalla, F. M.; Eissa, A. A. M.; Abou-Seri, S. M.; Hassan, G. S. *Bioorg. Med. Chem.* **2011**, *19*, 6087-6097.
18. Alam, O.; Khan, S. A.; Siddiqui, N.; Ahsan, W.; Verma, S. P.; Gilani, S. J. *Eur. J. Med. Chem.* **2010**, *45*, 5113-5119.
19. Naik, T. A.; Chikhaliya, K. H. *E. J. Chem.* **2007**, *4*, 60-66.
20. Kandeel, M. M.; Mounir, A. A.; Refaat, H. M.; Kassab, A. E. *Int. J. Pharm. Pharm. Sci.* **2012**, *4*, 438-448.
21. Jennings, L. D.; Kincaid, S. L.; Wang, Y. D.; Krishnamurthy, G.; Beyer, C. F.; McGinnis, J. P. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 4731-4735.
22. Horiuchi, T.; Chiba, J.; Uoto, K.; Soga, T. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 305-308.
23. Wang, Y. D.; Johnson, S.; Powell, D.; McGinnis, J. P.; Miranda, M.; Rabindran, S. K. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 3763-3766.
24. Salman, A. A.; Tamara, S. N.; Farooq I. M. *Journal of Al-Nahrain University - Science* **2013**, *16*, 84-92.
25. Jain, K. S.; Chitre, T. S.; Miniyaar, P. B.; Kathiravan, M. K.; Bendre, V. S.; Veer, V. S.; Shahane, S. R.; Shishoo, C. J. *Curr. Sci.* **2006**, *90*, 793-803.
26. Miranda, C. L.; Stevens, J. F.; Ivanov, V.; McCall, M.; Frei, B.; Deinzer, M. L. *J. Agric. Food. Chem.* **2000**, *48*, 3876-3884.
27. Hsieh, H. K.; Tsao, L. T.; Wang, J. P.; Lin, C. N. *J. Pharm. Pharmacol.* **2000**, *52*, 163-170.
28. Liu, M.; Wilairat, P.; Go, M. L. *J. Med. Chem.* **2001**, *44*, 4443-4452.

29. Nielsen, S. F.; Chen, M.; Theander, T. G.; Kharazmi, A.; Christensen, S. B. *Bioorg. Med. Chem. Lett.* **1995**, *5*, 449-452.
30. Pati, H. N.; Holt, H. L.; Blanc, R. L.; Dickson, J.; Stewart, M.; Brown, T. *Med. Chem. Res.* **2005**, *14*, 19-25.
31. Wu, J.; Li, J.; Cai, Y.; Pan, Y.; Ye, F.; Zhang, Y.; Zhao, Y.; Yang, S.; Li, X.; Liang, G. *J. Med. Chem.* **2011**, *54*, 8110-8123.
32. Abdelhafez, O. M.; Abdel-Latif, N. A.; Badria, F. A. *Arch. Pharm. Res.* **2011**, *34*, 1623-1632.
33. Abdel-Latif, N. A. *Sci. Pharm.* **2005**, *74*, 173-216.
34. Gomha, S.; Abdallah, M.; Abd El-Aziz, M.; Serag, N. *Turk. J. Chem.* **2016**, *40*, 484-498.
35. Abbas, I.; Gomha, S.; Elaasser, M.; Bauomi, M. *Turk. J. Chem.* **2015**, *39*, 334-346.
36. Winter, E.; Neuenfeldt, P. D.; Chiaradia-Delatorre, L. D.; Gauthier, C.; Yunes, R. A.; Nunes, R. J.; Creczynski-Pasa, T. B.; Pietro, A. D. *J. Med. Chem.* **2014**, *57*, 2930-2941.
37. Gürdere, M. B.; Özbek, O.; Ceylan, M. *Synth. Commun.* **2016**, *46*, 322-331.
38. El-Rayyes, N.; Ai-Johary, A. *J. Chem. Eng. Data.* **1985**, *30*, 500-502.
39. Kanagarajana, V.; Ezhilarasi, M. R.; Gopalakrishnan, M. *Spectrochim. Acta. A.* **2011**, *78*, 635-639.
40. Kachroo, M.; Panda, R.; Yadav, Y. *Der Pharma Chemica* **2014**, *6*, 352-359.
41. Sahin Yaglioglu, A.; Demirtas, I.; Goren, N. *Phytochem. Lett.* **2014**, *8*, 213-219.
42. Demirtas, I.; Sahin Yaglioglu, A. *J. Chem.* **2013**, *2013*, 1-6.