Synthesis, physical properties and microbiological activities of more water soluble silver sulfadiazine derivatives[1]

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ABSTRACT

In this study the preparation of five hydrophilic derivatives of sulfadiazine is reported. The common structural element in the compounds is the 2-sulfonamidopyrimidine moiety. Three of these compounds are suitable for the preparation of a photostable 1 : 1 silver compound. These silver compounds are five to ten times more water soluble than silver sulfadiazine. The IR, ^{1}H- and ^{13}C-NMR data point to a similar co-ordination of silver in these compounds as with silver sulfadiazine. The microbiological activity of these silver compounds against *St. aureus* is slightly lower. (Pharm Weekbl [Sci] 1983;5:298-301)

INTRODUCTION

Recently one of us (BULT 1982) has given an evaluation of the microbiological and medicinal chemical properties of silver sulfadiazine (AgSD) and related metal sulfanilamides in relation to their use in burn treatment. One of the conclusions was that substitution of the sulfadiazine part (SD) in the therapeutically useful topical antimicrobial silver sulfadiazine by a biologically inactive component can result in a safer drug. The following parameters seem to be of importance for the substitution of sulfadiazine (SD) in AgSD by an anion L:

- the conditional stability constant log K of the compound AgL needs to possess a medium value comparable with AgSD (about 4). A value too low causes chloride depletion, possibly black staining and a fast reaction with silver-binding wound components (proteins *etc.*); a log K value that is too high prevents the liberation of Ag by dissociation of AgL;
- AgL needs to be photostable;
- a somewhat better solubility, as with AgSD, is favourable (AgSD: 0.2 mg/100 ml water) for the disposal of the silver ion under wound conditions.

In this study we report the synthesis and microbiological evaluation of a number of silver compounds with sulfadiazine derivatives. As it is known from structure analysis that the 2-sulfonamidopyrimidine moiety is involved in silver co-ordination (COOK and TURNER 1975), this part of the molecule was retained in all new compounds. Preservation of this structural element in the derivatives (Fig. 1: HSD) may result in silver compounds with log K values comparable to AgSD. The preparation of compounds with increased water solubility was realized by

- the introduction of a hydrophilic group at the 4-amino group of HSD (Fig. 1: HMSSD, HOSD, HMOSD);
- the substitution of the 4-amino group (Fig. 1: HNSAP);
- the substitution of the 4-aminophenyl group by a small organic substituent (Fig. 1: HMSAP).

EXPERIMENTAL

Methods

The melting points were taken in a Büchi-Tottoli apparatus and are uncorrected. The elemental analyses were performed by Pascher and Pascher, Mikroanalytisches Laboratorium, Bonn, FRG. Silver is analyzed by the Volhard procedure after decomposition of the silver compound with concentrated HNO_3. The IR spectra (KBr) were recorded on a Beckman IR 10 and the direct inlet mass spectra on an LKB 2091 mass spectrometer, electron impact ionization at 70 eV. The ^{1}H-NMR spectra were recorded on a Bruker WM-300 at 300.13 MHz or a Jeol-PS-100 NMR spectrometer at 99.5 MHz. The samples were dissolved in DMSO-d_6 using Me_4Si as an internal standard. ^{13}C-NMR spectroscopy was performed on a Bruker WM-300 at 75.46 MHz or a Jeol PFT-100 NMR spectrometer at 25.15 MHz. DMSO-d_6 and Me_4Si were used as solvent and internal standard respectively. Proton noise decoupling and proton-gated decoupling were used to obtain the long-range ^{13}C-^{1}H coupling constants for peak assignment. In case of the silver compounds solubility problems were encountered. The solubility of the silver compounds in water was determined as described before (BULT and KLASEN 1980). The silver content was analysed by AAS (Perkin Elmer 460, acetylene-air flame, lamp: 3 UAX/Ag-Cathodeon Ltd.: 328.1 nm) using a silver nitrate solution in distilled water for calibration.

Measurement of the minimum inhibitory concentration (MIC)

An overnight culture of *Staphylococcus aureus* (ATCC 6538) was diluted 1 : 500 in saline and 0.1 ml of this suspension was used to inoculate tubes with 5 ml Bacto Nutrient Broth, pH = 6.8 (Difco Lab., Detroit, USA) + glucose (1.0 mg/ml) + thymol blue (80 µg/ml) containing graded concentrations of the silver compounds (concentration range 100-0.78 µg/ml). All tubes were incubated at 37°C. Turbidity and/or a change in the colour of the

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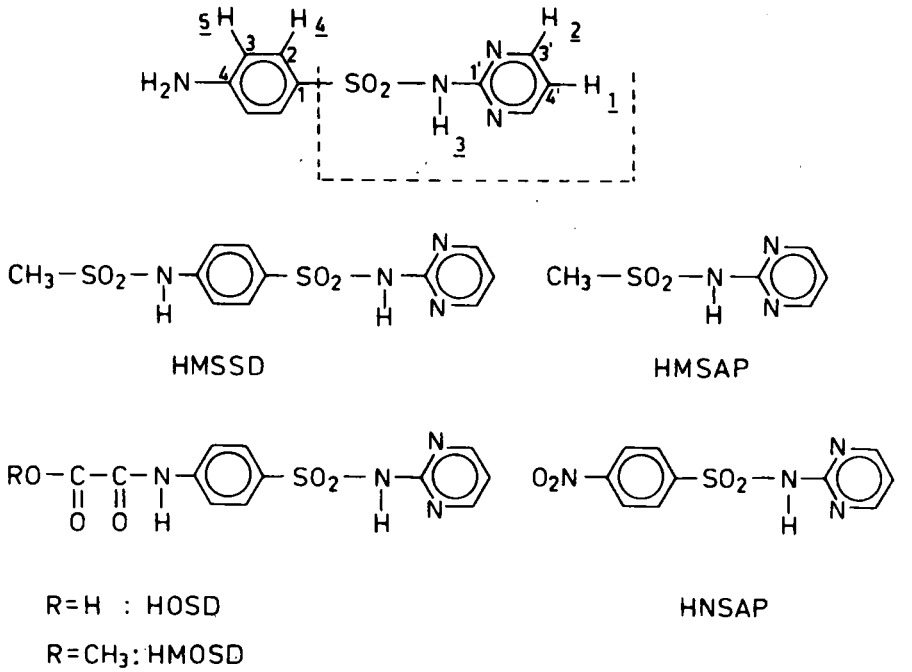

FIGURE 1. Structure formulae of sulfadiazine (HSD) and its derivatives. The common structural element in the compounds, 2-sulfonamidopyrimidine, is indicated in HSD. The numbering of the H and C atoms given in HSD is used in NMR assignment. HMOSD: N4-methyloxalylsulfadiazine; HMSAP: 2-methylsulfonamidopyrimidine; HMSSD: N4-methylsulfonamidosulfadiazine; HNSAP: 2-(4-nitrobenzene)sulfonamidopyrimidine; HOSD: N4-oxalylsulfadiazine; HSD: sulfadiazine

indicator were recorded. Final readings were made after 7 days. The lowest concentration which showed no turbidity or change in colour was taken as the MIC. Experiments were carried out in duplicate.

Preparation of N4-methylsulfonamidosulfadiazine (HMSSD)

To a solution of sulfadiazine (7.51 g, 30 mmol) in dry pyridine (50 ml) was added slowly (15 min) with cooling (0°C) a solution of methanesulfonyl chloride (3.67 g, 32 mmol) in dry pyridine (10 ml). After 1 h heating at 60°C the red coloured reaction mixture was concentrated to 20 ml by evaporation in vacuo. To the residue was added 50 ml of a cooled 2 N HCl solution (0°C). The precipitate formed was filtered off and washed with 2 N HCl. The product was light yellow, yield: 9.20 g (93.4% calculated to sulfadiazine), m.p.: 237-238°C. Crystallization from pyridine/H_2O resulted in a solid with m.p. 240-241°C. IR: 1322 cm^{-1} [$\nu_{as}(SO_2)$], 1140 cm^{-1} [$\nu_s(SO_2)$]; ^{1}H-NMR: δ 3.13 (s, 3H, CH_3–SO_2), 7.05 (t, 1H, H1), 7.32 (d, 2H, H5), 7.95 (d, 2H, H4), 8.51 (d, 2H, H2), 10.43 (s, 1H, H3 or N4–H); ^{13}C-NMR: δ 40.1 (q, CH_3–SO_2), 115.8 (m, C4'), 117.5 (d, C3), 129.5 (d, C2), 134.2 (t, C1), 142.7 (t, C4), 156.9 (t, C1'), 158.4 (d, C3').

Preparation of N4-methyloxalylsulfadiazine (HMOSD)

To dimethyl oxalate (26.4 g, 200 mmol) heated to 70°C was added sulfadiazine (12.52 g, 50 mmol). The reaction mixture was heated during 2 h at 130-150°C with stirring. The solid reaction product (MOSD) was filtered off at 70°C and washed with boiling methanol (50 ml) and 2 N HCl (50 ml) respectively to remove the unreacted dimethyloxalate and sulfadiazine. The solid product was refluxed with 200 ml of methanol during 0.5 h and filtered off warm. A white solid resulted, yield: 9.82 g (58.4% calculated to sulfadiazine), m.p.: 240-241°C. IR: 1335 cm^{-1} [$\nu_{as}(SO_2)$], 1158 cm^{-1} [$\nu_s(SO_2)$]; ^{1}H-NMR: δ 3.91 (s, 3H, OCH_3), 7.05 (t, 1H, H1), 7.98 (d, 4H, H4 + H5), 8.48 (d, 2H, H2), approx. 11.0 (broad, H3); ^{13}C-NMR: δ 53.4 (q, OCH_3), 115.8 (m, C4'), 120.1 (d, C3), 128.7 (d, C2), 135.8 (t, C1), 141.4 (t, C4), 156.9 (t, C1'), 158.4 (d, C3'), 155.6, 160.6 (2 × C=O).

Preparation of N4-oxalylsulfadiazine (HOSD)

HMOSD (7.00 g, 20 mmol) was dissolved in 50 ml of 1 N NaOH solution and warmed during 45 min at 40-50°C in the presence of activated charcoal. After filtration and addition of 2 N HCl a white solid was precipitated. The solid was isolated, yield: 5.76 g (89.4% calculated to HMOSD), m.p.: 253-255°C. IR: 1338 cm^{-1} [$\nu_{as}(SO_2)$]; 1155 cm^{-1} [$\nu_s(SO_2)$]; ^{1}H-NMR: δ 7.05 (t, 1H, H1), 8.00 (d, 4H, H4 + H5), 8.56 (d, 2H, H2); ^{13}C-NMR: δ 115.7 (m, C4'), 119.7 (d, C3), 128.6 (d, C2), 135.4 (t, C1), 141.6 (t, C4), 156.7 (t, C1'), 158.2 (d, C3'), 157.8, 161.7 (2 × C=O).

Preparation of 2-(4-nitrobenzene)sulfonamidopyrimidine (HNSAP)

4-Nitrobenzenesulfonyl chloride was prepared according to the procedure described by WERTHEIM (1946) for 2-nitrobenzenesulfonyl chloride. The product was light yellow, yield: 65.4%, m.p. 70-71°C (literature: 79.5-80.5°C). The crude product was used for the following synthesis of 2-(4-nitrobenzene)sulfonamidopyrimidine.

To a cooled solution (0°C) of 2-aminopyrimidine

(3.33 g, 35 mmol) in dry pyridine (150 ml) was added slowly in portions 4-nitrobenzenesulfonyl chloride (8.00 g, 36 mmol). Heating at a steam bath for 2 h resulted in a red solution. The solution was concentrated to 30 ml by evaporation in vacuo. After addition of 100 ml of 2 N HCl (cooling to 0°C) a precipitate was formed. The precipitate was filtered off, suspended in 1 N NaOH (50 ml) and heated to 70°C for 30 min in the presence of activated charcoal. The suspension was filtered off and the filtrate was acidified with 2 N HCl. The light yellow solid formed was isolated, yield: 3.20 g (32.6% calculated to 2-aminopyrimidine), m.p.: 254-256°C. IR: approx. 1300 cm^{-1} [$\nu_{as}(SO_2)$], 1166 cm^{-1} [$\nu_s(SO_2)$]; 1523 cm^{-1} [$\nu_{as}(NO_2)$]; 1348 cm^{-1} [$\nu_s(NO_2)$]; ^{1}H-NMR: δ 7.07 (t, 1H, H1), 8.22 (m, 2H, H4), 8.40 (m, 2H, H5), 8.52 (d, 2H, H2); ^{13}C-NMR: δ 115.0 (m, C4'), 124.1 (d, C3), 129.0 (d, C2), 146.3 (t, C1), 149.5 (t, C4), 156.3 (t, C1'), 158.2 (d, C3').

Preparation of 2-methylsulfonamidopyrimidine (HMSAP)

To a cooled solution (0°C) of 2-aminopyrimidine (9.99 g, 105 mmol) in dry pyridine (100 ml) was added slowly methanesulfonyl chloride (13.17 g, 115 mmol). After 2 h stirring at room temperature the yellow solution was quickly evaporated in vacuo at 40 to 50°C. To the residue was added ethanol (50 ml) and the white solid precipitate was filtered off, yield: 7.00 g (38.5% calculated to 2-aminopyrimidine), m.p.: 238-239°C. The solid was recrystallized by the following procedure. Dissolution in 1 N NaOH and heating for 0.5 h at 70°C in the presence of activated charcoal. After filtration and acidification of the filtrate the solid product formed was filtered off (m.p.: 246-247°C). IR: 1322 cm^{-1} [$\nu_{as}(SO_2)$], 1138 cm^{-1} [$\nu_s(SO_2)$]; ^{1}H-NMR: δ 3.36 (s, 3H, CH_3–SO_2), 7.15 (t, 1H, H1), 8.63 (d, 2H, H2), 11.34 (s, 1H, H3); ^{13}C-NMR: δ 41.3 (q, CH_3SO_2), 115.7 (m, C4'), 157.5 (t, C1'), 158.5 (d, C3').

Preparation of the silver compounds

About 4 g of the sulfonamide derivative was dissolved in water (60 ml) by adding an equimolar amount of 1 N NaOH and gently warming (50°C). In case of incomplete dissolution the solution was filtered. After dilution to 150 ml a solution containing an equimolar amount of silver nitrate (80 ml) was added dropwise with stirring. After standing 1 h in the dark the precipitate was filtered off, washed with water (100 ml) and ethanol (50 ml). The product was dried in vacuo. Yields: AgMSAP (95.8%), AgNSAP (91.4%), AgMSSD (78.2%).

AgMSAP: Analysis ($C_5H_6N_3SO_2Ag$): calculated C 21.44, H 2.16, N 15.00, S 11.45, Ag 38.52; found C 21.2, H 2.2, N 15.0, S 11.3, Ag 38.3; IR: 1228 cm^{-1} [$\nu_{as}(SO_2)$], 1114 cm^{-1} [$\nu_s(SO_2)$]; ^{1}H-NMR: δ 3.20 (CH_3–SO_2), 6.91 (t, 1H, H1), 8.55 (d, 2H, H2); ^{13}C-NMR: δ approx. 40 (CH_3SO_2), 111.3 (C4'), 159.2 (C3').

AgNSAP: Analysis ($C_{10}H_7N_4O_4SAg$): calculated C 31.03, H 1.82, N 14.47, S 8.28, Ag 27.86; found C 30.8, H 1.9, N 14.5, S 18.1, Ag 27.3; IR: 1260 cm^{-1} [$\nu_{as}(SO_2)$], 1132 cm^{-1} [$\nu_s(SO_2)$]; 1520, 1348 cm^{-1} [$\nu(NO_2)$]; ^{1}H-NMR: δ 6.90 (s, 1H, H1), 8.22 (d, 2H, H4), 8.31 (d, 2H, H5), 8.44 (d, 2H, H2); ^{13}C-NMR: δ 112.1 (C4'), 123.3 (C3), 129.0 (C2), 148.6 (C4), 149.5 (C1), 159.1 (C3'), 161.5 (C1').

AgMSSD: Analysis ($C_{11}H_{11}N_4O_4S_2Ag$): calculated C 30.36, H 2.55, N 12.87, S 14.73, Ag 24.78; found: C 29.7, H 2.7, S 14.3, Ag 24.7. IR: 1325 cm^{-1}, approx. 1260 cm^{-1}; approx. 1230 cm^{-1}, 1130 cm^{-1} [2 × $\nu(SO_2)$]; ^{1}H-NMR: δ 3.07 (s, 3H, CH_3–SO_2), 6.86 (t, 1H, H1), 7.24 (d, 2H, H5), 7.95 (d, 2H, H4), 8.44 (d, 2H, H2), 11.15 (s, broad, N4-H); ^{13}C-NMR: δ 39.6 (CH_3SO_2), 111.7 (C4'), 117.3 (C3), 129.3 (C2), 136.9 (C1), 141.2 (C4), 159.2 (C3'), 160.9 (C1').

AgMOSD: Analysis ($C_{13}H_{11}N_4O_5SAg$): calculated Ag 24.34; found Ag 31.9. Ag_2MOSD: calculated Ag 39.22.

AgOSD: Analysis ($C_{12}H_9N_4O_5SAg$): calculated Ag 25.14; found Ag 32.7. Ag_2OSD: calculated Ag 40.3.

RESULTS AND DISCUSSION

The sulfadiazine derivatives are prepared by well-known general methods of synthesis. In the mass spectra of the compounds the peaks corresponding with the fragments M–$SO_2^{+\cdot}$ (M–64) and M–SO_2H^+ (M–65) are found. This is in accordance with expectation (STOBER and DE WITTE 1982). Only for HMSAP the molecular ion peak (M^+) is found. The IR spectra indicate the presence of –SO_2–. The ^{1}H-NMR and ^{13}C-NMR spectra agree in number of bands, band positions and multiplicity and intensity ratios with expectation (data see *Experimental*). The ^{13}C chemical shifts of the substituted benzenesulfonamido part of the molecules measured mostly agree well with the calculated values (MIYAJIMA *et al.* 1971; STOTHERS 1972). The interpretation of the NMR spectra is based on the assignments of HSD given in the literature (^{1}H-NMR: TURCZAN and MEDWICK 1972; ^{13}C-NMR: CHANG *et al.* 1975). Also the elemental composition of the compounds is supported by the analytical data of the corresponding silver compounds.

Of the five sulfadiazine derivatives prepared only three could be converted into the corresponding 1:1 silver compound with well defined composition. (AgMSAP, AgMSSD, AgNSAP). The silver compounds with HMOSD and HOSD had a silver content corresponding with a composition intermediate between 1:1 and 2:1 (Ag:ligand). The first three silver compounds mentioned are photostable to daylight, the other two are unstable to light (turning to grey-red). Most probably the silver ion in MOSD and OSD is at least partly bound to the oxalyl moiety of the molecules. The same phenomenon of photo-instability was seen with the disilver compound of *N*-4-succinylsulfadiazine (BULT and KLASEN 1980).

It is of interest to get insight into the structures of AgMSAP, AgMSSD and AgNSAP. The common structural element in these compounds suitable for silver co-ordination is 2-sulfonamidopyrimidine. Thus, a structure similar to AgSD is obvious. Only in case of AgMSSD two potential co-ordination sites are available (both SO_2–NH groups). Studies on the structure of AgSD in the solid state are made with X-ray diffraction (COOK and TURNER 1975) and IR (BULT and KLASEN 1978). The IR spectra reveal that the shifts of $\nu(SO_2)$ upon co-ordination of Ag with HMSSD and HMSAP are comparable with AgSD. With AgNSAP a deviating shift pattern is found. Also the chemical shifts in the NMR spectra change in position more or less upon co-ordination of the silver

TABLE I. *Changes in chemical shift [δ(HL)–δ(AgL)] upon co-ordination of the silver ion with the free ligand HL*

NMR signal[1]	δ(HL)–δ(AgL)[2]			
	HSD	HMSAP	HNSAP	HMSSD
H1	+0.19	+0.24	+0.17	+0.19
H2	+0.06	+0.08	+0.08	+0.07
H4	−0.03		0	0
H5	+0.05		+0.09	+0.08
C1′	−[3]	−[3]	−5.2	−4.0
C3′	−0.5	0.7	−0.9	−0.8
C4′	+4.5	+4.4	+2.9	+4.1
C1	−[3]		−3.2	−2.7
C2	+0.5		0	+0.2
C3	+0.6		+0.8	+0.2
C4	+1.9		+0.9	+1.5

[1]The numbering of the atoms is given in Figure 1.
[2]The change in chemical shift upon co-ordination of silver with the free ligand (HL). The values are in ppm. The first section of the table contains the ^{1}H-NMR chemical shift change data; the second section is concerned with the ^{13}C-NMR data.
[3]Signals missing because of insufficient solubility of the silver compound.

ion with the ligands. Because of the poor solubility of the silver compounds in DMSO the quality of the ^{13}C-NMR-spectra was marginal. In Table I the chemical shift changes are summarized. The changes in chemical shift of the four compounds are fairly coherent in direction and magnitude. This may be indicative of a similar way of silver co-ordination. Anyhow, this disfavours co-ordination of silver in AgMSSD with the SO_2–N_4H group. Comparison of the NMR spectra of AgSD and NaSD demonstrates significant differences in band positions and excludes a considerable dissociation of AgSD in DMSO. In conclusion, co-ordination of silver with the 2-sulfonamidopyrimidine part of the molecules HMSAP, HNSAP and HMSSD in a similar way as with AgSD is very likely.

TABLE II. *Solubility and minimum inhibitory concentration (MIC) of the silver compounds*

AgL	Solubility[1] (μg Ag/ml)	MIC (μg AgL/ml)
AgMSAP	3.8	25
AgMSSD	2.1	25
AgNSAP	2.5	50
AgSD	0.4[2]	12.5

[1]Mean of three determinations.
[2]BULT and KLASEN (1980) found 0.5 μg Ag/ml.

In Table II the water solubility data and MIC values of the silver compounds are summarized. The solubility of the silver compounds as compared with AgSD increases by a factor of 5 to 10 times. The MIC values of the three silver compounds as compared with AgSD are slightly higher against *St. aureus*. The MIC value of 12.5 μg/ml for AgSD is in good agreement with the values observed by CARR *et al.* (1973). It is of interest to note that the MIC values are much higher than the water solubilities. As was discussed by BULT (1982) dissociated Ag is the primary active species of AgSD and it is obvious that this holds true for the other silver compounds.

This study demonstrates that it is possible to prepare more water soluble silver compounds closely related to sulfadiazine. For the study of the therapeutic utility of these silver compounds an extensive examination of the microbiological and pharmacological properties is necessary. Also it is possible to extend the series of more water soluble sulfadiazine derivatives. Especially HMSAP is a promising compound suitable for derivatization *e.g.* substitution of the methyl group by other small (hydrophilic) groups.

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