Pergamon

Bioorganic & Medicinal Chemistry Letters 11 (2001) 1099–1103

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

2-[N^1-2-Pyrimidyl-aminobenzenesulfonamido] Ethyl 4-Bis(2-chloroethyl) Aminophenyl Butyrate: A Potent Antitumor Agent

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Received 6 December 2000; accepted 20 January 2001

Abstract—2-[N^1-2-Pyrimidyl-aminobenzenesulfonamido] ethyl 4-bis(2-chloroethyl) aminophenyl butyrate has been prepared by reaction of chlorambucil with sulfadiazine derivative. Schiff's base has been used as the protective group of the aromatic amine in the synthesis. It can be completely removed by the irradiation of 365 nm UV light at room temperature. The title compound exhibits a high antitumor activity with a therapeutic index (TI) of 47.55 which is twice that of chlorambucil's (TI: 22.84).

In the long history of drug discovery, an interesting phenomenon has been noted that compounds with the same structural feature show diverse biological activities. For instance, the sulfonamides with different substituted groups display a wide variety of pharmacological activities such as antibacterial, insulin-releasing antidiabetic, carbonic anhydrase inhibitory, high-ceiling diuretic, and antithyroid.[1] Recently more papers have reported several kinds of antitumor agents possessing the feature structure of sulfonamide.[2–5] It is well known that sulfadiazine is a useful antibacterial drug with the typical sulfonamide structure. It has been shown to concentrate selectively in the Yoshida sarcoma.[6] This finding has aroused considerable interest and great efforts have been made to design new antitumor agents by combining sulfadiazine and antitumor agents in one compound. Generally, there are two reactive sites for the modification of sulfadiazine, one is the aromatic amine, the other is sulfonamide. Heretofore, the main work of modified sulfadiazine has been focused on the aromatic amine due to its relatively high reaction activity.[7–11]

As shown in Scheme 1, ^{14}C labeled sulfadiazine mustard has been synthesized by Nguyen,[10] and sulfadiazine-acrylamide and its polymer by Abel et al.[11] Unfortunately, all these compounds lost selectivity for the tumors that concentrate sulfadiazine. Further work in our group shows that if the polymers, such as polyethylene oxide, were used as matrix, and the sulfadiazine and antitumor drugs were fastened on its two ends, then the selectivity was recovered, although the mechanism of selectivity is still unclear.[12–16] Inspired by these results, we believe that more potent antitumor agents may be prepared by combining sulfadiazine and a conventional antitumor agent such as chlorambucil (CBL) in one molecule through an appropriate way. Thus, we have designed and synthesized compound **12**, which is the combination of sulfadiazine and CBL.

In the design of **12** (Scheme 3), we considered three factors. First, instead of the aromatic amine, sulfonamide has been chosen as the reaction site for modification since those modified on the aromatic amine lost selectivity toward tumor cells. Second, sulfadiazine should be connected with CBL in an appropriate way such that both the selectivity of sulfadiazine toward tumor cells and the antitumor activity of CBL are not destroyed. Third, CBL and **10** were connected by an ester bond, so that free CBL can be released in the body.

To prepare **12**, 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 acidic or alkaline conditions and can be

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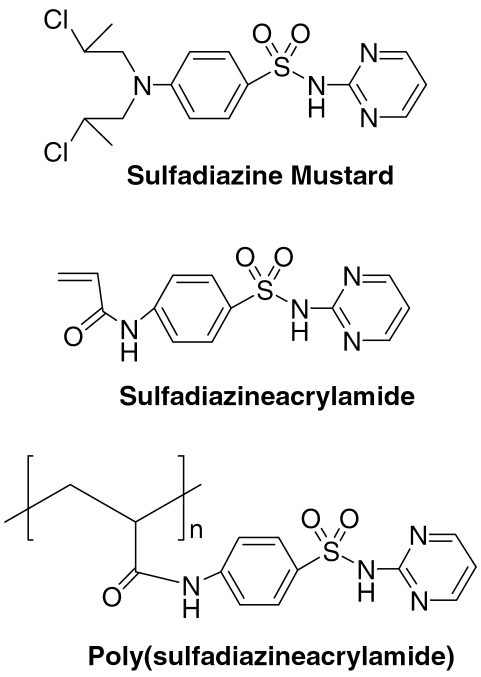
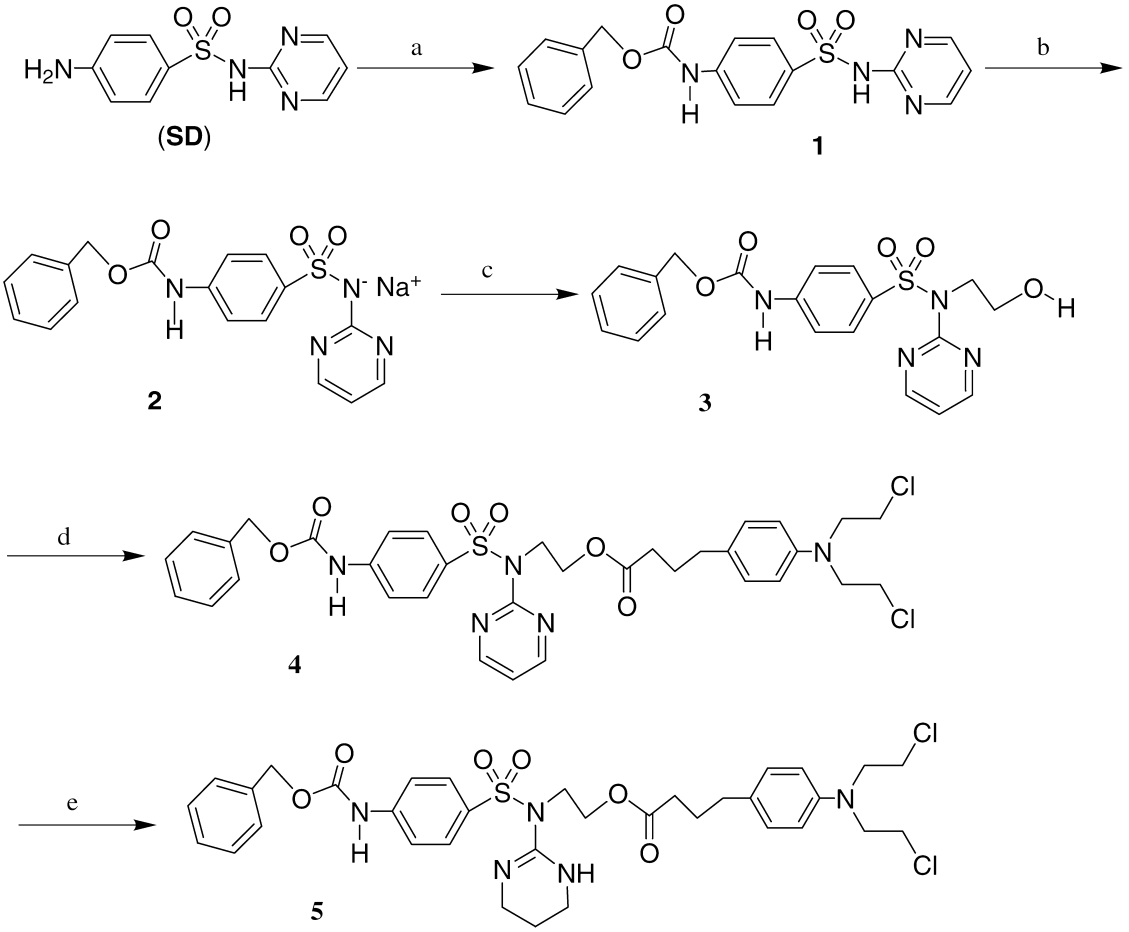
0960-894X/01/$ - see front matter
PII: S0960-894X(01)00157-3



Scheme 1. ^{14}C labeled sulfadiazine derivatives.

easily removed by catalytic hydrogenation. However, as shown in Scheme 2, this synthetic route led to **5** instead of the desired **12**. Compound **1** was prepared by reacting sulfadiazine with benzyl chloroformate. Treatment of **1** with aqueous sodium hydroxide led to compound **2**. The reaction of **2** with 2-bromoethanol in DMF afforded **3**. Compound **4** was obtained by the coupling of **3** with CBL in the presence of DCC at room temperature. The hydrogenation of **4** was 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 **5**. But, when hydrogen gas or ammonium formate was used as hydrogen donor, the product was a mixture of **5** and its deprotected compound. Thus, the target molecule **12** cannot be prepared by this method.

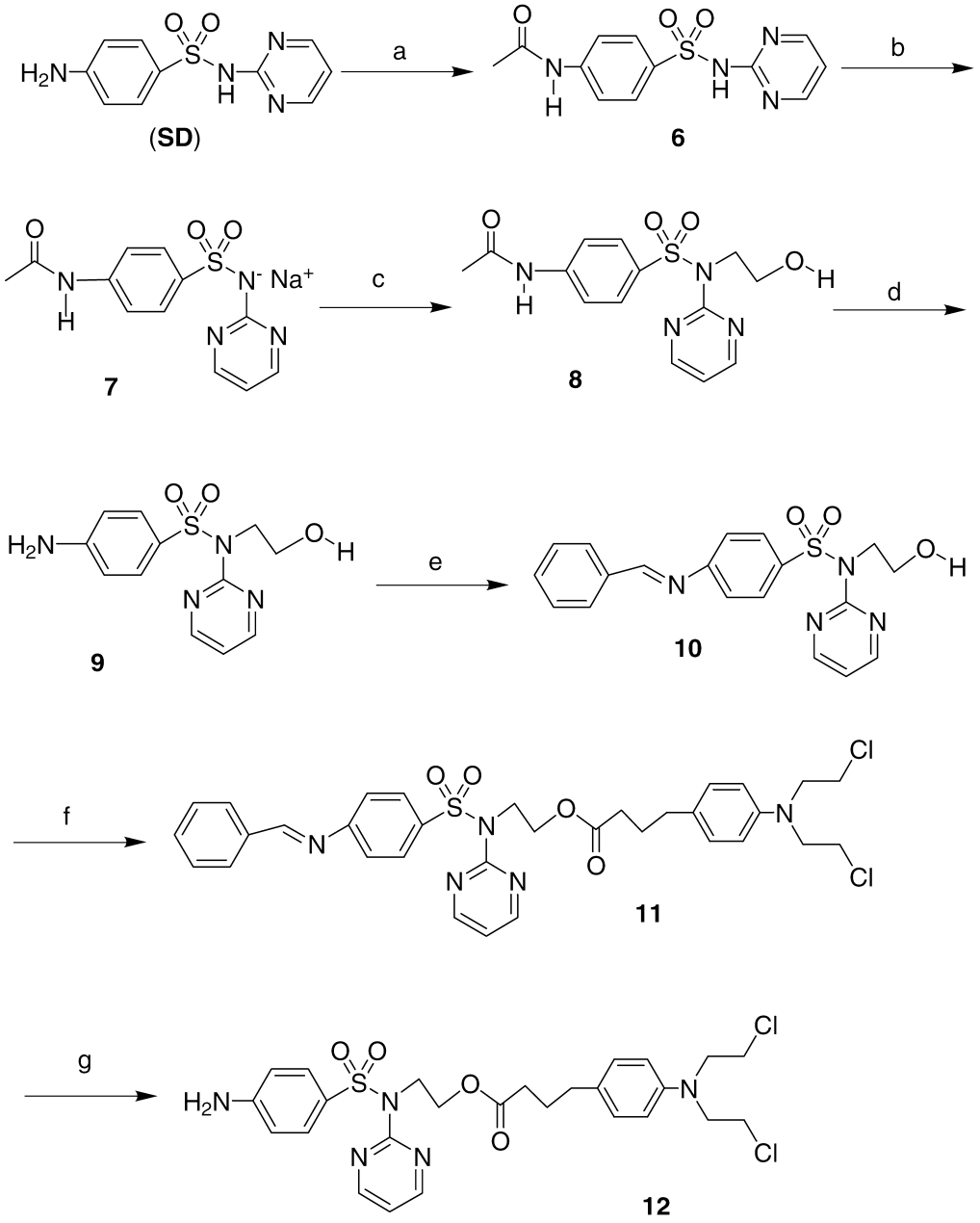
Since **12** is our target compound, we developed another synthetic route for it, which is shown in Scheme 3. In this route, firstly acetyl was used as the protective group of the aromatic amine, then **7** and **8** were synthesized according



Scheme 2. Reagents and conditions: (a) benzyl chloroformate, pyridine, 0 °C, 8 h; (b) NaOH (aq, 1.0 equiv); (c) DMF, $BrCH_2CH_2OH$ (1.0 equiv), 80 °C, 8 h; (d) chlorambucil (1.0 equiv), DCC (1.1 equiv), pyridine, rt, 48 h; (e) $CHCl_3$, cyclohexene (excessive), 10% Pd–C, reflux, 18 h.

to the synthetic procedures for **2** and **3**, respectively. And the acetyl group was removed by refluxing **8** in HCl (1.2 M) for about 1 h. The reaction of **9** with benzaldehyde afforded Schiff's base protected compound **10**. Compound **11** was prepared according to the procedure of **4**. Irradiation of **11** with 365 nm UV light at rt afforded **12** in high yield (98%), which exhibits great advantage over the conventional deprotection method. Compound **12** was obtained only in 43% yield when **11** was hydrolyzed in dilute hydrochloric acid. In this route, two different protective groups had been applied, the acetyl and the Schiff's base. Acetyl as well as Cbz is stable under all the reaction conditions, but can not be removed without destroying the target molecule. Schiff's base can be easily removed by the irradiation of UV light that won't destroy the target molecule (**12**). Therefore, after the preparation of **8**, the acetyl group was replaced by the Schiff's base, which is unstable in the preceding reactions, but stable in the following reactions. All the new compounds have been fully characterized by IR, NMR, MS and elemental analysis.[17]

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



Scheme 3. Reagents and conditions: (a) acetyl chloride, pyridine, 0 °C, 2 h; (b) NaOH (aq, 1.0 equiv); (c) DMF, $BrCH_2CH_2OH$ (1.0 equiv), 80 °C, 8 h; (d) HCl (1.2 M), reflux, 1 h; (e) benzaldehyde, 100 °C, 6 h; (f) chlorambucil (1.0 equiv), DCC (1.1 equiv), pyridine, rt, 48 h; (g) acetone, 365 nm UV light, rt, 1 h.

Table 1. In vivo antitumor activity of CBL[d] and **12** against murine S-180 sarcoma

Drug	Dose (mg/kg)	Schedule	Mice In.[a]/Fi.[b]	Body wt. In./Fi.(g)	Tumor 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
12	13	ip×7qd	10/9	19.8/18.0	0.41±0.14	81.45	<0.01
12	9.6	ip×7qd	10/9	19.6/18.3	0.51±0.11	76.92	<0.01
12	6.5	ip×7qd	10/10	19.8/19.5	0.57±0.10	74.21	<0.01
12	4.8	ip×7qd	10/10	19.8/20.7	0.81±0.21	63.35	<0.01
12	3.3	ip×7qd	10/10	19.6/22.5	1.09±0.20	50.68	<0.01
12	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.

Table 2. Antitumor activity comparison of **12** with CBL at dose of equal toxicity

Drug	LD_{50}×1/10	LD_{50}×1/20	LD_{50}×1/40
12b	81.5%	74.2%	50.7%
CBL	73.3%	52.9%	37.1%

Table 3. Antitumor activity comparison of **12** with CBL at dose of equal molarity

Drug	0.022 (mmol/kg)	0.016 (mmol/kg)	0.008 (mmol/kg)	0.004 (mmol/kg)
12	81.5%	76.9%	63.4%	47.9%
CBL	77.4%	73.3%	52.9%	37.1%

the behavior and the death distribution of the test mice were recorded. LD_{50} was calculated by using the Bliss method. The in vivo antitumor activity was tested against the mouse solid tumor S-180 cell line which was maintained by intraperitoneal passage at weekly intervals in male TA1 mice. 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.

According to the LD_{50} values of **12** (130.76 mg/kg ip in mice, 0.225 mmol/kg) and CBL (49.56 mg/kg ip in mice, 0.163 mmol/kg), it can be concluded that the acute toxicity of **12** is lower than that of its mother compound CBL. The results of in vivo antitumor activity of **12** and CBL against murine S-180 sarcoma are listed in Table 1. The antitumor activity comparison of **12** with CBL was listed in Table 2 (at equal toxicity) and Table 3 (at equal molarity), respectively.

The data in Tables 2 and 3 indicate that **12** is more potent than CBL when the tested mice were administered at doses of either equal toxicity or equal molarity, especially at relatively low dose range. This effect may be partly attributed to the targeting action of **12** that may lead to a relatively high drug concentration in the tumor cells. Since the active moiety of **12** is still CBL which demonstrated a strong dose–effect relationship mainly at low dose range as shown in Table 1, the inhibition increased by **12** at relatively low dose is more obvious than that at high dose. However, compared with sulfadiazine, the concentration effect of **12** isn't obvious as we expected. It may due to the hydrolysis of the ester bond in the body that some free CBL had already been released before the concentration of **12** in tumor cells. The TI (therapeutic index) of **12** calculated from the data in Table 1 is 47.55, which is about the twice of CBL's (TI: 22.84, calculated from the data obtained in the same test system as **12**). Thus, **12** is much safer and more useful than its mother compound CBL when used as antitumor agents.

In summary, we have synthesized **12** as a potent antitumor agent by combining sulfadiazine and CBL in one molecule through an ester bond. **12** is more potent and safer than its mother compound CBL. This class of targeting agents may be further developed to form candidate drugs, which may have advantages over the currently available anticancer agents.

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17. Selected data for compound **12**: mp: 97–98 °C; IR ν_{max} (KBr, cm^{-1}) 3469, 3348 (NH_2), 1735 (C=O), 1593, 1546, 1514 (phenyl), 1272 (SO_2N); ^1H NMR (300 MHz, $CDCl_3$) δ 1.81 (m, 2H, $CH_2CH_2CH_2$), 2.23 (t, 2H, $J=7.2$ Hz, $O{=}CCH_2CH_2CH_2$), 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), 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.