



## A facile synthesis and biological activity of novel tetrahydrobenzo[4,5']thieno[3',2':5,6]pyrido[4,3-d]pyrimidin-4(3H)-ones

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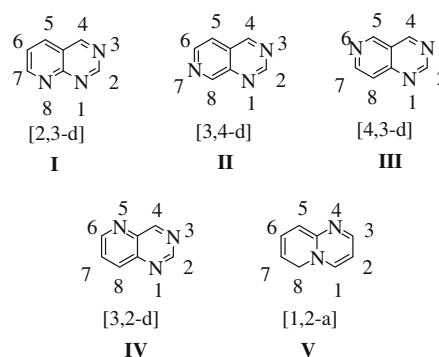
### ABSTRACT

The synthesis and biological activity of 2-substituted-8,9,10,11-tetrahydrobenzo[4,5']thieno[3',2':5,6]pyrido[4,3-d]pyrimidin-4(3H)-ones are described. Bioassay results indicated that these compounds have antifungal activity against *Botrytis cinerea* at a concentration of 50 mg/L. In addition, compounds **5m** and **5n** were effective to both KB cells and their parent multidrug resistant KBv200 cells with the overexpression of ABCB1. For example, compound **5m** showed the best inhibition against KB and KBv200 cells with IC<sub>50</sub> values of 17.4 and 25.4 μM, respectively.

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Derivatives of pyridopyrimidines have received much attention because of their potential biological activity as isosteres of quinoxalines or pteridines. For example, some related 4-(phenylamino)pyrido[d]pyrimidines have been reported as selective inhibitors of tyrosine phosphorylation by the epidermal growth-factor receptor (EGFR), and have become an important class of potential anticancer drugs.<sup>1,2</sup> All five possible pyridopyrimidine systems, pyrido[2,3-d]pyrimidine **I**, pyrido[3,4-d]pyrimidine **II**, pyrido[4,3-d]pyrimidine **III**, pyrido[3,2-d]pyrimidine **IV** and pyrido[1,2-a]pyrimidine **V** have been reported. The pyrido[2,3-d]pyrimidine system has been studied in more detail because examples have medicinal applications as inhibitors of tyrosine kinase<sup>3</sup> (AK) or dihydrofolate reductase<sup>3,4</sup> (DHFR). However, few reports<sup>1,5</sup> are available so far on the biological activity of pyrido[4,3-d]pyrimidine derivatives.

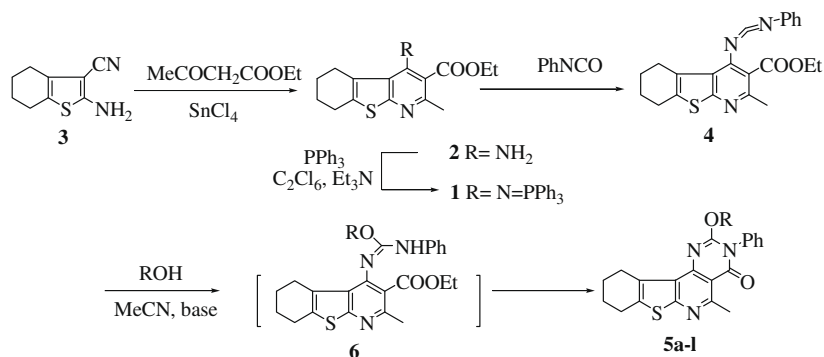
For the reason given above, the synthesis of pyridopyrimidine derivatives is challenging. The methods described so far for the preparation of some representative derivatives of this ring system involve two general routes: (a) formation of the pyridine ring by cyclization of suitable substituents of a pyrimidine; (b) formation of the pyrimidine ring by cyclization of a suitable pyridine derivative.<sup>6</sup> However, these methods often require forcing conditions, long reaction times and complex synthetic pathways.



The aza-Wittig reactions of iminophosphoranes have received increasing attention in view of their utility in the synthesis of N-heterocyclic compounds.<sup>7</sup> Recently, we have become interested in the synthesis of new bioactive heterocycles such as thiazolopyrimidinones,<sup>8</sup> thienopyrimidinones,<sup>9</sup> and triazolopyrimidinones,<sup>10</sup> all prepared from various iminophosphoranes, with the aim of evaluating their biological activity. Herein, we would like to describe a facile synthesis of fused pyrido[4,3-d]pyrimidine derivatives from the easily accessible iminophosphorane **1**. The preliminary results of an in vitro bioassay indicated that some of these compounds display favorable cytotoxic and moderate antifungal activity.

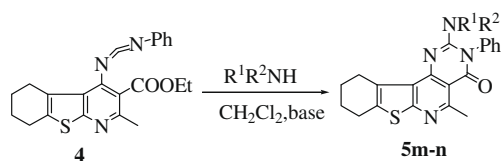
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The 2-amino-4,5,6,7-tetrahydro[1]benzothiophene-3-carbonitrile **3** was converted into 5,6,7,8-tetrahydro[1]benzothieno[2,3-*b*]pyridine **2** via reaction with ethyl acetoacetate and tin tetrachloride under heating.<sup>11</sup> The iminophosphorane **1** was subsequently obtained in a satisfactory yield when **2** was treated with triphenylphosphine, hexachloroethane and Et<sub>3</sub>N.<sup>12</sup> Iminophosphorane **1** reacted with phenyl isocyanate and gave carbodiimide **4**. The direct reaction of carbodiimide **4** with phenols, in the absence of a catalyst, did not produce 2-aryloxy-thieno[3',2':5,6]pyrido[4,3-*d*]pyrimidin-4(3*H*)-ones **5**. However, it was found that the reaction gave **5a-l** in good yields when heated for 1–2 h in the presence of a catalytic amount of K<sub>2</sub>CO<sub>3</sub> (Table 1).<sup>13</sup> The formation of **5** can be rationalized in terms of an initial nucleophilic addition of phenoxides to the carbodiimides **4** to give the intermediate **6** which cyclizes to give **5a-l**. The cyclization took place smoothly, whether the substituents on the phenols were electron-withdrawing or electron-releasing.

In refluxing toluene, **4** did not react with primary alkylamines to give the target compounds. However, when the solvent was changed to CH<sub>2</sub>Cl<sub>2</sub> and in the presence of a catalytic amount of EtONa, compound **4** was converted smoothly to the 2-alkylamino-8,9,10,11-tetrahydrobenzo[4',5']thieno[3',2':5,6]pyrido[4,3-*d*]pyrimidin-4(3*H*)-ones **5m-n** in satisfactory yields at room temperature.<sup>14</sup>



All the compounds of the **5** series were obtained as white or yellow solids after recrystallization from ethanol. Their structures were fully characterized by IR, <sup>1</sup>H NMR, EI-MS and elemental analysis. For example, the IR spectrum of **5m** revealed absorption bands at 1677 (C=O), 3145 (C<sub>6</sub>H<sub>5</sub>), and 3381 (NH) cm<sup>-1</sup>. The corresponding <sup>1</sup>H NMR spectrum showed the 5-Me group at δ(H) 2.96 (s), and the CH<sub>2</sub> signals of the cyclohexenyl ring appeared at δ(H) 1.91, 2.88, and 3.31. The other signals resonated at δ(H) 7.26–7.65 (m, 5 arom. H), 0.88 (t, *J* = 7.2 Hz, Me), 1.60 (q, *J* = 7.2 Hz, CH<sub>2</sub>), and 3.41–3.44 (m, NCH<sub>2</sub>). The mass spectrum of **5m** showed the molecular ion peak at *m/z* 404 as the base peak (100%). The structures of **5m** and the other analogs were further confirmed on the basis of elemental analysis. In the case of **5k**, single-crystal X-ray diffraction was employed to confirm the structure.<sup>15</sup>

The biological activity of the series of compounds **5** was investigated, and the results showed that some of them exhibited cytotoxicity against various KB and KBv200 cancer lines.<sup>16</sup> As indicated in Table 1, most of the compounds showed good to moderate cytotoxicity against KB. Compounds **5m** showed the best inhibitory

Table 1

Yields and in vitro cytotoxicity (IC<sub>50</sub>, μM) of pyrido[4,3-*d*]pyrimidine **5**

| Compds       | RO or NR <sup>1</sup> R <sup>2</sup>                    | Yield <sup>a</sup> (%) | Cytotoxicity against KB <sup>b,c</sup> | Cytotoxicity against KBv200 <sup>b,c</sup> |
|--------------|---|------------------------|--|--|
| <b>5a</b>    | 4-Cl-3-CH <sub>3</sub> -C <sub>6</sub> H <sub>3</sub> O | 82                     | 84.3                                   | 72.2                                       |
| <b>5b</b>    | 4-Br-C <sub>6</sub> H <sub>4</sub> O                    | 87                     | 68.9                                   | >100                                       |
| <b>5c</b>    | 2-Cl-5-CH <sub>3</sub> -C <sub>6</sub> H <sub>3</sub> O | 90                     | >100                                   | >100                                       |
| <b>5d</b>    | 2,4-Di-F-C <sub>6</sub> H <sub>3</sub> O                | 88                     | >100                                   | >100                                       |
| <b>5e</b>    | C <sub>6</sub> H <sub>5</sub> O                         | 91                     | 54.2                                   | 61.9                                       |
| <b>5f</b>    | 4-CH <sub>3</sub> -C <sub>6</sub> H <sub>4</sub> O      | 98                     | 48.3                                   | 67.3                                       |
| <b>5g</b>    | 3-F-C <sub>6</sub> H <sub>4</sub> O                     | 88                     | >100                                   | >100                                       |
| <b>5h</b>    | 3-CH <sub>3</sub> -C <sub>6</sub> H <sub>4</sub> O      | 88                     | >100                                   | >100                                       |
| <b>5i</b>    | 2-Cl-4-F-C <sub>6</sub> H <sub>3</sub> O                | 87                     | >100                                   | >100                                       |
| <b>5j</b>    | 4-NO <sub>2</sub> -C <sub>6</sub> H <sub>4</sub> O      | 97                     | >100                                   | >100                                       |
| <b>5k</b>    | 3,5-2F-C <sub>6</sub> H <sub>3</sub> O                  | 92                     | 52.5                                   | 39.9                                       |
| <b>5l</b>    | 3-OCH <sub>3</sub> -C <sub>6</sub> H <sub>4</sub> O     | 83                     | >100                                   | >100                                       |
| <b>5m</b>    | CH <sub>3</sub> (CH <sub>2</sub> ) <sub>2</sub> NH      | 65                     | 17.4                                   | 25.4                                       |
| <b>5n</b>    | CH <sub>3</sub> (CH <sub>2</sub> ) <sub>3</sub> NH      | 72                     | 32.0                                   | 32.8                                       |
| Fluorouracil |   | 12.5                   | —                                      | —  |

<sup>a</sup> Isolated yields based on iminophosphorane **1**.

<sup>b</sup> IC<sub>50</sub> is the concentration of compound required to inhibit the cell growth by 50% compared to an untreated control.

<sup>c</sup> KB cells were the drug sensitive human oral carcinoma cells and KBv200 cells were the multidrug resistant cells with overexpression of ABCB1 cells induced by vincristine.

activity against KB and KBv200 with IC<sub>50</sub> 17.4 and 25.4 μM, respectively (Table 1). Although its inhibitory activity against KB is lower than that of fluorouracil, the cytotoxicity against KB of title compounds could be further improved by incorporating appropriate functional groups.

The preliminary antifungal activity of compounds **5** series was also measured at a concentration of 50 mg/L using a reported procedure<sup>5</sup> and the inhibition rates are listed in Table 2. It was found that most of the compounds induced a good inhibition effect against *Botrytis cinerea* comparable to a commercial fungicide triadimefon, with the inhibitory rate of 98%, 99% and 100% for compounds **5g**, **5h** and **5n**, respectively. They did have any obvious

**Table 2**  
Antifungal activity (relative inhibition (%), 50 mg/L) of pyrido[4,3-d]pyrimidine **5**

| Compds      | <i>Rhizoctonia solani</i> | <i>Botrytis cinerea</i> | <i>Gibberella zeae</i> | <i>Botryosphaeria berengera</i> | <i>Bipolaris maydis</i> |
|-------------|---------------------------|-------------------------|------------------------|---------------------------------|-------------------------|
| <b>5a</b>   | 22                        | 73                      | 31                     | 39                              | 52                      |
| <b>5b</b>   | 68                        | 83                      | 65                     | 83                              | 81                      |
| <b>5c</b>   | 63                        | 88                      | 23                     | 50                              | 48                      |
| <b>5d</b>   | 63                        | 78                      | 38                     | 50                              | 71                      |
| <b>5e</b>   | 65                        | 85                      | 15                     | 56                              | 76                      |
| <b>5f</b>   | 61                        | 80                      | 31                     | 56                              | 76                      |
| <b>5g</b>   | 84                        | 98                      | 58                     | 78                              | 90                      |
| <b>5h</b>   | 91                        | 99                      | 65                     | 78                              | 81                      |
| <b>5i</b>   | 79                        | 88                      | 54                     | 67                              | 67                      |
| <b>5j</b>   | 68                        | 85                      | 46                     | 61                              | 67                      |
| <b>5k</b>   | 74                        | 83                      | 31                     | 61                              | 48                      |
| <b>5l</b>   | 76                        | 66                      | 42                     | 67                              | 76                      |
| <b>5m</b>   | 24                        | 57                      | 20                     | 29                              | 47                      |
| <b>5n</b>   | 89                        | 100                     | 83                     | 76                              | 79                      |
| Triadimefon | 91                        | 78                      | 59                     | —                               | —                       |

influence on the growth of *Rhizoctonia solani*, *Gibberella zeae*, *Bipolaris maydis* and *Botryosphaeria berengera*.

In summary, we have developed a novel approach for the synthesis of 2-substituted-8,9,10,11-tetrahydrobenzo[4',5']thieno[3',2'-5,6]pyrido[4,3-d]pyrimidin-4(3H)-ones **5** via tandem aza-Wittig and annulation reactions. The biological evaluation showed that some compounds have fungicidal activities at 50 mg/L and some of them were effective to both KB cells and KBv200 cells.

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- Preparation of iminophosphorane 1*: To a soln of **2** (1.06 g, 4 mmol) in MeCN (15 ml), PPh<sub>3</sub> (1.31 g, 5 mmol), C<sub>2</sub>Cl<sub>6</sub> (1.19 g, 5 mmol) and, in this order, NEt<sub>3</sub> (8.0 ml) were added. The mixture was stirred for 18–24 h at 0 °C. Then, the soln was concentrated, and the residue was crystallized from EtOH afforded **1** in 90.2% yield. Mp 225–226 °C. <sup>1</sup>H NMR: 0.99 (t, *J* = 7.2, Me); 1.40–1.64 (m, CH<sub>2</sub>CH<sub>2</sub>); 2.41 (s, Me); 2.50–2.54 (m, CH<sub>2</sub>); 2.65–2.69 (m, CH<sub>2</sub>); 3.38 (q, *J* = 7.2, CH<sub>2</sub>O); 7.44–7.62 (m, 15 arom. H). MS (70 eV) *m/z* (%): 552 (M<sup>+</sup>+1 1.3), 551 (M<sup>+</sup> 3.49), 521 (100), 443 (6.2), 262 (9.5), 201 (40.5), 183 (81.4), 108 (12.4).
- Preparation of compounds 5a–l*: To a soln of **1** (1.1 g, 2 mmol) in anhyd CH<sub>2</sub>Cl<sub>2</sub> (10 ml), phenyl isocyanate (0.24 g, 2 mmol) was added under N<sub>2</sub> at rt. The mixture was left standing for 30–40 min. Then, the solvent was removed in vacuum, and Et<sub>2</sub>O/petroleum ether (1:2) was added to precipitate triphenylphosphine oxide. Filtration and removal of the solvent gave the crude carbodiimides **4**, which were used directly without further purification. To the solution of **4** in CH<sub>3</sub>CN (15 ml), substituted phenol (2 mmol) and catalytic solid K<sub>2</sub>CO<sub>3</sub> (0.024 g, 0.2 mmol) were added. The mixture was stirred for 3–6 h at 75 °C and filtered, the filtrate was condensed and the residual was

recrystallized from EtOH to give the target compounds.

**Compound 5a**. A white solid. Mp 233–234 °C. IR (KBr)  $\nu$ : 1702 (C=O), 1617, 1561, 1400 cm<sup>-1</sup>. <sup>1</sup>H NMR (400 MHz CDCl<sub>3</sub>)  $\delta$  (ppm): 6.91–7.62 (m, 8H, ArH), 3.04 (s, 3H, MeC(5)), 2.80–2.83 (m, 2H, CH<sub>2</sub>), 2.47–2.51 (m, 2H, CH<sub>2</sub>), 2.40 (s, 3H, Me), 1.81–1.84 (m, 2H, CH<sub>2</sub>), 1.63–1.66 (m, 2H, CH<sub>2</sub>). MS (70 eV) *m/z* (%): 489 (34), 488 (28), 487 (M<sup>+</sup>, 100), 472 (12), 362 (18), 346 (27), 215 (14). Anal. Calcd for C<sub>27</sub>H<sub>22</sub>ClN<sub>3</sub>O<sub>2</sub>S: C, 66.45; H, 4.54; N, 8.61. Found: C, 66.11; H, 4.57; N, 8.45.

**Compound 5b**. A white solid. Mp 246–247 °C. IR (KBr)  $\nu$ : 1701 (C=O), 1613, 1560, 1401 cm<sup>-1</sup>. <sup>1</sup>H NMR (400 MHz CDCl<sub>3</sub>)  $\delta$  (ppm): 7.03–7.60 (m, 9H, ArH), 3.04 (s, 3H, MeC(5)), 2.80–2.83 (m, 2H, CH<sub>2</sub>), 2.43–2.47 (m, 2H, CH<sub>2</sub>), 1.81–1.84 (m, 2H, CH<sub>2</sub>), 1.63–1.66 (m, 2H, CH<sub>2</sub>). <sup>13</sup>C NMR (150 MHz CDCl<sub>3</sub>)  $\delta$  (ppm): 163.5, 162.7, 157.7, 154.2, 149.9, 149.5, 137.3, 135.6, 134.5, 131.6, 129.7, 129.6, 128.1, 125.1, 124.6, 120.7, 109.6, 26.4, 25.7, 22.8, 22.4, 20.1. MS (70 eV) *m/z* (%): 519 (84), 518 (43), 517 (M<sup>+</sup>, 100), 363 (18), 243 (14), 215 (14), 77 (92). Anal. Calcd for C<sub>26</sub>H<sub>20</sub>BrN<sub>3</sub>O<sub>2</sub>S: C, 60.24; H, 3.89; N, 8.11. Found: C, 60.51; H, 3.85; N, 8.04.

**Compound 5c**. A white solid. Mp 237–238 °C. IR (KBr)  $\nu$ : 1695 (C=O), 1618, 1561, 1400 cm<sup>-1</sup>. <sup>1</sup>H NMR (400 MHz CDCl<sub>3</sub>)  $\delta$  (ppm): 7.05–7.60 (m, 8H, ArH), 3.05 (s, 3H, MeC(5)), 2.78–2.81 (m, 2H, CH<sub>2</sub>), 2.34–2.37 (m, 5H, CH<sub>3</sub>+CH<sub>2</sub>), 1.78–1.80 (m, 2H, CH<sub>2</sub>), 1.56–1.59 (m, 2H, CH<sub>2</sub>). MS (70 eV) *m/z* (%): 487 (M<sup>+</sup>, 100), 473 (13), 451 (18), 361 (24), 316 (24), 214 (48), 77 (99). Anal. Calcd for C<sub>27</sub>H<sub>27</sub>ClN<sub>3</sub>O<sub>2</sub>S: C, 66.45; H, 4.54; N, 8.61. Found: C, 66.68; H, 4.76; N, 8.75.

**Compound 5d**. A white solid. Mp 25–226 °C. IR (KBr)  $\nu$ : 1699 (C=O), 1615, 1560, 1402 cm<sup>-1</sup>. <sup>1</sup>H NMR (400 MHz CDCl<sub>3</sub>)  $\delta$  (ppm): 6.93–7.62 (m, 8H, ArH), 3.05 (s, 3H, MeC(5)), 2.80–2.83 (m, 2H, CH<sub>2</sub>), 2.43–2.46 (m, 2H, CH<sub>2</sub>), 1.80–1.83 (m, 2H, CH<sub>2</sub>), 1.61–1.64 (m, 2H, CH<sub>2</sub>). MS (70 eV) *m/z* (%): 477 (M<sup>+</sup>+2, 4), 475 (M<sup>+</sup>, 14), 398 (3), 215 (6), 128 (22), 119 (17), 77 (100). Anal. Calcd for C<sub>26</sub>H<sub>19</sub>F<sub>2</sub>N<sub>3</sub>O<sub>2</sub>S: C, 65.67; H, 4.03; N, 8.84. Found: C, 65.75; H, 4.19; N, 8.95.

**Compound 5e**. A white solid. Mp 217–218 °C. IR (KBr)  $\nu$ : 1698 (C=O), 1613, 1560, 1398 cm<sup>-1</sup>. <sup>1</sup>H NMR (400 MHz CDCl<sub>3</sub>)  $\delta$  (ppm): 7.13–7.60 (m, 8H, ArH), 3.06 (s, 3H, MeC(5)), 2.79–2.81 (m, 2H, CH<sub>2</sub>), 2.41–2.44 (m, 2H, CH<sub>2</sub>), 1.77–1.79 (m, 2H, CH<sub>2</sub>), 1.56–1.59 (m, 2H, CH<sub>2</sub>). <sup>13</sup>C NMR (150 MHz CDCl<sub>3</sub>)  $\delta$  (ppm): 162.1, 157.6, 152.8, 149.1, 146.0, 135.6, 134.8, 134.4, 133.3, 132.5, 131.6, 130.0, 129.6, 128.2, 128.0, 125.0, 124.8, 26.1, 25.8, 25.5, 22.4, 22.2. MS (70 eV) *m/z* (%): 441 (3), 440 (11), 439 (M<sup>+</sup>, 34), 424 (7), 363 (8), 91 (26), 77 (100). Anal. Calcd for C<sub>26</sub>H<sub>21</sub>N<sub>3</sub>O<sub>2</sub>S: C, 71.05; H, 4.82; N, 9.56. Found: C, 70.96; H, 5.05; N, 9.62.

**Compound 5f**. A white solid. Mp 246–247 °C. IR (KBr)  $\nu$ : 1699 (C=O), 1616, 1559, 1402 cm<sup>-1</sup>. <sup>1</sup>H NMR (400 MHz CDCl<sub>3</sub>)  $\delta$  (ppm): 7.00–7.61 (m, 9H, ArH), 3.04 (s, 3H, MeC(5)), 2.78–2.81 (m, 2H, CH<sub>2</sub>), 2.43–2.45 (m, 2H, CH<sub>2</sub>), 2.39 (s, 3H, Me), 1.78–1.81 (m, 2H, CH<sub>2</sub>), 1.57–1.60 (m, 2H, CH<sub>2</sub>). MS (70 eV) *m/z* (%): 453 (M<sup>+</sup>, 100), 438 (24), 362 (21), 346 (25), 215 (18), 91 (44), 77 (53). Anal. Calcd for C<sub>27</sub>H<sub>23</sub>N<sub>3</sub>O<sub>2</sub>S: C, 71.50; H, 5.11; N, 9.26. Found: C, 71.62; H, 5.10; N, 9.32.

**Compound 5g**. A white solid. Mp 213–214 °C. IR (KBr)  $\nu$ : 1701 (C=O), 1619, 1562, 1402 cm<sup>-1</sup>. <sup>1</sup>H NMR (400 MHz CDCl<sub>3</sub>)  $\delta$  (ppm): 6.94–7.62 (m, 9H, ArH), 3.04 (s, 3H, MeC(5)), 2.79–2.83 (m, 2H, CH<sub>2</sub>), 2.50–2.53 (m, 2H, CH<sub>2</sub>), 1.78–1.84 (m, 2H, CH<sub>2</sub>), 1.62–1.66 (m, 2H, CH<sub>2</sub>). MS (70 eV) *m/z* (%): 458 (7), 457 (M<sup>+</sup>, 45), 429 (12), 362 (19), 215 (10), 111 (33), 77 (100). Anal. Calcd for C<sub>26</sub>H<sub>20</sub>FN<sub>3</sub>O<sub>2</sub>S: C, 68.25; H, 4.41; N, 9.18. Found: C, 68.39; H, 4.53; N, 9.35.

**Compound 5h**. A white solid. Mp 206–207 °C. IR (KBr)  $\nu$ : 1697 (C=O), 1616, 1560, 1399 cm<sup>-1</sup>. <sup>1</sup>H NMR (400 MHz CDCl<sub>3</sub>)  $\delta$  (ppm): 6.92–7.61 (m, 9H, ArH), 3.05 (s, 3H, MeC(5)), 2.79–2.82 (m, 2H, CH<sub>2</sub>), 2.48–2.51 (m, 2H, CH<sub>2</sub>), 2.38 (s, 3H, CH<sub>3</sub>), 1.79–1.82 (m, 2H, CH<sub>2</sub>), 1.60–1.63 (m, 2H, CH<sub>2</sub>). MS (70 eV) *m/z* (%): 455 (36), 453 (M<sup>+</sup>, 100), 438 (14), 362 (10), 346 (13), 215 (4), 89 (34). Anal. Calcd for C<sub>27</sub>H<sub>23</sub>N<sub>3</sub>O<sub>2</sub>S: C, 71.50; H, 5.11; N, 9.26. Found: C, 71.52; H, 5.48; N, 9.17.

**Compound 5i**. A white solid. Mp 26.6 °C. IR (KBr)  $\nu$ : 1701 (C=O), 1620, 1563, 1403 cm<sup>-1</sup>. <sup>1</sup>H NMR (400 MHz CDCl<sub>3</sub>)  $\delta$  (ppm): 7.07–7.63 (m, 8H, ArH), 3.05 (s, 3H, MeC(5)), 2.79–2.82 (m, 2H, CH<sub>2</sub>), 2.36–2.39 (m, 2H, CH<sub>2</sub>), 1.79–1.82 (m, 2H, CH<sub>2</sub>), 1.60–1.62 (m, 2H, CH<sub>2</sub>). MS (70 eV) *m/z* (%): 493 (3), 492 (4), 491 (M<sup>+</sup>, 7), 363 (8), 240 (16), 215 (17), 77 (100). Anal. Calcd for C<sub>26</sub>H<sub>19</sub>ClFN<sub>3</sub>O<sub>2</sub>S: C, 63.48; H, 3.89; N, 8.54. Found: C, 63.72; H, 4.21; N, 8.65.

**Compound 5j**. A yellow solid. Mp 176–178 °C. IR (KBr)  $\nu$ : 1703 (C=O), 1620, 1560, 1401 cm<sup>-1</sup>. <sup>1</sup>H NMR (400 MHz CDCl<sub>3</sub>)  $\delta$  (ppm): 1.56–1.58 (m, 2H, CH<sub>2</sub>), 1.78–1.82 (m, 2H, CH<sub>2</sub>), 2.40–2.43 (m, 2H, CH<sub>2</sub>), 2.79–2.82 (m, 2H, CH<sub>2</sub>), 3.06 (s, 3H, MeC(5)), 7.27–8.35 (m, 9H, ArH). MS (70 eV) *m/z* (%): 486 (8), 485 (24), 484 (M<sup>+</sup>, 100), 469 (11), 456 (16), 362 (15), 77 (25). Anal. Calcd for C<sub>26</sub>H<sub>20</sub>N<sub>4</sub>O<sub>4</sub>S: C, 64.45; H, 4.16; N, 11.56. Found: C, 64.36; H, 4.04; N, 11.74.

**Compound 5k**. A white solid. Mp 237–238 °C; IR (KBr)  $\nu$ : 1694 (C=O), 1615, 1561, 1401, 1259 cm<sup>-1</sup>. <sup>1</sup>H NMR (400 MHz CDCl<sub>3</sub>)  $\delta$  (ppm): 6.78–7.61 (m, 8H, ArH), 3.05 (s, 3H, MeC(5)), 2.81–2.84 (m, 2H, CH<sub>2</sub>), 2.60–2.63 (m, 2H, CH<sub>2</sub>), 1.82–1.85 (m, 2H, CH<sub>2</sub>), 1.68–1.71 (m, 2H, CH<sub>2</sub>). MS (70 eV) *m/z* (%): 477 (8), 476 (28), 475 (M<sup>+</sup>, 100), 460 (15), 447 (22), 362 (18), 77 (87). Anal. Calcd for C<sub>26</sub>H<sub>19</sub>F<sub>2</sub>N<sub>3</sub>O<sub>2</sub>S: C, 65.67; H, 4.03; N, 8.84. Found: C, 65.75; H, 4.12; N, 8.89.

**Compound 5l**. A white solid. Mp 228–229 °C. IR (KBr)  $\nu$ : 1701 (C=O), 1618, 1560, 1400 cm<sup>-1</sup>. <sup>1</sup>H NMR (400 MHz CDCl<sub>3</sub>)  $\delta$  (ppm): 6.91–7.60 (m, 9H, ArH), 3.84 (s, 3H, OCH<sub>3</sub>), 3.05 (s, 3H, MeC(5)), 2.79–2.82 (m, 2H, CH<sub>2</sub>), 2.50–2.53 (m, 2H, CH<sub>2</sub>), 1.78–1.81 (m, 2H, CH<sub>2</sub>), 1.61–1.63 (m, 2H, CH<sub>2</sub>). MS (70 eV) *m/z* (%): 471 (43), 470 (100), 469 (M<sup>+</sup>, 95), 363 (12), 350 (14), 123 (43), 77 (26). Anal. Calcd for C<sub>27</sub>H<sub>23</sub>N<sub>3</sub>O<sub>3</sub>S: C, 69.06; H, 4.94; N, 8.95. Found: C, 69.20; H, 5.27; N, 9.05.

- Preparation of compounds 5m–n*: To a soln of **4** in CH<sub>2</sub>Cl<sub>2</sub> (10 ml), the appropriate alkylamine (2 mmol) was added and the mixture was stirred for 30 min. The solvent was removed and anhyd EtOH (10 ml) containing several drops of a solution of EtONa in EtOH was added. The mixture was stirred for

11–14 h at rt. After solvent had been removed, the residue was crystallized from EtOH and afforded the target compounds.

Compound **5m**. A pale yellow solid. Mp 243–244 °C. IR (KBr)  $\nu$ : 3381 (N–H), 1672 (C=O), 1557, 1517, 1450  $\text{cm}^{-1}$ .  $^1\text{H}$  NMR (400 MHz  $\text{CDCl}_3$ )  $\delta$  (ppm): 7.26–7.65 (m, 5H, Ar–H), 4.36 (s, 1H, NH), 3.41–3.43 (m, 2H,  $\text{NCH}_2$ ), 2.96 (s, 3H, MeC(5)), 2.88–3.31 (m, 4H, 2  $\text{CH}_2$ ), 1.88–1.92 (m, 4H, 2  $\text{CH}_2$ ), 1.58–1.62 (m, 2H,  $\text{CH}_2$ ), 0.88 (t,  $J$  = 7.2 Hz, 3H, Me).  $^{13}\text{C}$  NMR (150 MHz  $\text{CDCl}_3$ )  $\delta$  (ppm): 162.9, 162.0, 157.4, 152.7, 148.1, 134.4, 134.1, 131.8, 130.7, 129.7, 128.9, 124.7, 124.6, 41.8, 27.2, 26.9, 22.9, 22.7, 21.1, 19.0, 13.7. MS (70 eV)  $m/z$  (%): 406 (8), 405 (24), 404 ( $\text{M}^+$ , 100), 389 (14), 363 (39), 285 (12), 216 (7), 91 (8). Anal. Calcd for  $\text{C}_{23}\text{H}_{24}\text{N}_4\text{O}_5$ : C, 68.29; H, 5.98; N, 13.85. Found: C, 68.11; H, 6.06; N, 14.00.

Compound **5n**. A pale yellow solid. Mp 233–234 °C. IR (KBr)  $\nu$ : 3445 (N–H), 1677 (C=O), 1558, 1510, 1401  $\text{cm}^{-1}$ .  $^1\text{H}$  NMR (400 MHz  $\text{CDCl}_3$ )  $\delta$  (ppm): 7.25–7.65 (m,

5H, Ar–H), 4.34 (s, 1H, NH), 3.44–3.48 (m, 2H,  $\text{NCH}_2$ ), 2.96 (s, 3H, MeC(5)), 2.87–3.32 (m, 4H, 2  $\text{CH}_2$ ), 1.90–1.93 (m, 4H, 2  $\text{CH}_2$ ), 1.52–1.60 (m, 2H,  $\text{CH}_2$ ), 1.27–1.33 (m, 2H,  $\text{CH}_2$ ), 0.91 (q,  $J$  = 7.2 Hz, 3H, Me). MS (70 eV)  $m/z$  (%): 418 ( $\text{M}^+$ , 100), 403 (34), 389 (43), 376 (78), 362 (99), 346 (42), 285 (44), 91 (23). Anal. Calcd for  $\text{C}_{24}\text{H}_{26}\text{N}_4\text{O}_5$ : C, 68.87; H, 6.26; N, 13.39. Found: C, 69.04; H, 6.62; N, 13.57.

15. Ren, Q. Y.; He, H. W.; Meng, X. G. *Acta Crystallogr., Sect. E* **2006** 62, 5029 (The crystallographic data have been deposited at the Cambridge Crystallographic Data Center (E-mail: deposit@ccdc.cam.ac.uk), the deposition number is CCDC-627452).
16. Cytotoxic activity was evaluated using a standard MTT assay after exposure of cells to the tested compounds for 72 h. The results were presented as mean values of three independent experiments done in quadruplicates. Coefficients of variation were <10%.