Original article

A novel kind of antitumour drugs using sulfonamide as parent compound

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Received 6 September 2001; revised 17 October 2001; accepted 17 October 2001

Abstract – To obtain potent antitumour agents with low toxicity, sulfonamide derivatives containing 5-flurouracil and nitrogen mustard, respectively are designed and synthesised. 1-(3-(4-Acetylaminobenzenesulfonamido)-3-oxopropyl)-5-fluoropyrimidine-2,4-dione (4) was obtained by the coupling of p-acetamidobenzenesulfonamide with 3-(5-fluorouracil-1-yl) propionic acid. The hydrolysis of 4 led to 1-(3-(4-aminobenzenesulfonamido)-3-oxopropyl)-5-fluoropyrimidine-2,4-dione (5). Treatment of p-acetamidobenzenesulfonyl chloride with bis(2-chloroethyl) amine led to 4-acetylamino-N,N-bis(2-chloroethyl)benzenesulfonamide (6). Subsequent hydrolysis of 6 in hydrochloric acid led to 4-amino-N,N-bis(2-chloroethyl)benzenesulfonamide hydrochloride (7). Two different synthetic route were investigated in the synthesis of 2-[N1-2-pyrimidyl-aminobenzenesulfonamido] ethyl 4-bis(2-chloroethyl) aminophenyl butyrate (12b). Carbobenzyloxy was proved to be unsuitable for the protection of the aromatic amino group of sulfadiazine since the pyrimidine ring was also hydrogenated at the last step of the first route under the deprotection condition. In another route, acetyl was firstly used as the protective group, then it was replaced by the Schiff’s base. The reaction of chlorambucil with 2-[N1-2-pyrimidyl-(p-acetyl)aminobenzenesulfonamido] ethanol (10b) afforded 2-[N1-2-pyrimidyl-(p-benzylidene)aminobenzenesulfonamido] ethyl 4-bis(2-chloroethyl) aminophenyl butyrate (11b). Compound 12b was obtained by the hydrolysis of 11b. The acute toxicity and antitumour activity of 5, 7 and 12b have been investigated in mice. Compound 12b exhibited high antitumour activity and low toxicity with a therapeutic index (TI) of 47.55. © 2001 Éditions scientifiques et médicales Elsevier SAS

sulfonamide / antitumour activity / targeting / nitrogen mustard

1. Introduction

Conventional cancer chemotherapy is highly inadequate as a result of the lack of selectivity between cancer cells and normal cells. This calls for novel cancer therapies for selectively targeting cancers without toxicity to normal tissues. Sulfapyrazine has been shown to concentrate selectively in the Walker carcinoma growing in rats [1]. Similarly sulfadiazine was found concentrated in the Yoshida sarcoma [2]. These findings have aroused considerable interest and great efforts have been made to design new antitumour agents by combining sulfadiazine and antitumour agents in one compound. Heretofore, the main work of modified sulfadiazine has been focused on the aromatic amine due to its relatively high reaction activity [3–7]. As shown in figure 1, 14C labelled sulfadiazine mustard has been synthesised by Nguyen et al. [6], and sulfadiazine acrylamide and its polymer by Abel et al. [7].

The surprising finding that all these compounds were only taken up poorly by those tumours which concentrate sulfadiazine led to a reconsideration of the drug design strategy. Unfortunately, there was not any successful report about the application of the targeting function of sulfadiazine. In 1996, Huang suggested a new idea to design polymeric medicine using sulfadiazine as targeting compound [8]. The work in Huang’s group showed if polyethyleneoxide (Mn = 2000) were used as matrix, and the sulfadia-
azine was attached on its end at the site of sulfonamido group rather than the aromatic amino group, then the selectivity was recovered although the mechanism is still unclear [8–13]. Recently, more papers have reported several types of antitumour agents possessing the feature structure of sulfonamide [14–24]. Therefore, the sulfonamide moiety appears to be a crucial functionality to interact with cellular targets. From this point of view, we have synthesised several sulfonamide derivatives containing 5-FU and nitrogen mustard moiety respectively, in order to get potent antitumour drugs with low toxicity.

2. Chemistry

As shown in figure 2, compound 2 was prepared from 5-FU and ethyl acrylate by the method of Ouchi and Yuyama [25], m.p. 186–187 °C (lit., 194–195 °C). The structure of 2 was corroborated by the 1H-NMR analyses. Compound 4 was obtained by the coupling of p-acetimidobenzensulfonamide (3) with compound 2 in the presence of DCC and DMAP at room temperature. The unreacted 3 was removed by adjusting the pH value of the solution since 3 precipitated completely at pH of 5.5 while most of 4 was still remained in the solution. Hydrolysis of 4 in aqueous sodium hydroxide under given conditions afforded compound 5. The synthetic route of compound 7 was shown in figure 3. Treatment of p-acetimidobenzensulfonyl chloride with bis(2-chloroethyl) amine led to compound 6. Subsequent hydrolysis of 6 in hydrochloric acid led to 7.

To synthesis our target compound 12b (figure 5), it is important to choose an appropriate protective group for the aromatic amine of sulfadiazine because of its relatively high reactivity. We first selected Cbz (carbobenzyloxy) as the protective group since it is relatively stable at acidic or alkaline conditions and can be easily removed by catalytic hydrogenation. However, as shown in figure 4, this synthetic route led to 12a instead of the desired 12b. Compound 8a was prepared by reacting sulfadiazine with benzyl chloroformate. Treatment of 8a with aqueous sodium hydroxide led to compound 9a. The reaction of 9a with 2-bromoethanol in DMF afforded 10a. Compound 11a was obtained by the coupling of 10a with CBL (chlorambucil) in the presence of DCC at room temperature. The hydrogenation of 11a had been investigated under several kinds of reaction conditions using different hydrogen donors, such as hydrogen gas, cyclohexene and ammonium formate. Unfortunately, the pyrimidine ring was hydrogenated before the hydrogenation of the Cbz group under all these conditions. When using cyclohexene as hydrogen donor, the product was mainly 12a. But, when hydrogen gas or ammonium formate was used as hydrogen donor, the product was a mixture of 12a and its deprotected compound. Thus, 12b cannot be prepared by this method.

Since 12b is our target compound, we had developed another synthetic route for it, which is shown in figure 5. In this route, first acetyl was used as the protective group of the aromatic amine, then 9b and
target molecule (12b). Therefore, after the preparation of 10b, the acetyl group was replaced by the Schiff’s base, which is unstable under the preceding reaction conditions, but relatively stable under the subsequent reaction conditions.

3. Results and discussion

As we mentioned above, the aim of our work is to get potent antitumour drugs with low toxicity by combining conventional antitumour drugs and sulfonamides in one molecule. With this in mind, we had designed 5 and 7 at first, which contain the simplest sulfonamide structure. The acute toxicity of 5 and 7 were investigated in mice and the results were listed in table I. As we had expected, 5 showed very low toxicity that no sign of toxicity was observed even when the mice were treated with a dose as high as 2 g kg$^{-1}$ by the intraperitoneal route. Unfortunately, the antitumour activity of 5 was reduced markedly by comparison with the mother compound 5-FU. The results of in vivo antitumour activity of 5 and 7 against murine S-180 sarcoma were listed in table II. The low cytotoxicity of 5 may be attributed to the high stability of 2 that free 5-FU cannot be easily released. The LD$_{50}$ of 7 is 281.28 mg...
**Figure 5. Reagents and conditions:** (a) acetyl chloride, pyridine, 0 °C, 2 h; (b) NaOH (aq., 1.0 equivalent); (c) DMF, BrCH₂CH₂OH (1.0 equivalent), 80 °C, 8 h; (d) HCl (1.2 M), reflux, 1 h; (e) benzaldehyde, 100 °C, 6 h; (f) chlorambucil (1.0 equivalent), DCC (1.1 equiv.), pyridine, r.t., 48 h; (g) HCl (1 M), r.t., 10 h.

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**Table I.** LD₅₀ of the test compounds (ip in mice).

<table>
<thead>
<tr>
<th>Compound</th>
<th>LD₅₀ (mg kg⁻¹)ᵃ</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>&gt;2000ᵇ</td>
</tr>
<tr>
<td>7</td>
<td>281.28 (253.45–312.17)</td>
</tr>
<tr>
<td>12b</td>
<td>130.76 (118.53–144.28)</td>
</tr>
<tr>
<td>CBL</td>
<td>49.56 (44.82–54.83)</td>
</tr>
</tbody>
</table>

ᵃ P < 0.05.
ᵇ No sign of toxicity was observed.

kg⁻¹ i.p. in mice, which is less toxic than the highly toxic N-methyl-mustard (LD₅₀, 1.1 mg kg⁻¹), and nor-N-mustard (LD₅₀, 100 mg kg⁻¹) [26]. The potent doses of 7 (inhibition >30%, P < 0.05) for the S-180 were 60 and 30 mg kg⁻¹, respectively. However, compared with nor-N-mustard at equal molar dose, 7 (6 mg kg⁻¹) only exhibited 19% inhibition while nor-N-mustard showed 43.43% inhibition.

Despite the disappointing results, we still believe that desirable antitumour agents may be obtained. Thus, we have designed compound 12b, which is composed of sulfadiazine and CBL (chlorambucil). In the design of 12b, three points had been considered. First, instead of the simplest sulfonamide, sulfadiazine was selected as the targeting moiety, which had been proved to concentrate in the Yoshida sarcoma [2]. Second, sulfadiazine should be connected with CBL in an appropriate way that both the selectivity of sulfadiazine toward tumour cells and the antitumour activity of CBL are reserved. Third, CBL and 14 should be connected by an appropriate bond, so that free CBL can be released in body, preferably in the tumour cells.

According to the LD₅₀ values of 12b (130.76 mg kg⁻¹ ip in mice, 0.225 mmol kg⁻¹) and CBL (49.56 mg kg⁻¹ ip in mice, 0.163 mmol kg⁻¹), it can be concluded that the acute toxicity of 12b is lower than that of its mother compound CBL. The results of in vivo antitumour activity of 12b and CBL against murine S-180 sarcoma were listed in table III. And the antitumour activity comparison of 12b with CBL was listed in table IV (at equal toxicity) and table V (at equal molarity), respectively.

The data in tables III and IV indicate that 12b is more potent than CBL at dose of either equal toxicity or equal molarity. It is also noted that this effect is more obvious at relatively low dose range. This effect may be partly attributed to the targeting action of 12b that may lead to a relatively high drug concentration in the tumour cells. Since the active moiety of 12b is still CBL which demonstrated strong dose–effect relationship mainly at low dose range as shown in table I, the inhibition increased by 12b at relatively low dose is more obvious than that at high dose. However, compared with sulfadiazine, the concentration effect of 12b is not obvious as we expected. It may due to the hydrolysis of the ester bond in body that some free CBL had already been released before the concentration of 12b in tumour cells. The TI (therapeutic index) of 12b figured out from the data is 47.55, which is about the twice of CBL’s (TI: 22.84). Thus, 12b is much safer than its mother compound CBL when used as antitumour agents.

The rationale of the accumulation of sulfonamides in tumours is still not very clear. It was ever thought to be related to the difference in pH between tumours...
and the normal tissue [3]. It is well known, the sulfonamide group of sulfadiazine is on acid, it can react with sodium hydroxide to form the salt. On the other hand, the aromatic amine of sulfadiazine is on basic, it is easy to react with acidic compound. As we mentioned above, the early unsuccessful investigations about the sulfadiazine all focused on the modification of the aromatic amino group [3–7], the basic aromatic amine was reacted and the acidic sulfonamide was remained, it led to the loss of selectivity toward tumour cells. In our continuous study of this kind of drugs, it was noticed that the aromatic amino group of sulfadiazine should be kept unsubstituted or the selectivity will be lost [8, 11]. It may be explained by this way that the tumour cells on acid show selective affinity toward the basic aromatic amine rather than the acidic sulfonamide.

Table II. In vivo antitumour activity of (5) and (7) against murine S-180 sarcoma.

<table>
<thead>
<tr>
<th>Drug</th>
<th>Dose (mg kg(^{-1}))</th>
<th>Schedule</th>
<th>Mice In.(^{a})/Fi. (^{b})</th>
<th>Body weight In./Fi. (g)</th>
<th>Tumour WT. X ± SD (g)</th>
<th>Inhibition (%)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.2 (^{c})</td>
<td>ip × 7qd</td>
<td>20/20</td>
<td>19.7/26.5</td>
<td>2.98 ± 0.31</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>5</td>
<td>500</td>
<td>ip × 7qd</td>
<td>10/10</td>
<td>19.7/24.9</td>
<td>1.97 ± 0.23</td>
<td>33.89</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>5</td>
<td>100</td>
<td>ip × 7qd</td>
<td>10/10</td>
<td>19.9/25.7</td>
<td>2.37 ± 0.31</td>
<td>20.45</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>5-Fu</td>
<td>35</td>
<td>ip × 7qd</td>
<td>10/10</td>
<td>19.9/23.9</td>
<td>1.57 ± 0.19</td>
<td>47.32</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Control</td>
<td>0.2 (^{c})</td>
<td>ip × 7qd</td>
<td>20/20</td>
<td>20.3/26.5</td>
<td>2.21 ± 0.29</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>7</td>
<td>60</td>
<td>ip × 7qd</td>
<td>10/10</td>
<td>20.5/25.1</td>
<td>1.17 ± 0.23</td>
<td>47.06</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>7</td>
<td>30</td>
<td>ip × 7qd</td>
<td>10/10</td>
<td>20.1/25.7</td>
<td>1.37 ± 0.34</td>
<td>38.01</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>7</td>
<td>6</td>
<td>ip × 7qd</td>
<td>10/10</td>
<td>20.3/25.9</td>
<td>1.79 ± 0.28</td>
<td>19.00</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>NNM (^{d})</td>
<td>3</td>
<td>ip × 7qd</td>
<td>10/10</td>
<td>20.3/24.8</td>
<td>1.25 ± 0.31</td>
<td>43.43</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

\(^{a}\) Initial stage of experiment.  
\(^{b}\) Final stage of experiment.  
\(^{c}\) mL/mouse.  
\(^{d}\) Nor-nitrogen mustard.

Table III. In vivo antitumour activity of CBL \(^{d}\) and (12b) against murine S-180 sarcoma.

<table>
<thead>
<tr>
<th>Drug</th>
<th>Dose (mg kg(^{-1}))</th>
<th>Schedule</th>
<th>Mice In.(^{a})/Fi. (^{b})</th>
<th>Body weight In./Fi. (g)</th>
<th>Tumour WT. X ± SD (g)</th>
<th>Inhibition (%)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>CBL</td>
<td>6.8</td>
<td>ip × 7qd</td>
<td>10/9</td>
<td>19.8/18.4</td>
<td>0.50 ± 0.14</td>
<td>77.38</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>CBL</td>
<td>5</td>
<td>ip × 7qd</td>
<td>10/9</td>
<td>19.7/19.1</td>
<td>0.59 ± 0.16</td>
<td>73.30</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>CBL</td>
<td>2.5</td>
<td>ip × 7qd</td>
<td>10/10</td>
<td>20.0/21.3</td>
<td>1.04 ± 0.16</td>
<td>52.94</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>CBL</td>
<td>1.3</td>
<td>ip × 7qd</td>
<td>10/10</td>
<td>19.9/23.4</td>
<td>1.39 ± 0.22</td>
<td>37.10</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>12b</td>
<td>13</td>
<td>ip × 7qd</td>
<td>10/9</td>
<td>19.8/18.0</td>
<td>0.41 ± 0.14</td>
<td>81.45</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>12b</td>
<td>9.6</td>
<td>ip × 7qd</td>
<td>10/9</td>
<td>19.6/18.3</td>
<td>0.51 ± 0.11</td>
<td>76.92</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>12b</td>
<td>6.5</td>
<td>ip × 7qd</td>
<td>10/10</td>
<td>19.8/19.5</td>
<td>0.57 ± 0.10</td>
<td>74.21</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>12b</td>
<td>4.8</td>
<td>ip × 7qd</td>
<td>10/10</td>
<td>19.8/20.7</td>
<td>0.81 ± 0.21</td>
<td>63.35</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>12b</td>
<td>3.3</td>
<td>ip × 7qd</td>
<td>10/10</td>
<td>19.6/22.5</td>
<td>1.09 ± 0.20</td>
<td>50.68</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>12b</td>
<td>2.5</td>
<td>ip × 7qd</td>
<td>10/10</td>
<td>19.9/23.4</td>
<td>1.15 ± 0.23</td>
<td>47.96</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Control</td>
<td>0.2 (^{c})</td>
<td>ip × 7qd</td>
<td>20/20</td>
<td>20.1/25.8</td>
<td>2.21 ± 0.22</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

\(^{a}\) Initial stage of experiment.  
\(^{b}\) Final stage of experiment.  
\(^{c}\) mL/mouse.  
\(^{d}\) Chlorambucil.

Table IV. Antitumour activity comparison of 12b with CBL at dose of equal toxicity.

<table>
<thead>
<tr>
<th>Drug</th>
<th>LD(_{50}) × 1/10 (%)</th>
<th>LD(_{50}) × 1/20 (%)</th>
<th>LD(_{50}) × 1/50 (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>12b</td>
<td>81.5</td>
<td>74.2</td>
<td>50.7</td>
</tr>
<tr>
<td>CBL</td>
<td>73.3</td>
<td>52.9</td>
<td>37.1</td>
</tr>
</tbody>
</table>
Table V. Antitumour activity comparison of 12b with CBL at dose of equal molarity.

<table>
<thead>
<tr>
<th>Drug</th>
<th>0.022 mmol kg⁻¹ (%)</th>
<th>0.016 mmol kg⁻¹ (%)</th>
<th>0.008 mmol kg⁻¹ (%)</th>
<th>0.004 mmol kg⁻¹ (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>12b</td>
<td>81.5</td>
<td>76.9</td>
<td>63.4</td>
<td>47.9</td>
</tr>
<tr>
<td>CBL</td>
<td>77.4</td>
<td>73.3</td>
<td>52.9</td>
<td>37.1</td>
</tr>
</tbody>
</table>

4. Conclusions

In conclusion, we have synthesised three new compounds (5, 7, 12b) by combining sulfonamides and conventional anticancer agents in one molecule. Instead of 12b, 12a was obtained when using carbobenzyloxy as protective group. Compound 12b demonstrates high antitumour activity and is more potent and safer than its mother compound CBL. The concentration of sulfonamides in tumour cells may be related to the basicity of the aromatic amino group and the acidity of the tumour cells. This class of targeting agents may be further developed to form candidate drugs, which may have advantages over the currently available anticancer agents.

5. Experimental

5.1. Chemistry

Melting points were determined with a Thiele apparatus in open capillary tubes and are uncorrected. IR spectra were measured on a Magna-550 FTIR spectrometer. ¹H-NMR spectra were recorded on a Bruker MSL-300 spectrometer with tetramethylsilane (TMS) as the internal standard and are reported as parts-per-million (ppm). The following abbreviations are used: singlet (s), doublet (d), triplet (t), quartet (q), multiplet (m), broad (br), relative intensity (RI). Mass spectra were taken with a Perkin–Elmer 2400 Series II. Analysis indicated by the symbols of the elements were within ±0.4% of the theoretical values. Analytical thin layer chromatography was performed on precoated silica gel plates (GF-254). Sulfadiazine (SD) was purchased from Shanghai Sanwei Pharmaceutical Ltd. Co. and used directly. Solvents and reagents were purified by standard methods as necessary.

5.1.1. 1-(3-(4-Acetylaminobenzenesulfonyl)amido)-3-oxopropyl)-5-fluoropyrimidine-2,4-dione (4)

To a solution of 2 (20.2 g, 0.1 mol) and 3 (21.4 g, 0.1 mol) in DMF (180 mL), was added DCC (22 g, 0.11 mol) and DMAP (1.23 g, 0.01 mol). The mixture was stirred under nitrogen atmosphere at room temperature (r.t.) for 48 h. Glacial acetic acid (5 mL) was then added to destroy the excessive DCC and the precipitate was filtrated off. The filtrate was concentrated to 1/6 of its original volume, then poured into 250 mL water with vigorous stirring. The crude product obtained by suction filtration was dissolved in dilute aq. NaOH, and the pH value of the solution was adjusted to about 5.5 by dilute HCl. Then the precipitate was filtered off, and the pH value of the filtrate was adjusted to about 1.5. The white precipitate was collected and rinsed with water for several times, then dried to constant weight. The yield was 53.9%. m.p. 252−253 °C; IR νmax (KBr, cm⁻¹) 3429 (NH), 1707 (C=O), 1593, 1538, 1479 (phenyl); ¹H-NMR (300 MHz, DMSO-d₆) δ 2.09 (s, 3H, CH₃CO), 2.63 (t, 2H, J = 6.6 Hz, NCH₂CH₂CONH), 3.73 (t, 2H, J = 6.6 Hz, NCH₂CH₂CONH), 7.78 (d, 2H, J = 9 Hz, phenyl), 7.83 (d, 2H, J = 9 Hz, phenyl), 7.86 (d, 1H, J = 6.9 Hz, 6-H of 5-Fu), 10.42 (s, 1H, CH₂CONH), 11.78 (d, 1H, J = 5.1 Hz, CONH of 5-Fu), 12.18 (s, 1H, SO₂NH); MS (EI) m/z (RI) 399 [(M+H)⁺, 3], 257 (58), 198 (56), 130 (43), 109 (100), 43 (94). Anal. C₁₃H₁₃FN₄O₆S (C, H, N, S).

5.1.2. 1-(3-(4-Aminobenzenesulfonyl)amido)-3-oxopropyl)-5-fluoropyrimidine-2,4-dione (5)

The mixture of 4 (10 g, 25 mmol) and aq. sodium hydroxide (2.5 M, 100 mL) in a 250-mL round-bottom flask was stirred at 70−74 °C for 30 min. After the solution was cooled to the r.t., the pH value of it was adjusted to 3.5−4.0 by HCl. The precipitate was collected and rinsed with water for several times. The dry product was obtained in 78% yield. m.p. 237−238 °C; IR νmax (KBr, cm⁻¹) 3459, 3360 (NH₂), 1722, 1692 (C=O), 1593 (phenyl); ¹H-NMR (300 MHz, DMSO-d₆) δ 2.58 (t, 2H, J = 6.6 Hz, NCH₂CH₂CONH), 3.73 (t, 2H, J = 6.6 Hz, NCH₂CH₂CONH), 6.16 (s, 2H, NH₂), 6.60(d, 2H, J = 9 Hz, phenyl), 7.52 (d, 2H, J = 9 Hz, phenyl), 7.86 (d, 1H, J = 6.9 Hz, 6-H of 5-Fu), 11.76 (d, 1H, J = 5.1 Hz, CONH of 5-Fu), 11.81 (s, 1H, SO₂NH); MS (EI) m/z (RI) 356 [M⁺, 7], 156 (78), 130 (40), 109 (100), 100 (87), 44 (48). Anal. C₁₃H₁₃FN₄O₆S (C, H, N, S).
5.1.3. 4-Acetylamino-N,N-bis(2-chloroethyl)-benzenesulfonamide (6)

The solution of triethylamine (9.6 mL, 82.7 mmol) in pyridine (65 mL) was added dropwise into bis(2-chloroethyl) amine hydrochloride (12.44 g, 69.7 mmol) at 0 °C with stirring. Then p-acetamidobenzenesulfonyl chloride (17.9 g, 76.7 mmol) was added to the mixture by small portions to keep the reaction temperature not higher than 15 °C. After the addition, the mixture was stirred at 38–42 °C for 3 h, then poured into 550 mL water with stirring. The filter cake was washed with water, and dried in vacuo. White crystal was obtained by recrystallisation from toluene in 85% yield. m.p. 116–118 °C; IR \( \nu_{\text{max}} \) (KBr, cm\(^{-1}\)) 3306 (NH\(_2\)), 1677 (C=O), 1598, 1546 (phenyl), 1335 (SO\(_2\)N); \( ^{1}\text{H-NMR} \) (300 MHz, DMSO-\( d_6 \)) \( \delta \) 2.23 (s, 3H, CH\(_3\)CO), 3.47 (t, 4H, J = 6.6 Hz, NCH\(_2\)CH\(_2\)Cl), 3.68 (t, 4H, J = 6.6 Hz, NCH\(_2\)CH\(_2\)Cl), 7.70 (d, 2H, J = 5.7 Hz, phenyl), 7.74 (s, 1H, CONH), 7.72 (d, 2H, J = 5.7 Hz, phenyl); MS (EI) \( m/z \) (RI) 339 [M+H\(^+\)], 319 (82), 211 (100), 185 (29), 108 (12), 91 (77), 79 (14). Anal. C\(_{18}\)H\(_{16}\)N\(_2\)O\(_3\)S (C, H, N, S).

5.1.4. 4-Amino-N,N-bis(2-chloroethyl)benzenesulfonamide hydrochloride (7)

The mixture of 6 (10 g, 29.5 mmol) and hydrochloric acid (6 M, 100 mL) was refluxed for 40 min. Then it was cooled to r.t. and kept for 10 h. The crystal was obtained in 92% yield. IR \( \nu_{\text{max}} \) (KBr, cm\(^{-1}\)) 3380, 2618 (ArNH\(_2\)Cl), 1593, 1498 (phenyl), 1341(SO\(_2\)N); \( ^{1}\text{H-NMR} \) (300 MHz, DMSO-\( d_6 \)) \( \delta \) 3.39 (t, 4H, J = 6.9 Hz, NCH\(_2\)CH\(_2\)Cl), 3.70 (t, 4H, J = 6.9 Hz, NCH\(_2\)CH\(_2\)Cl), 5.83 (br, s, 3H, NH\(_2\)), 6.88 (d, 2H, J = 8.7 Hz, phenyl), 7.59 (d, 2H, J = 8.7 Hz, phenyl); MS (EI) \( m/z \) (RI) 296 [M–HCl, 8], 247 (26), 156 (100), 108 (27), 92 (36), 65 (19). Anal. C\(_{16}\)H\(_{15}\)Cl\(_2\)N\(_2\)O\(_2\)S (C, H, N, S).

5.1.5. N\(^{4}\)-2-Pyrimidyl-(p-benzyloxy carbonyl)aminobenzenesulfonamide (8a)

To a stirred solution of sulfadiazine (25 g, 0.1 mol) in pyridine (300 mL) was added dropwise benzyl chloroformate (20.5 g, 0.12 mol) at 0–5 °C. The reaction mixture was stirred for 8 h at r.t., then the solution was concentrated to about 40 mL and poured into excessive water. The precipitate was washed with 1 M HCl and water successively, then dried to constant weight. Recrystallisation from acetonitrile yielded the title compound 8a (32.6 g, 85%) as a white solid. m.p. 219–220 °C; IR \( \nu_{\text{max}} \) (KBr, cm\(^{-1}\)) 3399 (NH), 1732 (C=O), 1588 (phenyl), 1507 (phenyl), 1225 (SO\(_2\)N); \( ^{1}\text{H-NMR} \) (300 MHz, DMSO-\( d_6 \)) \( \delta \) 5.17 (s, 2H, ArCH\(_2\)OH), 7.04 (t, 1H, J = 4.8 Hz, pyrimidinyl), 7.39 (m, 5H, phenyl), 7.62 (d, 2H, J = 7.2 Hz, phenyl), 7.90 (d, 2H, J = 7.2 Hz, phenyl), 8.50 (d, 2H, J = 4.8 Hz, pyrimidinyl), 10.26 (s, 1H, ArNHCO), 11.69 (br s, 1H, SO\(_2\)NH); MS (EI) \( m/z \) (RI) 385 (M+H\(^+\)), 319 (82), 211 (100), 185 (29), 108 (12), 91 (77), 79 (14). Anal. C\(_{18}\)H\(_{16}\)N\(_2\)O\(_3\)S (C, H, N, S).

5.1.6. N\(^{1}\)-2-Pyrimidyl-(p-benzyloxy carbonyl)aminobenzenesulfonamide sodium salt (9a)

To an aq. solution of sodium hydroxide (0.2 g in 200 mL), 8a (19.2 g, 0.05 mol) was added, the solution was stirred and gently warmed until all of 8a was dissolved and the pH of the solution dropped to about 8. After filtration the filtrate was condensed by rotary evaporation to a paste. The residue was purified by recrystallisation in 95% EtOH, and 9a (17.3 g, 85%) was obtained as a white solid. IR \( \nu_{\text{max}} \) (KBr, cm\(^{-1}\)) 1701 (C=O), 1603 (phenyl), 1532 (phenyl), 1236 (SO\(_2\)N); \( ^{1}\text{H-NMR} \) (300 MHz, DMSO-\( d_6 \)) \( \delta \) 5.17 (s, 2H, ArCH\(_2\)OH), 6.39 (t, 1H, J = 4.8 Hz, pyrimidinyl), 7.39 (m, 5H, phenyl), 7.62 (d, 2H, J = 7.2 Hz, phenyl), 7.90 (d, 2H, J = 7.2 Hz, phenyl), 8.50 (d, 2H, J = 4.8 Hz, pyrimidinyl), 10.26 (s, 1H, ArNHCO).

5.1.7. N\(^{1}\)-2-Pyrimidyl-(p-acetylaminobenzene sulphonamide sodium salt (9b)

According to the procedure for the synthesis of 9a, 9b was prepared in 80% yield. IR \( \nu_{\text{max}} \) (KBr, cm\(^{-1}\)) 3361 (NH), 1665 (C=O), 1588, 1532, 1445 (phenyl), 1236 (SO\(_2\)N); \( ^{1}\text{H-NMR} \) (300 MHz, DMSO-\( d_6 \)) \( \delta \) 2.04 (s, 3H, CH\(_3\)C=O), 6.37 (t, 1H, J = 4.8 Hz, pyrimidinyl), 7.52 (d, 2H, J = 8.7 Hz, phenyl), 7.68 (d, 2H, J = 8.7 Hz, phenyl), 8.08 (d, 2H, J = 4.8 Hz, pyrimidinyl), 10.05 (s, 1H, ArNHCO=O).

5.1.8. 2-[N\(^{4}\)-2-Pyrimidinyl-(p-benzyloxy carbonyl)aminobenzenesulfonamido] ethanol (10a)

To a solution of 9a (4.07 g, 10 mmol) in DMF (30 mL), was added 2-bromoethanol (1.25 g, 10 mmol). Then the solution was heated to 80 °C with stirring and maintained at this temperature for 8 h. The reaction mixture was concentrated to about 10 mL and poured into 200 mL water. Recrystallisation of the precipitate from acetonitrile yielded 10a (2.1 g, 43%) as a white solid. m.p. 200–201 °C; IR \( \nu_{\text{max}} \) (KBr, cm\(^{-1}\)) 3489 (OH), 3305 (NH), 1737 (C=O), 1607, 1588, 1503 (phenyl), 1206 (SO\(_2\)N); \( ^{1}\text{H-NMR} \) (300 MHz, DMSO-\( d_6 \)) \( \delta \) 3.73 (t, 2H, J = 4.5 Hz, CH\(_2\)CH\(_2\)OH), 4.12 (t, 2H, J = 4.5 Hz, CH\(_2\)CH\(_2\)OH), 5.04 (t, 1H, J = 4.5 Hz,
CH₂CH₂OH), 5.16 (s, 2H, ArCH₂O), 6.80 (dd, 1H, J = 2.4 Hz, J = 4.2 Hz, pyrimidinyl), 7.39 (m, 5H, phenyl), 7.53 (d, 2H, J = 9 Hz, phenyl), 7.70 (d, 2H, J = 9 Hz, phenyl), 8.27 (dd, 1H, J = 2.4 Hz, J = 4.2 Hz, pyrimidinyl), 8.59 (q, 1H, J = 2.4 Hz, pyrimidinyl), 9.08 (s, 1H, ArNHC=O). Anal. C₂₃H₂₇Cl₂N₅O₆S (C, H, N, S).

5.1.11. 2-[N'-2-(1,4,5,6-Tetrahydropyrimidyl)- (p-benzoxycarbonyl) aminobenzenesulfonamido] ethyl 4-bis-(2-chloroethyl) aminophenyl butyrate (12a)

To a stirred suspension of 11a (2 g, 2.8 mmol) and 10% Pd–C (2 g) in chloroform (60 mL), cyclohexene (5 mL) was added under nitrogen. The reaction mixture was refluxed for 18 h. Then the catalyst was removed by filtration. The filtrate was concentrated under reduced pressure, and the residue was purified by column chromatography over silica gel (dichloromethane–chloroform, 1:1) to give 12a (1.8 g, 90%) as a yellow solid. m.p. 96–98 °C; ¹H-NMR (300 MHz, acetone-d₆) δ 1.69 (m, 2H, CH₂CH₂CH₂), 1.87 (m, 2H, CH₂CH₂CH₂), 2.31 (t, 2H, J = 7.2 Hz, O–C–H(CH₂CH₂), 2.56 (t, 2H, J = 7.2 Hz, CH₂CH₂CH₂Ph), 2.87 (m, 4H, NCH₂CH₂), 3.62 (t, 2H, J = 2.4 Hz, NCH₂CH₂), 3.78 (m, 8H, N(CH₂CH₂CH₂), 4.22 (t, 2H, J = 2.4 Hz, NCH₂CH₂), 5.25 (2H, PhCH₂O), 6.77 (d, 2H, J = 7.5 Hz, phenyl), 7.12 (d, 2H, J = 7.5 Hz, phenyl), 7.44 (m, 5H, phenyl), 7.75 (d, 2H, J = 7.2 Hz, phenyl), 7.79 (s, 1H, NH), 7.83 (dd, 2H, J = 7.2 Hz, phenyl), 9.14 (s, 1H, ArNHC=O); MS (EI) m/z (RI) 718 [M⁺, 4], 305 (9), 303 (13), 256 (37), 254 (100), 118 (20), 91 (22), 79 (19). Anal. C₃₄H₄₁Cl₂N₅O₆S (C, H, N, S).

5.1.12. 2-(N'-2-Pyrimidinyl-aminobenzenesulfonamido) ethanol (13)

In a 150 mL round-bottom flask provided with reflux condenser, a mixture of 10b (10 g, 30 mmol) and hydrochloric acid (1.2 M, 60 mL) was refluxed for about 1 h. When the solution obtained was cooled to r.t., aq. NaOH was added to neutralise the solution. The precipitate was isolated and washed with water, then dried in vacuo to yield 13 (7.8 g, 88%) as an orange solid. m.p. 224–226 °C; IR νmax (KBr, cm⁻¹) 3448 (OH), 3351, 3249 (NH₂), 1599, 1507 (phenyl), 1241 (SO₂N); δ₁H (300 MHz, DMSO-d₆) 3.73 (t, 2H, J = 4.5 Hz, CH₂CH₂OH), 4.07 (t, 2H, J = 4.5 Hz, CH₂CH₂OH), 5.02 (t, 1H, J = 5.4 Hz, CH₂CH₂OH), 5.66 (br s, 2H, NH₂), 6.51 (d, 2H, J = 8.7 Hz, phenyl), 6.73 (dd, 1H, J = 2.4 Hz, J = 4.2 Hz, pyrimidinyl), 7.51 (d, 2H, J = 8.7 Hz, phenyl), 8.23 (dd, 1H, J = 2.4 Hz, J = 4.2 Hz, pyrimidinyl), 8.38 (q, 1H, J = 2.4 Hz, pyrimidinyl); MS (EI) m/z (RI) 295 ([M+H⁺, 15), 172 (60), 156 (100), 108 (29), 108 (83), 92 (81), 65 (68). Anal. C₁₂H₁₄N₂O₄S (C, H, N, S).
5.1.13. 2-[N¹-2-Pyrimidinyl-(p-benzyldiene)amino-benzenesulfonamido] ethanol (14)

To 40 mL freshly distilled benzaldehyde in 100 mL round-bottom flask, was added 13 (10 g, 34 mmol). Then the mixture was heated to about 100 °C, and maintained at this temperature under nitrogen atmosphere for about 6 h until the mixture became a clear solution. The solution was cooled to r.t., and kept at refrigerator for 3 h. The crude product collected by suction filter was washed with small portion of cold EtOH for twice, then dried in vacuo to give 14 (10.8 g, 83%) as a yellow solid. m.p. 187 °C; IR νmax (KBr, cm⁻¹) 3428 (NH₂), 1735 (C=O), 1593, 1546, 1514 (phenyl), 1348 (SO₂N); ¹H-NMR (300 MHz, CDCl₃) δ 1.81 (m, 2H, CH₂CH₂), 4.23 (t, 2H, NCH₂C), 3.77 (t, 2H, C=CH₂), 2.32 (t, 2H, CH₂CH₂OH), 4.15 (t, 2H, CH₂CH₂), 5.06 (t, 1H, CH₂CH₂O), 2.50 (t, 2H, CH₂CH₂), 7.04 (d, 2H, phenyl), 6.64 (dd, 4H, J₂H₂ = 2.4 Hz, pyrimidyl); 13C-NMR δ 130.11, 131.68, 144.38, 149.57, 154.06, 164.08, 172.71.

5.1.14. 2-[N¹-2-Pyrimidinyl-(p-benzyldiene)amino-benzenesulfonamido] ethyl 4-bis-(chloroethyl)aminophenyl butyrate (11b)

According to the procedure for the synthesis of 11a, 11b was prepared in the yield of 65%. m.p. 141–142 °C; ¹H-NMR (300 MHz, acetone-d₆) δ 1.83 (m, 2H, CH₂CH₂), 2.32 (t, 2H, J = 7.2 Hz, O–CCH₂–CH₃), 2.50 (t, 2H, J = 7.2 Hz, CH₂CH₂CH₂Ph), 3.80 (m, 8H, N(CH₂CH₂Cl)₂), 4.51 (t, 2H, J = 2.1 Hz, NCH₂CH₂O), 4.59 (t, 2H, J = 2.1 Hz, NCH₂CH₂O), 6.74 (d, 2H, J = 8.7 Hz, phenyl), 6.79 (dd, 1H, J = 2.4 Hz, J = 4.2 Hz, pyrimidyl), 7.04 (d, 2H, J = 8.7 Hz, phenyl), 7.32 (d, 2H, J = 8.7 Hz, phenyl), 7.59 (m, 3H, phenyl), 8.02 (d, 2H, J = 3 Hz, phenyl), 8.07 (d, 2H, J = 3 Hz, phenyl), 3.83 (dd, 1H, J = 2.4 Hz, J = 4.2 Hz, pyrimidyl), 8.60 (s, 1H, ArCH=NR), 8.62 (q, 1H, J = 2.4 Hz, pyrimidyl). Anal. C₃₅H₃₃Cl₂N₂O₄S (C, H, N, S).

5.1.15. 2-[N¹-2-Pyrimidyl-amino-benzenesulfonamido] ethyl 4-bis-(chloroethyl) aminophenyl butyrate (12b)

A mixture of hydrochloric acid (1 M, 10 mL) and 11b (1 g) was stirred at r.t. for 10 h, then was neutralised by aq. sodium hydroxide (1 M). The precipitate was separated by filtration and rinsed with water, then dried in vacuo. Compound 12b (0.43 g, 53%) was obtained as a white solid. m.p. 97–98 °C; IR νmax (KBr, cm⁻¹) 3469, 3348 (NH₂), 1735 (C=O), 1593, 1546, 1514 (phenyl), 1272 (SO₂N); ¹H-NMR (300 MHz, CDCl₃) δ 1.81 (m, 2H, CH₂CH₂), 2.23 (t, 2H, J = 7.2 Hz, O–CCH₂–CH₂), 2.53 (t, 2H, J = 7.2 Hz, CH₂CH₂CH₂Ph), 3.60 (t, 4H, J = 6.0 Hz, N(CH₂CH₂Cl)₂), 3.70 (t, 4H, J = 6.0 Hz, N(CH₂CH₂Cl)₂), 4.25 (t, 2H, J = 4.5 Hz, NCH₂CH₂O), 4.43 (t, 2H, J = 4.5 Hz, NCH₂CH₂O), 5.64 (br s, 2H, NH₂), 6.46 (dd, 1H, J = 2.4 Hz, phenyl), 7.00 (d, 2H, J = 6.9 Hz, phenyl), 7.66 (dd, 1H, J = 2.4 Hz, J = 4.2 Hz, pyrimidyl), 7.81 (d, 2H, J = 6.9 Hz, phenyl), 8.58 (q, 1H, J = 2.4 Hz, pyrimidyl); ¹³C-NMR (75 MHz, CDCl₃) δ 26.27, 33.07, 33.71, 40.56, 52.93, 53.51, 60.14, 106.85, 112.19, 113.51, 129.28, 129.57, 130.11, 131.68, 144.38, 149.57, 154.06, 164.08, 172.71. Anal. C₂₅H₂₃Cl₂N₂O₂S (C, H, N, S).

5.2. Biological studies

5.2.1. Animal studies

TA1 mice (propagated at the animal supply centre of the Shanghai Institute of Pharmaceutical Industry), 6–8 weeks age, weighing 18–20 g of either sex were used. They were maintained under controlled temperature and humidity with sterile bedding and food and water ad libitum.

5.2.2. Toxicity assessment

For the assessment of the acute toxicity, compounds were injected ip×1 into the TA1 mice at five different dose levels on day 0. Then the behaviour and the death distribution of the test mice were recorded. The highest death rate appeared on day 1 and the condition of the survivals was good after 2 weeks. LD₅₀ was calculated by using the Bliss method.

5.2.3. In vivo antitumour activity

The mouse solid tumour S-180 cell line was maintained by intraperitoneal passage at weekly intervals in male TA1 mice. In experiments with solid S-180 cells, 1×10⁶ cells in 0.2 mL were injected intraperitoneal into the right axilla of TA1 mice (10 mice each group) on day 0. The test compound was suspended in 0.5% sodium carboxymethyl cellulose containing 3.5% Tween 80 and was administered intraperitoneal with a consecutive (qd×7) schedule at various doses from day 1. Control mice were given saline alone with the same schedule. The mice were weighed twice a week to monitor the toxic effects. The mice were killed on day 14, and
the tumours were removed and weighed. The antitumour activity was evaluated as the percentage of the tumour weight inhibition (TWI) compared with the mean tumour weight in the control group, according to the following formula.

\[
TWI\% = (1 - \frac{T}{C}) \times 100\%
\]

\( T \) and \( C \) represent the mean tumour weight in the test group and the control group, respectively.

5.2.4. Statistical analyses

Results are expressed as the mean±SD. The significance of difference between groups and/or drugs was assessed by using Student’s \( t \)-test. \( P<0.05 \) was taken as significant.

References