AMIDES OF ANTIBIOTIC STREPTONIGRIN AND AMINO DICARBOXYLIC ACIDS OR AMINOSUGARS

SYNTHESIS AND BIOLOGICAL EVALUATION

Sir:

Streptonigrin has been shown to be a potent cytotoxic agent, with broad spectrum activity in a number of murine tumor models[1]. Recently it was shown that streptonigrin is a nonintercalative antibiotic, which induces mammalian topoisomerase II dependent DNA cleavage[2]. It is a strong inhibitor of the reverse transcriptase associated with avian myeloblastosis virus and human immunodeficiency virus[3]. However, the severe side effects of streptonigrin, mainly bone marrow depression, have resulted in the discontinuation of its clinical use as an antitumor compound.

Here we report on the preparation and biological evaluation of the amides of streptonigrin and amino dicarboxylic acids or aminosugars, which may be of interest, as they should be more water-soluble and possibly less toxic.

In the synthesis of streptonigrin amides we used the *N*-hydroxysuccinimide ester of streptonigrin (**2**), which represents a convenient agent for the preparation of polyfunctional derivatives as in this case there is no need to protect hydroxy groups of aminosugars.

Streptonigrin (**1**) and *N*-hydroxysuccinimide in THF in the presence of DCC provided **2** (Tables 1 and 2). **2** was purified by column chromatography on Sephadex LH-20 using chloroform - methanol (7 : 3) as the eluant. Interaction of **2** with dibenzyl L-aspartate or dibenzyl L-glutamate *p*-toluene sulfonates in DMF in the presence of *N*-methylmorpholine gave amides **3** or **4**, which were purified by column chromatography (Kieselgel 60) with chloroform - petroleum ether (3 : 1) as the eluant. **3** and **4** were deblocked by hydrogenation over Pd to give *N*-streptonigrin-2′-oyl-L-aspartic (**5**) or *N*-streptonigrin-2′-oyl-L-glutamic (**6**) acid (Scheme 1).

The amides of streptonigrin and aminosugars (**7**～**11**) were obtained by the interaction of **2** with 2-amino-2-deoxy-D-glucose, 2-amino-2-deoxy-D-galactose, 6-amino-D-fucose, methyl 2,6-dideoxy-6-amino-β-D-glucopyranoside or *N*-methyl-1-deoxy-1-amino-D-glucitol in a 1 : 1 mixture of methanol with DMF (Scheme 2). After 72 hours the reaction mixture was evaporated *in vacuo* at 30°C and azeotroped with toluene (several times). The residue was purified by gel filtration on a Sephadex LH-20 column using chloroform - methanol (7 : 3) as eluant (Table 1). Compound **2** did not interact with 2,3,4,6-tetra-*O*-acetyl-1-mercapto-D-galactose or 2,3,4,6-tetra-*O*-benzoyl-β-D-galactopyranosyl amine, which suggests that these mercapto or amino groups do not possess the necessary nucleophilicity.

The structures of amides **3**, **4**, **5** and **6** were confirmed by ^{1}H NMR and EI-MS. Previously we used a similar method for the preparation of amides of streptonigrin and anthracycline antibiotics daunorubicin, carminomycin or doxorubicin[4], as well as for the amides of D- and L-CH_3SCH_2-$SCH_2CH(NH_2)COOCH_3$[5]. All these compounds

Table 1. Preparation and physical properties of compounds **2**～**11**.

Compound	Yield (%)	MP (°C)	Calcd			Molecular formula	Found			Rf[a] (System)	EI-MS[b] $(M)^+$
			C	H	N		C	H	N		
2	85.0	260	57.71	4.17	11.60	$C_{29}H_{25}N_5O_{10}$	57.63	4.19	11.52	0.65 (A)	
3	50.0	112								0.83 (B)	
4	37.4	90～92								0.80 (B)	
5	46.0	159～161				$C_{30}H_{29}N_5O_{11}$				0.18 (B)	635
6	44.0	181～183				$C_{29}H_{27}N_5O_{11}$				0.23 (B)	621
7	63.5	108～110	55.77	4.98	10.49	$C_{31}H_{33}N_5O_{12}$	55.25	4.99	10.23	0.25 (B)	
8	60.0	116～118	55.77	4.98	10.49	$C_{31}H_{33}N_5O_{12}$	55.39	5.02	10.15	0.22 (B)	
9	65.0	164～166	55.77	4.98	10.49	$C_{31}H_{33}N_5O_{12}$	55.32	5.01	10.27	0.44 (B)	
10	79.0	156～158	57.74	5.30	10.52	$C_{32}H_{35}N_5O_{11}$	57.23	5.38	10.29	0.45 (B)	
11	85.0	142～145 (dec)	56.22	5.46	10.25	$C_{32}H_{37}N_5O_{12}$	56.08	5.50	10.11	0.20 (B)	

[a] Kieselgel 60 plates (Merck, BRG), in chloroform - acetone - methanol 16 : 1 : 1 (A system) or 8 : 1 : 1 (B system).
[b] Varian-MAT-112 spectrometer at 210～230°C and 70 eV electron energy.

Table 2. ^{1}H NMR spectral data of derivatives **2**~**11** (VXR-400 Varian instrument).

Compound (isomer)	Ratio of a/b or α/β isomers	δ ppm (J Hz) 4-H	3-H	12′-H	11′-H	CH_2CH_2 or α-CH or 1″-H ($J_{1'',2''}$)	CH_3O	CH_3N	3′-CH_3	Solvent
2		9.01 (8.0) d 1H	8.38 d 1H	6.80 (8.5) d 1H	6.67 d 1H	2.92 s 4H	4.07 3.99 3.95 s 3H each		2.35 s 3H	$CDCl_3$
3		8.98 (8.4) d 1H	8.41 d 1H	6.98 (8.7) d 1H	6.70 d 1H	4.78 dt 1H	3.98 3.87 3.80 s 3H each		2.43 s 3H	$CDCl_3$
4		8.74 (8.4) d 1H	8.40 d 1H	6.94 (8.7) d 1H	6.44 d 1H	4.77 dt 1H	4.03 3.99 3.85 s 3H each		2.40 s 3H	$CDCl_3$
5	3:1									DMSO-d_6
5 (a)		8.96 (8.4) d 1H	8.34 d 1H	6.93 (8.7) d 1H	6.70 d 1H	4.75 dt 1H	3.84 3.81 3.75 s 3H each		2.25 s 3H	
5 (b)		8.98 (8.4) d 1H	8.36 d 1H	6.95 (8.7) d 1H	6.71 d 1H					
6	2:1									DMSO-d_6
6 (a)		8.92 (8.4) d 1H	8.32 d 1H	6.91 (8.7) d 1H	6.68 d 1H	4.51 dt 1H	3.86 3.82 3.76 s 3H each		2.43 s 3H	
6 (b)		8.94 (8.4) d 1H	8.34 d 1H	6.92 (8.7) d 1H	6.69 d 1H					
7	1:2									DMSO-d_6 + D_2O
7 (α)		8.75 (8.4) d 1H	8.39 d 1H	6.94 (8.7) d 1H	6.70 d 1H	5.17 (4.0) dd 0.35H	3.79 3.75 3.72 s 3H each		2.23 s 3H	
7 (β)						5.06 (7.2) dd 0.65H				
8	1:3									DMSO-d_6 + D_2O
8 (α)		8.80 (8.4) d 1H	8.38 d 1H	6.85 (8.7) d 1H	6.66 d 1H	5.23 (3.9) dd 0.25H	3.80 3.76 3.72 s 3H each		2.25 s 3H	
8 (β)						5.17 (7.0) dd 0.75H				

Table 2. (Continued)

Compound (isomer)	Ratio of a/b or α/β isomers	δ ppm (J Hz) 4-H	3-H	12'-H	11'-H	CH_2CH_2 or α-CH or 1''-H ($J_{1'',2''}$)	CH_3O	CH_3N	3'-CH_3	Solvent
9	1:2									DMSO-d_6+
9 (α)		8.95 (8.4) d 1H	8.39 d 1H	6.90 (8.7) d 1H	6.72 d 1H	4.82 (3.9) dd 0.35H	3.85 3.81 3.75 s 3H each		2.23 s 3H	D_2O
9 (β)						4.76 (7.0) dd 0.65H				
10		8.86 (8.4) d 1H	8.30 d 1H	6.90 (8.7) d 1H	6.70 d 1H	4.48 ($J_{1'',2''a}$ 8.9) ($J_{1'',2''b}$ 0.9) t 1H	3.86 3.80 3.75 3.38 s 3H each		2.26 s 3H	MDSO-d_6+ CF_3COOH
11	3:2									DMSO-d_6+
11 (a)		8.79 (8.4) d 0.6H	8.37 d 0.6H	6.79 (8.7) d 0.6H	6.73 d 0.6H		3.88 3.86 3.78 s 3H each	2.98 s 1.8H	1.915 s 1.8H	CF_3COOH
11 (b)		8.72 (8.4) d 0.4H	8.30 d 0.4H	6.76 (8.7) d 0.4H	6.70 d 0.4H			3.10 s 1.2H	1.907 s 1.2H	

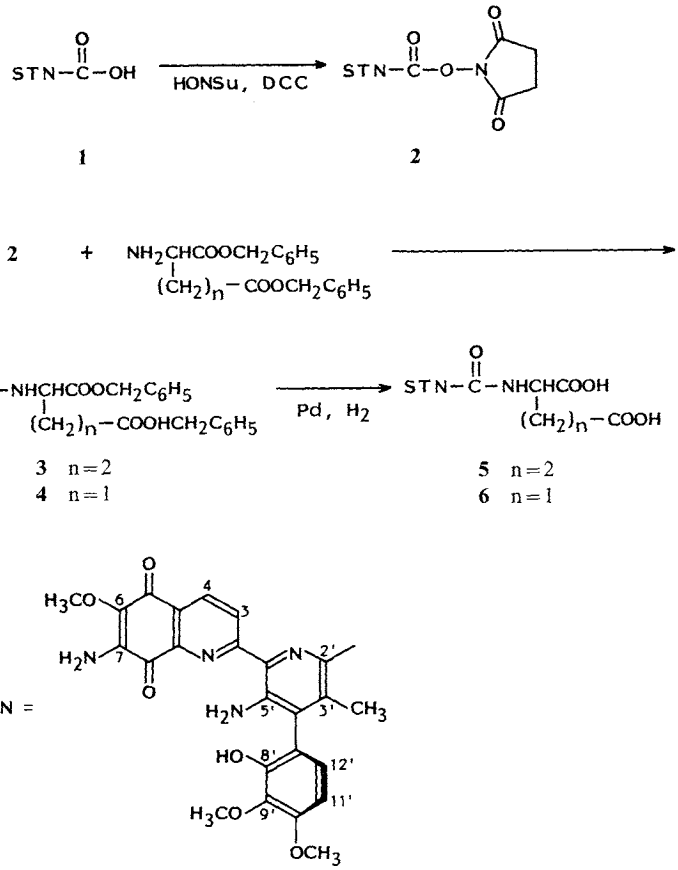
were isolated as individual stereoisomers, as confirmed by TLC and ^{1}H NMR methods. Recently, TAKE *et al.*[6] described an α-amino acid derivative of streptonigrin that consisted of two isomers which could not be distinguished in terms of biological activity and physicochemical properties such as ^{1}H NMR, UV and IR spectra, although they had different Rf values. CD spectra of these compounds demonstrated that they were diastereoisomers. It was postulated that these isomers differed in the chirality of the core portion of the streptonigrin moiety.

We did not succeed in the chromatographic separation of the isomeric amides of **1** with amino dicarboxylic acids or aminosugars. However, ^{1}H NMR spectra of amides **5** and **6** (with amino dicarboxylic acids) and non-mutarotating sugars (**10** and **11**) demonstrate the presence of two isomers. These amides were obtained under mild conditions in aprotic solvents. Racemization of the streptonigrin moiety in assymmetric amides is in agreement with the data of TAKE *et al.*[6].

^{1}H NMR spectra indicated that the amides **7**, **8** and **9** obtained from mutarotating sugars were mixtures of α- and β-anomers. Therefore it proved difficult to identify signals of the isomers differing in streptonigrin chirality. For the amide **11** the presence of two isomers as detected by the ^{1}H NMR spectrum, could be explained not only by racemization of the streptonigrin core but also by hindered rotation around C–N amide bond. It is known that rotation barrier values for secondary amides (V= 19~22 kcal/mol) are similar to rotation barrier values for diphenic acid derivatives (V=19~30 kcal/mol)[7].

The inhibitory effects of streptonigrin (**1**) and compounds **5**~**11** on the proliferation of murine leukemia (L1210), human T-lymphoblast (MOLT-4F) and human T-lymphocyte (MT-4) cells are shown in Table 3. The assays for measuring inhibition of tumor cell growth and HIV-1 cytopathicity in MT-4 cells were performed as previously described[8,9]. All new amides (**5**~**11**) were markedly less cytostatic than **1**. Interestingly, these amides were 50- to 100-fold more inhibitory to MT-4 than L1210 or MOLT-4F cell proliferation. In fact, the cytostatic data obtained for L1210 or MOLT-4F cells have been obtained upon

Scheme 1.



Scheme 2.

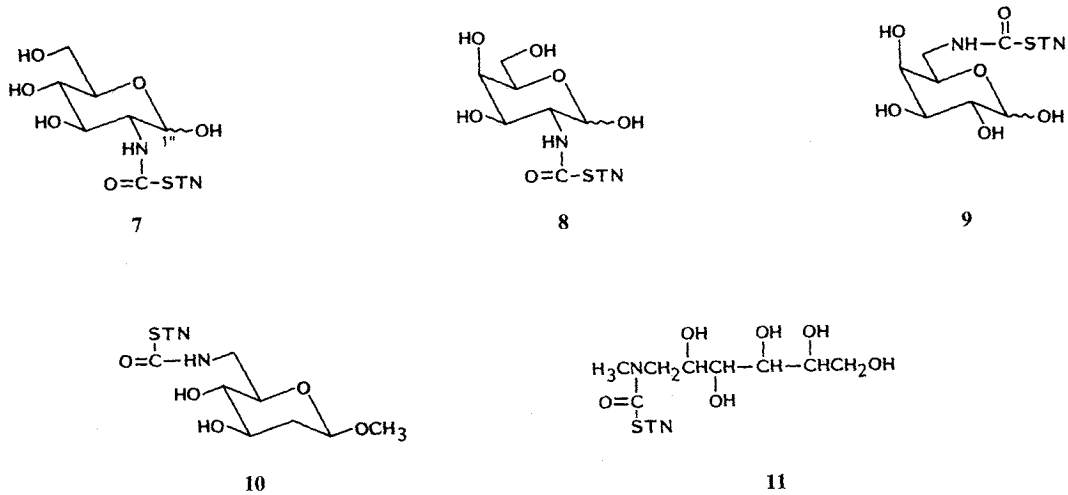


Table 3. Inhibitory effects of streptonigrin (**1**) and compounds **5**~**11** on the proliferation of murine leukemia (L1210), human T-lymphoblast (MOLT-4F) and human T-lymphocyte (MT-4) cells.

Compound	Inhibition of cell proliferation IC* (μM)		
	L1210	MOLT-4F	MT-4
1	0.044±0.009	0.004±0.0004	0.0002±0.0001
5	42±3.8	>100	2.0±0.48
6	53±3.8	>100	1.7±0.73
7	>100	>100	1.6±0.59
8	48±17.7	57±7.1	1.1±0.13
9	34±5.1	57±13.1	1.2±0.02
10	2.3±0.08	2.81±0.01	0.32±0.26
11	>100	>100	2.3±0.42

* 50% inhibitory concentrations, or concentrations required to inhibit cell proliferation by 50%.

Table 4. Therapeutic efficacy of streptonigrin (**1**) and its amide **11** in rausher leukemia virus (RLV)-infected mice.

Compound	Dose μg/kg/day	Therapeutic effect		Toxic effect	
		Weight of spleen (mg)	Number of colonies per spleen	DRD (%)	Alteration in body weight (%)
		mean±SD (%)		mean±SD	
1 (STN-COOH)	12.5	280±79[a] (35)[b]	40±36 (40)[b]	0	101±2
	50.0	228±29[a] (47)	33±16[a] (51)	0	98±4
	200.0	132±28[a] (70)	11± 6 (83)[b]	0	89±6[a]
	800.0	No survivors	No survivors	100	Toxic
11	12.5	317±65[a] (26)	43±18 (36)	0	104±9
	50.0	410±55 (4)	33± 9[a] (50)	0	98±3
	200.0	292±114[a] (32)	56±15 (16)	0	96±3[a]
	800.0	325±53 (24)	58±20 (14)	0	96±5[a]
Control	—	429±118 (100)	67±18 (100)		107±2
Control (virus uninfected)		184±51 —	— —	—	102±2

RLV-containing serum was dissolved 1:10 in HANK's solution. Female BALB/c mice were inoculated intravenously (iv) with 0.2 ml of the viral inoculum on day 0. Drugs were dissolved in 0.9% saline solution and administered daily (by the intraperitoneal (ip) route) starting 5 hours after virus inoculation and continuing for 7 days. All the mice were sacrificed on the 7th day after virus inoculation and were examined for body weight, spleen weight and number of colonies (on the spleen surface).

DRD: drug related deaths. SD: standard deviation.

[a] Statistically significant difference between treated and control groups.

[b] In parentheses, % reduction in a spleen enlargement and the number of colonies.

incubation of tumor cells for 2 and 3 days, respectively. The cytostatic data obtained for MT-4 cells were calculated after a 5 day-incubation period. The longer exposure time of the test compounds to the cells may make them more susceptible to the cytostatic effects of the compounds. Alternatively and/or additionally, MT-4 cells might have a higher intrinsic sensitivity than the other tumor cell lines to the cell growth-inhibitory properties of the compounds. Compounds **5**~**11** did not inhibit the replication of HIV-1 or HIV-2 MT-4 cells at subtoxic concentrations (data not shown).

In vivo antiviral activity against rausher leukemia virus (RLV) was monitored by mortality (until the 7-day period), change of body weight over the 7-day period, spleen weight, and spleen colony formation (Table 4)[10]. Splenomegaly induced by RLV infection in BALB/c mice, as well as the number of colonies on the spleen surface, were suppressed following treatment with compound **1**. The effect of **1** was clearly dose-dependent in the range of doses varying from 12.25 μg/kg/day to

800 μg/kg/day. When administered at a dose as high as 800 μg/kg/day STN-COOH caused death of all mice within 7 days. Amide **11** brought about a significant reduction in spleen weight at doses of 12.5 and 200 μg/kg/day and a significant reduction in the number of colonies (on the spleen surface) at a dose of 50 μg/kg/day). In contrast with **1**, compound **11** was non-toxic at a dose of 800 μg/kg/day (Table 4).

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