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Original article

A novel kind of antitumour drugs using sulfonamide as parent compound

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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-[N^1-2-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-[N^1-2-pyrimidinyl-(*p*-acetyl)aminobenzenesulfonamido] ethanol (**10b**) afforded 2-[N^1-2-pyrimidinyl-(*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*, ^{14}C 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 sulfadi-

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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.

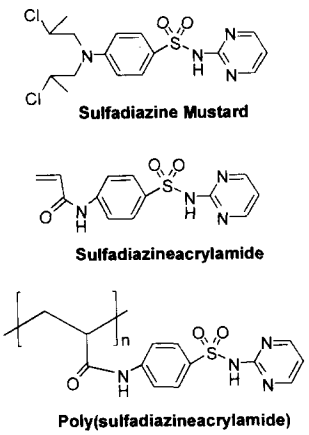

Figure 1. ^{14}C labelled sulfadiazine derivatives.

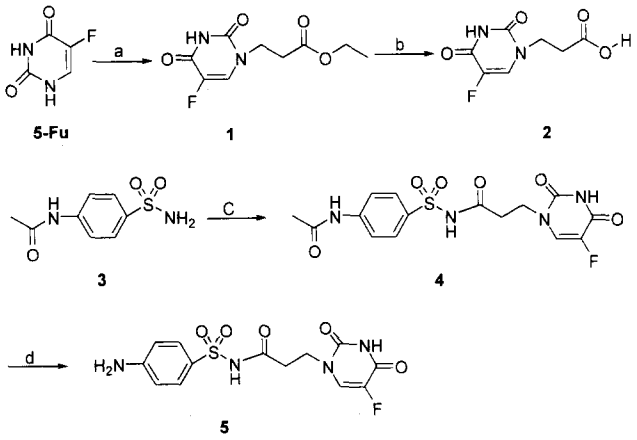

Figure 2. *Reagents and conditions*: (a) Na (catalytic amount), ethyl acrylate, ethanol, reflux, 56 h; (b) HCl (1.2 M), reflux, 1 h; (c) **2** (1.0 equivalent), DCC (1.1 equivalent), DMAP (0.1 equivalent), pyridine, r.t., 48 h; (d) NaOH (2.5 M), 70–74 °C, 0.5 h.

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 ^{1}H-NMR analyses. Compound **4** was obtained by the coupling of *p*-acetamidobenzenesulfonamide (**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*-acetamidobenzenesulfonyl 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

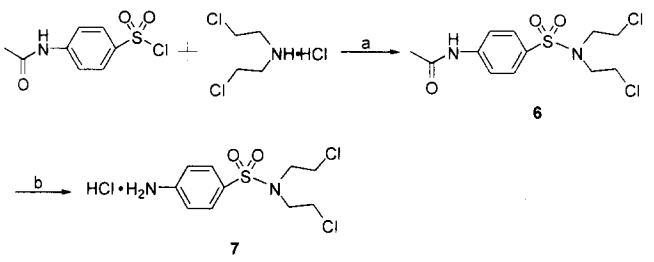

Figure 3. *Reagents and conditions*: (a) Et_3N (1.2 equivalent), pyridine, 0–40 °C, 3 h; (b) HCl (6 M), reflux, 40 min.

10b were synthesised according to the synthetic procedures for **9a** and **10a**, respectively. And the acetyl group was removed by refluxing **10a** in HCl (1.2 M) for about 1 h. The reaction of **13** with benzaldehyde afforded Schiff's base protected compound **14**. Compound **11b** was prepared according to the procedure of **11a**. Compound **12b** was obtained by the hydrolysis of **11b** in hydrochloric acid at room temperature. In this route, two different protective groups were applied, the acetyl and the Schiff's base. Acetyl as well as Cbz is stable under all the reaction conditions, but cannot be removed without destroying the target molecule. Schiff's base can be removed under mild condition that would not break the ester bond in the 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 sufonamides in one molecule. With this in mind, we had designed **5** and **7** at first, which contain the simplest sufonamide 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

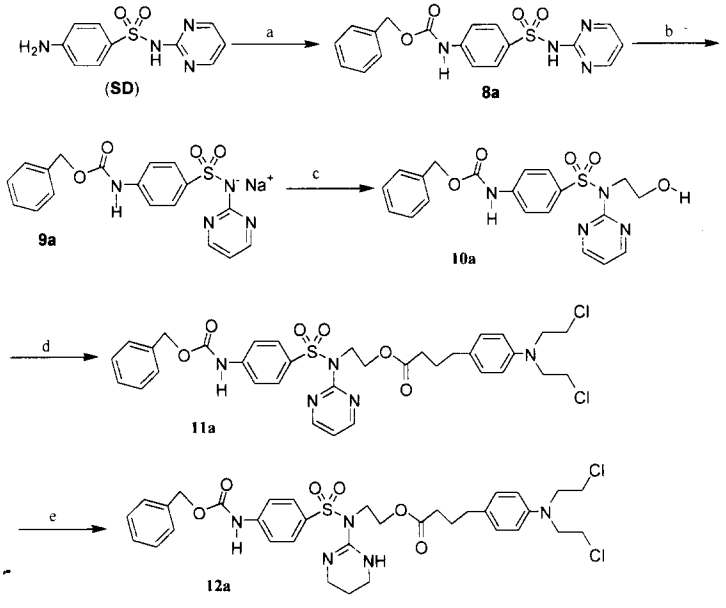

Figure 4. *Reagents and conditions*: (a) benzyl chloroformate, pyridine, 0 °C, 8 h; (b) NaOH (aq., 1.0 equivalent); (c) DMF, $BrCH_2CH_2OH$ (1.0 equivalent), 80 °C, 8 h; (d) chlorambucil (1.0 equivalent), DCC (1.1 equivalent), pyridine, r.t., 48 h; (e) $CHCl_3$, cyclohexene (excessive), 10% Pd–C, reflux, 18 h.

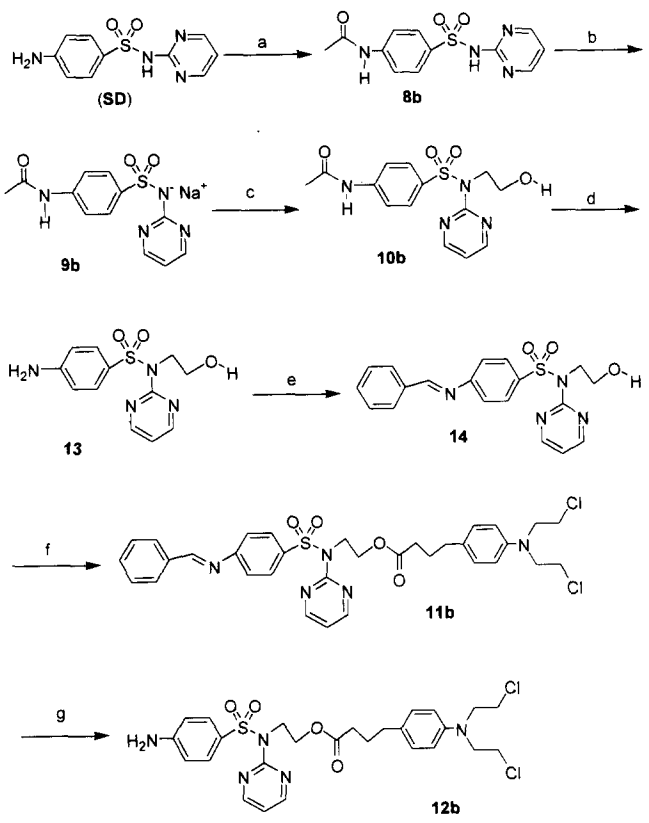

Figure 5. *Reagents and conditions*: (a) acetyl chloride, pyridine, 0 °C, 2 h; (b) NaOH (aq., 1.0 equivalent); (c) DMF, $BrCH_2CH_2OH$ (1.0 equivalent), 80 °C, 8 h; (d) HCl (1.2 M), reflux, 1 h; (e) benzaldehyde, 100 °C, 6 h; (f) chlorambucil (1.0 equivalent), DCC (1.1 equiv.), pyridine, r.t., 48 h; (g) HCl (1 M), r.t., 10 h.

Table I. LD_{50} of the test compounds (ip in mice).

Compound	LD_{50} (mg kg^{-1}) [a]
5	>2000 [b]
7	281.28 (253.45–312.17)
12b	130.76 (118.53–144.28)
CBL	49.56 (44.82–54.83)

[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 (LD_{50}, 1.1 mg kg^{-1}), and nor-*N*-mustard (LD_{50}, 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, sufadiazine 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 LD_{50} 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

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

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

[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.

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

[a] Initial stage of experiment.
[b] Final stage of experiment.
[c] mL/mouse.
[d] Chlorambucil.

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

Drug	$LD_{50} \times 1/10$ (%)	$LD_{50} \times 1/20$ (%)	$LD_{50} \times 1/50$ (%)
12b	81.5	74.2	50.7
CBL	73.3	52.9	37.1

Table V. Antitumour activity comparison of **12b** with CBL at dose of equal molarity.

Drug	0.022 mmol kg^{-1} (%)	0.016 mmol kg^{-1} (%)	0.008 mmol kg^{-1} (%)	0.004 mmol kg^{-1} (%)
12b	81.5	76.9	63.4	47.9
CBL	77.4	73.3	52.9	37.1

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. ^{1}H-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 HP5989A. Elemental analyses for C, H, N and S were determined on 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-Acetylaminobenzenesulfonamido)-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 filtered off. The filtrate was concentrate 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 ν_{max} (KBr, cm^{-1}) 3429 (NH), 1707 (C=O), 1593, 1538, 1479 (phenyl); ^{1}H-NMR (300 MHz, DMSO-d_6) δ 2.09 (s, 3H, CH_3C=O), 2.63 (t, 2H, $J = 6.6$ Hz, NCH_2CH_2CONH), 3.73 (t, 2H, $J = 6.6$ Hz, NCH_2CH_2CONH), 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, CH_3CONH), 11.78 (d, 1H, $J = 5.1$ Hz, CONH of 5-Fu), 12.18 (s, 1H, SO_2NH); MS (EI) *m/z* (RI) 399 ($[M+H]^+$, 3), 257 (58), 198 (56), 130 (43), 109 (100), 43 (94). Anal. $C_{15}H_{15}FN_4O_6S$ (C, H, N, S).

5.1.2. 1-(3-(4-Aminobenzenesulfonamido)-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 ν_{max} (KBr, cm^{-1}) 3459, 3360 (NH_2), 1722, 1692 (C=O), 1593 (phenyl); ^{1}H-NMR (300 MHz, DMSO-d_6) δ 2.58 (t, 2H, $J = 6.6$ Hz, NCH_2CH_2CONH), 3.73 (t, 2H, $J = 6.6$ Hz, NCH_2CH_2CONH), 6.16 (s, 2H, NH_2), 6.60(d, 2H, $J = 9$ Hz, phenyl), 7.52 (d, 2H, $J = 9$ Hz, phenyl), 7.86 (d, 1H, $J = 6.6$ Hz, 6-H of 5-Fu), 11.76 (d, 1H, $J = 5.1$ Hz, CONH of 5-Fu), 11.81 (s, 1H, SO_2NH); MS (EI) *m/z* (RI) 356 [M^+, 7], 156 (78), 130 (40), 109 (100), 100 (87), 44 (48). Anal. $C_{13}H_{13}FN_4O_5S$ (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 ν_{max} (KBr, cm^{-1}) 3306 (NH_2), 1677 (C=O), 1598, 1546 (phenyl), 1335 (SO_2N); ^{1}H-NMR (300 MHz, DMSO-d_6) δ 2.23 (s, 3H, CH_3CO), 3.47 (t, 4H, $J = 6.6$ Hz, NCH_2CH_2Cl), 3.68 (t, 4H, $J = 6.6$ Hz, NCH_2CH_2Cl), 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^+, 22], 289 (64), 198 (100), 134 (27), 43 (30). Anal. $C_{12}H_{16}Cl_2N_2O_3S$ (C, H, N, S).

5.1.4. 4-Amino-N,N-bis(2-chloroethyl)benzene-sulfonamide 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 ν_{max} (KBr, cm^{-1}) 3380, 2618 ($ArNH_3Cl$), 1593, 1498 (phenyl), 1341(SO_2N); ^{1}H-NMR (300 MHz, DMSO-d_6) δ 3.39 (t, 4H, $J = 6.9$ Hz, NCH_2CH_2Cl), 3.70 (t, 4H, $J = 6.9$ Hz, NCH_2CH_2Cl), 5.83 (br, s, 3H, NH_3), 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. $C_{10}H_{15}Cl_3N_2O_2S$ (C, H, N, S).

5.1.5. N^1-2-Pyrimidyl-(p-benzyloxycarbonyl)amino-benzenesulfonamide (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 ν_{max} (KBr, cm^{-1}) 3399 (NH), 1732 (C=O), 1588 (phenyl), 1507 (phenyl), 1225 (SO_2N); ^{1}H-NMR (300 MHz, DMSO-d_6) δ 5.17 (s, 2H, $ArCH_2O$), 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_2NH); MS (EI) *m/z* (RI) 385 ([M+H]$^+$, 8), 319 (82), 211 (100), 185 (29), 108 (12), 91 (77), 79 (14). Anal. $C_{18}H_{16}N_4O_4S$ (C, H, N, S).

5.1.6. N^1-2-Pyrimidyl-(p-benzyloxycarbonyl)amino-benzenesulfonamide 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 ν_{max} (KBr, cm^{-1}) 1701 (C=O), 1603 (phenyl), 1532 (phenyl), 1236 (SO_2N); ^{1}H-NMR (300 MHz, DMSO-d_6) δ 5.17 (s, 2H, $ArCH_2O$), 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-acetyl)aminobenzene-sulfonamide sodium salt (9b)

According to the procedure for the synthesis of **9a**, **9b** was prepared in 80% yield. IR ν_{max} (KBr, cm^{-1}) 3361 (NH), 1665 (C=O), 1588, 1532, 1445 (phenyl), 1236 (SO_2N); ^{1}H-NMR (300 MHz, DMSO-d_6) δ 2.04 (s, 3H, CH_3C=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, pyrimidyl), 10.05 (s, 1H, ArNHC=O).

5.1.8. 2-[N^1-2-Pyrimidinyl-(p-benzyloxycarbonyl)amino-benzenesulfonamido] 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 ν_{max} (KBr, cm^{-1}) 3489 (OH), 3305 (NH), 1737 (C=O), 1607, 1588, 1503 (phenyl), 1206 (SO_2N); ^{1}H-NMR (300 MHz, DMSO-d_6) δ 3.73 (t, 2H, $J = 4.5$ Hz, CH_2CH_2OH), 4.12 (t, 2H, $J = 4.5$ Hz, CH_2CH_2OH), 5.04 (t, 1H, $J = 4.5$ Hz,

CH_2CH_2OH), 5.16 (s, 2H, $ArCH_2O$), 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), 10.10 (s, 1H, ArNHCO); MS (EI) *m/z* (RI) 429 ($[M+H]^+$, 1), 333 (17), 108 (82), 91 (100), 79 (77). Anal. $C_{20}H_{20}N_4O_5S$ (C, H, N, S).

*5.1.9. 2-[N¹-2-Pyrimidinyl-(p-acetyl)aminobenzenesulfonamido] ethanol (**10b**)*

According to the procedure for the synthesis of **10a**, **10b** was prepared in the yield of 41%. White solid, m.p. 239–240 °C; IR ν_{max} (KBr, cm^{-1}) 3379 (br, OH, NH), 1697 (C=O), 1587, 1545, 1514 (phenyl), 1263 (SO_2N); ^{1}H-NMR (300 MHz, DMSO-d_6) δ 2.08 (s, 3H, CH_3C=O), 3.75 (t, 2H, $J = 4.5$ Hz, CH_2CH_2OH), 4.12 (t, 2H, $J = 4.5$ Hz, CH_2CH_2OH), 5.05 (t, 1H, $J = 4.8$ Hz, CH_2CH_2OH), 6.80 (dd, 1H, $J = 2.4$ Hz, $J = 4.2$ Hz, pyrimidinyl), 7.64 (d, 2H, $J = 7.2$ Hz, phenyl), 7.78 (d, 2H, $J = 7.2$ Hz, phenyl), 8.29 (dd, 1H, $J = 2.4$ Hz, $J = 4.2$ Hz, pyrimidyl), 8.60 (q, 1H, $J = 2.4$ Hz, pyrimidyl), 10.19 (s, 1H, ArNHC=O); MS (EI) *m/z* (RI) 337 ($[M+H]^+$, 23), 214 (29), 198 (78), 172 (96), 156 (71), 110 (100), 97 (72), 43 (95). Anal. $C_{14}H_{16}N_4O_4S$ (C, H, N, S).

*5.1.10. 2-[N¹-2-Pyrimidyl-(p-benzyloxycarbonyl) aminobenzenesulfonamido] ethyl 4-bis(2-chloroethyl) aminophenyl butyrate (**11a**)*

To a stirred solution of chlorambucil (0.3 g, 1 mmol) and **10a** (0.43 g, 1 mmol) in anhydrous pyridine (5 mL) was added DCC (0.22 g, 1.05 mmol) and catalytic amount of DMAP. The reaction was maintained at r.t. for 24 h before the precipitate was filtered off. Then the filtrate was poured into excessive water (80 mL). The precipitate was purified by recrystallization from acetone–ethanol (1:3/v:v). **11a** (0.46 g, 65%) was obtained as a white solid. m.p. 173–174 °C; IR ν_{max} (KBr, cm^{-1}) 3348 (NH), 1735 (C=O), 1593, 1546, 1514 (phenyl), 1272 (SO_2N); ^{1}H-NMR (300 MHz, acetone-d_6) δ 1.85 (m, 2H, $CH_2CH_2CH_2$), 2.32 (t, 2H, $J = 7.2$ Hz, O=C$CH_2CH_2CH_2$), 2.51 (t, 2H, $J = 7.2$ Hz, $CH_2CH_2CH_2Ph$), 3.81 (m, 8H, $N(CH_2CH_2Cl)_2$), 4.47 (t, 2H, $J = 2.4$ Hz, NCH_2CH_2O), 4.55 (t, 2H, $J = 2.4$ Hz, NCH_2CH_2O), 5.23 (s, 2H, $PhCH_2O$), 6.76 (d, 2H, $J = 8.7$ Hz, phenyl), 6.79 (dd, 1H, $J = 2.4$ Hz, $J = 4.2$ Hz, pyrimidinyl), 7.06 (d, 2H, $J = 8.7$ Hz, phenyl), 7.46 (m, 5H, phenyl), 7.68 (d, 2H, $J = 6.6$ Hz, phenyl), 7.95 (d, 2H, $J = 6.6$ Hz, phenyl), 8.34 (dd, 1H, $J = 2.4$ Hz, $J = 4.2$ Hz, pyrimidyl), 8.59 (q, 1H, $J = 2.4$ Hz, pyrimidyl), 9.08 (s, 1H, ArNHC=O). Anal. $C_{34}H_{37}Cl_2N_5O_6S$ (C, H, N, S).

*5.1.11. 2-[N¹-2-(1,4,5,6-Tetrahydropyrimidyl)-(p-benzyloxycarbonyl) 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; ^{1}H-NMR (300 MHz, acetone-d_6) δ 1.69 (m, 2H, $CH_2CH_2CH_2$), 1.87 (m, 2H, $CH_2CH_2CH_2$), 2.31 (t, 2H, $J = 7.2$ Hz, O=C$CH_2CH_2CH_2$), 2.56 (t, 2H, $J = 7.2$ Hz, $CH_2CH_2CH_2Ph$), 2.87 (m, 4H, NCH_2CH_2), 3.62 (t, 2H, $J = 2.4$ Hz, NCH_2CH_2O), 3.78 (m, 8H, $N(CH_2CH_2Cl)_2$), 4.22 (t, 2H, $J = 2.4$ Hz, NCH_2CH_2O), 5.25 (s, 2H, $PhCH_2O$), 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. $C_{34}H_{41}Cl_2N_5O_6S$ (C, H, N, S).

*5.1.12. 2-(N¹-2-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 ν_{max} (KBr, cm^{-1}) 3448 (OH), 3351, 3249 (NH_2), 1599, 1507, (phenyl), 1241 (SO_2N); δ_H (300 MHz, DMSO-d_6) 3.73 (t, 2H, $J = 4.5$ Hz, CH_2CH_2OH), 4.07 (t, 2H, $J = 4.5$ Hz, CH_2CH_2OH), 5.02 (t, 1H, $J = 5.4$ Hz, CH_2CH_2OH), 5.66 (br s, 2H, NH_2), 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, pyrimidyl), 8.58 (q, 1H, $J = 2.4$ Hz, pyrimidyl); MS (EI) *m/z* (RI) 295 ($[M+H]^+$, 15), 172 (60), 156 (100), 108 (29), 108 (83), 92 (81), 65 (68). Anal. $C_{12}H_{14}N_4O_3S$ (C, H, N, S).

*5.1.13. 2-[N¹-2-Pyrimidinyl-(p-benzylidene)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. 186–187 °C; IR ν_{max} (KBr, cm^{-1}) 3428 (OH), 1624 (CH=NAr), 1599, 1547, 1512 (phenyl), 1266 (SO_2N); ^{1}H-NMR (300 MHz, DMSO-d_6) δ 3.77 (t, 2H, CH_2CH_2OH), 4.15 (t, 2H, CH_2CH_2OH), 5.06 (t, 1H, CH_2CH_2OH), 6.84 (dd, 1H, pyrimidinyl), 7.30 (d, 2H, phenyl), 7.58 (m, 3H, phenyl), 7.89 (d, 2H, phenyl), 7.95 (dd, 2H, phenyl), 8.32 (dd, 1H, pyrimidyl), 8.61 (dd, 1H, pyrimidyl), 8.63 (s, 1H, ArCH=N); MS (EI) *m/z* (RI) 382 [M^+, 6], 348 (19), 279 (46), 260 (100), 244 (38), 196 (82), 180 (85), 152 (55), 109 (31). Anal. $C_{19}H_{18}N_4O_3S$ (C, H, N, S).

*5.1.14. 2-[N¹-2-Pyrimidinyl-(p-benzylidene)amino-benzenesulfonamido] ethyl 4-bis(2-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; ^{1}H-NMR (300 MHz, acetone-d_6) δ 1.83 (m, 2H, $CH_2CH_2CH_2$), 2.32 (t, 2H, $J=7.2$ Hz, O=CCH_2-CH_2CH_2), 2.50 (t, 2H, $J=7.2$ Hz, $CH_2CH_2CH_2Ph$), 3.80 (m, 8H, $N(CH_2CH_2Cl)_2$), 4.51 (t, 2H, $J=2.1$ Hz, NCH_2CH_2O), 4.59 (t, 2H, $J=2.1$ Hz, NCH_2CH_2O), 6.74 (d, 2H, $J=8.7$ Hz, phenyl), 6.79 (dd, 1H, $J=2.4$ Hz, $J=4.2$ Hz, pyrimidinyl), 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), 8.38 (dd, 1H, $J=2.4$ Hz, $J=4.2$ Hz, pyrimidyl), 8.60 (s, 1H, ArCH=NAr), 8.62 (q, 1H, $J=$ 2.4 Hz, pyrimidyl). Anal. $C_{33}H_{35}Cl_2N_5O_4S$ (C, H, N, S).

*5.1.15. 2-[N¹-2-Pyrimidyl-aminobenzenesulfonamido] ethyl 4-bis(2-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 ν_{max} (KBr, cm^{-1}) 3469, 3348 (NH_2), 1735 (C=O), 1593, 1546, 1514 (phenyl), 1272 (SO_2N); ^{1}H-NMR (300 MHz, $CDCl_3$) δ 1.81 (m, 2H, $CH_2CH_2CH_2$), 2.23 (t, 2H, $J=7.2$ Hz, O=C$CH_2CH_2CH_2$), 2.53 (t, 2H, $J=7.2$ Hz, $CH_2CH_2CH_2Ph$), 3.60 (t, 4H, $J=6.0$ Hz, $N(CH_2CH_2Cl)_2$), 3.70 (t, 4H, $J=6.0$ Hz, $N(CH_2CH_2Cl)_2$), 4.25 (t, 2H, $J=4.5$ Hz, NCH_2CH_2O), 4.43 (t, 2H, $J=4.5$ Hz, NCH_2CH_2O), 5.64 (br s, 2H, NH_2), 6.46 (dd, 1H, $J=2.4$ Hz, $J=4.2$ Hz, pyrimidinyl), 6.64 (dd, 4H, $J=8.4$ Hz, $J=2.4$ 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); ^{13}C-NMR (75 MHz, $CDCl_3$) 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. $C_{26}H_{31}Cl_2N_5O_4S$ (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. LD_{50} 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, 1×10^6 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.

$$\mathrm{TWI}\% = (1 - T/C) \times 100\%$$

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

[1] Stevens C.D., Quinlin P.M., Meinken M.A., et al., Science 112 (1950) 561–562.

[2] Calvert N., Connors T.A., Ross W.C.J., Eur. J. Cancer 4 (1968) 627.

[3] Abel G., Connors T.A., Ross W.C.J., Eur. J. Cancer 9 (1973) 49–54.

[4] Ringsdorf H., J. Polym. Sci. Symp. 51 (1975) 135–153.

[5] Bartulin J., Przybylski M., Ringsdorf H., Ritter H., Makromol. Chem. 175 (1974) 1007–1010.

[6] Nguyen H.N., Herbert M., Nguyen D.X., et al., J. Labelled Compounds 7 (1971) 299.

[7] Abel G., Connors Th.A., Hofmann V., Ringsdorf H., Makromol. Chem. 177 (1976) 2669–2674.

[8] Wang H.Y., Study on Synthesis, Characterization and Properties of Polymeric Targeting Medicines with Sulfadiazine End Group Using Polyethylene Oxide as Matrix, Ph.D. dissertation Fudan University, Shanghai, 1996.

[9] Chen S., Huang Z.H., Huang J.L., Eur. Polym. J. 36 (2000) 1703–1710.

[10] Huang J.L., Wang H.Y., Tian X.Y., J. Polym. Sci. Part A: Polym. Chem. 34 (1996) 1933–1940.

[11] Huang J.L., Chen S., Tan L.S., Wang H.Y., Sci. China (Series B) 41 (1998) 54–57.

[12] Huang Z.H., Chen S., Huang J.L., J. Appl. Polym. Sci. 73 (1999) 1379–1385.

[13] Chen S., Studies on Synthesis and Properties of Polymeric Targeting Antitumor Medicines Containing 5-FU and Nitrogen Mustard., Ph.D. dissertation Fudan University, Shanghai, 1998.

[14] Supuran C.T., Scozzafava A., Eur. J. Med. Chem. 35 (2000) 867–874.

[15] Scozzafava A., Supuran C.T., Bioorg. Med. Chem. Lett. 10 (2000) 1117–1120.

[16] Supuran C.T., Briganti F., Tilli S., et al., Bioorg. Med. Chem. Lett. 9 (2001) 703–714.

[17] Owa T., Okauchi T., Yoshimatsu K., et al., Bioorg. Med. Chem. Lett. 10 (2000) 1223–1226.

[18] Supuran C.T., Scozzafava A., J. Enzyme Inhib. 15 (2000) 597–610.

[19] Owa T., Nagasu T., Exp. Opin. Ther. Patents 10 (2000) 1725–1740.

[20] Chern J.W., Leu Y.L., Wang S.S., J. Med. Chem. 40 (1997) 2276.

[21] Medina J.C., Roche D., Shan B., et al., Bioorg. Med. Chem. Lett. 9 (1999) 1843.

[22] Yoshino H., Ueda N., Niijima J., et al., J. Med. Chem. 35 (1992) 2496.

[23] Owa T., Yoshino H., Okauchi T., et al., J. Med. Chem. 42 (1999) 3789.

[24] Scozzafava A., Mastrolorenzo A., Supuran C.T., Bioorg. Med. Chem. Lett. 10 (2000) 1887–1891.

[25] Ouchi T., Yuyama H., J. Macromol. Sci-Chem. 24 (1987) 1011–1014.

[26] Brock N., in: Reinhoudt D.N., Connors T.A., Pinedo H.M. (Eds.), Structure-Activity Relationships of Anti-tumor Agents, Martinus Nijhoff, London, 1983, p. 256.