Figure 1 is a computer-generated drawing of **5** showing its absolute stereochemistry. All bond distances and angles are within chemically reasonable limits. With the α carbon atom of the methoxyphenylthiolacetate group of **5** known to be *R*, the other chiral carbon atom (C-19) was found to have the *S* configuration.

Preparation of Enantiomeric Dithioacetals (+)-7 and (−)-7. To a −20 °C solution of **4** (0.5 mmol, 290 mg) in dry pyridine (3 mL) was added dropwise a 1 M solution of hydrazine in tetrahydrofuran (0.5 mmol, 500 μL). After 0.5 h *tert*-butyldiphenylsilyl acrylate (1.5 mmol, 465 mg) was added and the mixture was allowed to react for 4 h. It was then quenched with 25% aqueous ammonium acetate (20 mL), extracted with ethyl acetate, washed with brine, dried over sodium sulfate, filtered, and evaporated to dryness with use of toluene to remove pyridine to provide crude **6**. To the cooled (−5 °C) crude **6** in THF (5 mL) was added 1 M n-Bu_4NF in THF (500 μL) and the mixure was stirred 1 h. It was quenched with 25% aqueous ammonium acetate and the organic layer processed as before to give a residue which was purified by chromatography over SiO_2 using acetone–toluene–acetic acid (30:70:0.5) to yield the product (+)-7 (200 mg, 80%): $[\alpha]^{25}_D$ +1.2° (c = 1, acetone) (lit $[\alpha]^{25}_D$ −5.2° ((c = 2, CH_2CL_2)[9]).[11] Anal. ($C_{25}H_{24}ClNO_4S_2$) C, H, N, S.

Similarly **5** (58 mg) afforded (−)-7 (23 mg): $[\alpha]^{25}_D$ −2.7° (c = 1.5, acetone).

Preparation of Enantiomeric Dithioacetals (+)-8 and (−)-8. To a (−5 °C) solution of (+)-7 (0.1 mmol, 50 mg) in dichloroethane (1 mL) was added 1,1′-carbonyldiimidazole (0.11 mmol, 18 mg). After 0.5 h the mixture was warmed to room temperature for a further 0.25 h and then cooled again to −5 °C. A 1.7 M toluene solution of dimethylamine (1 mmol, 590 μL) was added and the mixture was stirred 0.75 h at −5 °C and then 0.25 h at room temperature. The reaction mixture was then evaporated to dryness and purified by chromatography over SiO_2 using ethyl acetate–hexanes (3:1) to yield the product (−)-8 (42 mg, 80%): $[\alpha]^{25}_D$ = −4.8° (c = 1.6, acetone) (lit. $[\alpha]^{25}_D$ = −4.2° (c = 1.28, acetone);[8] $[\alpha]^{25}_D$ = −5.1° (c = 2, THF)[9]).

Similarly (−)-7 (23 mg) afforded (+)-8 (16 mg): $[\alpha]^{25}_D$ = +4.1° (c = 1.6, acetone) (lit. $[\alpha]^{25}_D$ = +3.5° (c = 1.74, acetone);[8] $[\alpha]^{25}_D$ = +5.0° (c = 2, THF)[9]).

Supplementary Material Available: Tables containing the detailed X-ray experimental, fractional coordinates, temperature parameters, bond distances, and bond angles for 5 (6 pages). Ordering information is given on any current masthead page.

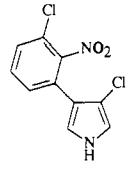
Synthesis and Antimicrobial and Cytotoxic Activities of Pyrrole-Containing Analogues of Trichostatin A

S. Massa,† M. Artico,*,† F. Corelli,‡ A. Mai,† R. Di Santo,† S. Cortes,§ M. E. Marongiu,§ A. Pani,§ and P. La Colla§

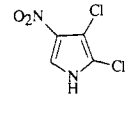
Dipartimento di Studi Farmaceutici, Università di Roma "La Sapienza", p.le Aldo Moro 5, 00185 Roma, Italy, Dipartimento Farmaco-Chimico-Tecnologico, Università di Siena, v. Banchi di Sotto 55, 53100 Siena, Italy, and Dipartimento di Biologia Sperimentale, Sezione di Microbiologia, Università di Cagliari, v. Porcell 4, 09124 Cagliari, Italy. *Received April 18, 1989*

A number of aroylpyrroleacrylic acid derivatives were synthesized by standard procedures and evaluated for cytotoxicity in Vero cells and for capacity to inhibit the multiplication of viruses, bacteria, and fungi. While none of the test compounds showed any activity against bacteria and fungi, most of them inhibited the replication of some DNA viruses at concentrations allowing the exponential growth of uninfected cells. In particular three compounds (**8**, **9c**, and **10h**) showed an antiviral activity at doses that were from 4- to >8-fold lower than the maximum nontoxic doses.

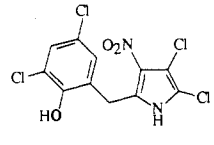
Various naturally occurring antibiotics have been found to display antimycotic activity. Pyrrolnitrin (**1**),[1] pyrrolomycins A (**2**) and B (**3**),[2,3] and the antibacterial pyoluteorin (**4**)[4] belong to the class of pyrrole derivatives that,



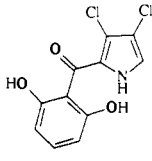
1 Pyrrolnitrin



2 Pyrrolomycin A



3 Pyrrolomycin B



4 Pyoluteorin

since 1965, has been subjected to extensive structural modifications. None of these analogues, however, showed better properties than pyrrolnitrin, which remains the sole therapeutically useful compound.

More recently, trichostatin A (**5**),[5] an antimycotic agent isolated from some strains of *Streptomyces hygroscopicus*, has attracted the attention of chemists[5,6] because of its peculiar structure possessing an unusual *p*-(dimethylamino)benzoyl group and an hydroxamic acid function. Interestingly, in addition to the antimycotic activity, trichostatin A, its glucoside trichostatin C (**6**), and trichostatic acid (**7**) have also been reported to be strong differentiation inducers of Friend leukemia cells.[6-10]

Since we have been engaged in the development of pyrrolnitrin analogues,[11,12] we examined the possibility of

†Università di Roma.
‡Università di Siena.
§Università di Cagliari.

(1) Imanaka, H.; Kousaka, M.; Tamura, G.; Arima, K. *J. Antibiot.* **1965**, *18*, 207.
(2) Ezaki, N.; Shomura, T.; Koyama, M.; Niwa, T.; Kojima, M.; Inouye, S.; Ito, T.; Nida, T. *J. Antibiot.* **1981**, *34*, 1363.
(3) Kaneda, M.; Nakamura, S.; Ezaki, N. *J. Antibiot.* **1981**, *34*, 1366.
(4) Tsuji, N.; Kobayashi, M.; Nagashima, K.; Wachisaka, Y.; Koizumi, K. *J. Antibiot.* **1976**, *29*, 1.
(5) Fleming, I.; Iqbal, J.; Krebs, E.-P. *Tetrahedron* **1983**, *31*, 1506.
(6) Mori, K.; Koseki, K. *Tetrahedron* **1988**, *44*, 6013.
(7) Yoshida, M.; Nomura, S.; Beppu, T. *Cancer Res.* **1987**, *47*, 3688.
(8) Morioka, H.; Ishihara, M.; Takezawa, M.; Shibai, H.; Komoda, Y. *Agric. Biol. Chem.* **1988**, *52*, 583.
(9) Yoshida, M.; Iwamoto, Y.; Uozumi, T.; Beppu, T. *Agric. Biol. Chem.* **1985**, *49*, 563.
(10) Morioka, H.; Ishihara, M.; Takezawa, M.; Hirayama, K.; Suzuki, E.; Komoda, Y.; Shibai, H. *Agric. Biol. Chem.* **1985**, *49*, 1365.
(11) Artico, M.; Nacci, V.; Filacchioni, G.; Chimenti, F. *Ann. Chim. (Rome)* **1968**, *58*, 1370.
(12) Artico, M.; Filacchioni, G.; Nacci, V.; Chimenti, F.; Giardina, M. C. *Farmaco, Ed. Sci.* **1970**, *25*, 651.

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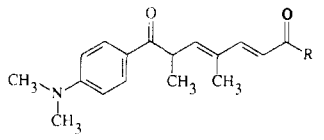
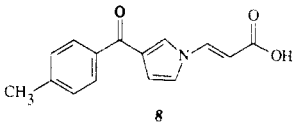


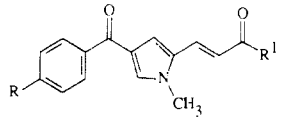
5 R = NHOH trichostatin

6 R = NHO-β-D-glucose trichostatin C

7 R = OH trichostatic acid



8



	R	R^1
9	H, NO_2, Cl, F, OCH_3, $N(CH_3)_2$, imidazole	OC_2H_5
10	H, NO_2, Cl, F, OCH_3, $N(CH_3)_2$, imidazole, NH_2	OH
11	H, NO_2, Cl, F	NHOH

incorporating a pyrrole moiety into the structure of trichostatin A in an attempt to enhance the antimycotic potency of each of the parent antibiotics and, possibly, to abate their toxicity.

For a better understanding of the structure–activity relationships of the trichostatin-like derivatives described in the present paper, the substituents R at benzene ring were chosen in a spectrum ranging from electron-donating to electron-withdrawing groups. Furthermore, in a project addressed at exploring new aspects of the biological potential of these compounds, in addition to antibacterial and antimycotic tests we also performed antiviral assays.

Chemistry

Ethyl *trans*-3-[1-methyl-4-(4-substituted-benzoyl)-1*H*-pyrrol-2-yl]propenoates (**9a–g**) were prepared by reacting 1-methyl-4-(4-substituted-benzoyl)-1*H*-pyrrole-2-carboxaldehydes (**12a–g**) with triethyl phosphonoacetate in absolute ethanol containing anhydrous potassium carbonate (Scheme I).

Aldehydes **12a–e** were obtained in one-pot Vilsmeier–Haack and Friedel–Crafts sequential reactions starting from 1-methylpyrrole in DMF with addition of oxalyl chloride, aluminum trichloride, and the appropriate aroyl chloride (Scheme II). Reductive methylation of **12b** gave aldehyde **12f**. The reaction of **12d** with imidazole in the presence of sodium hydride gave **12g**.

Alkaline hydrolysis of esters **9a–h** with 2 N potassium hydroxide in ethanol by heating the solution at 70 °C for 3 h afforded the corresponding acids **10a–h**.

The reaction of the acids **10a–d** with oxalyl chloride–DMF in methylene chloride followed by treatment with hydroxylamine hydrochloride in the presence of triethylamine led to the formation of derivatives **11a–d**.

The reduction with stannous chloride of nitro ester **9b** led to the related amino derivative **9h** (Scheme III).

Compound 8 was described previously by us.[13]

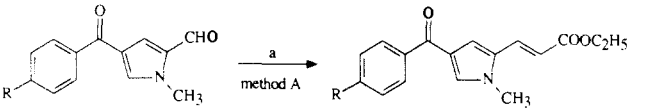
Results and Discussion

The aroylpyrroleacrylic acid derivatives described herein were subjected to the following in vitro assays: cytostatic activity in Vero cells (both the MNTD and the TD_{50} were determined), antiviral activity against four DNA viruses and one RNA virus (two reference antiviral drugs, acycoguanosine (ACG) and adenine arabinoside (ara-A), have been also included), antibacterial activity against two Gram-positive and one Gram-negative bacteria, antimycotic activity against a recent clinical isolate of *Candida albicans*.

Table II summarizes the results obtained in cytotoxicity and antiviral assays. As shown, a good correlation could be found between the cytotoxicity of the compounds and the type of substituents R and R^1.

No matter what the type of substituent R was, the most toxic compounds were those with $R^1 = OC_2H_5$ and NHOH (TD_{50} = 4–30 μg/mL), while the least toxic compounds were those with R^1 = OH (TD_{50} = 60–>1000 μg/mL). The sole exception to the correlation between cytotoxicity and

Scheme I[a]

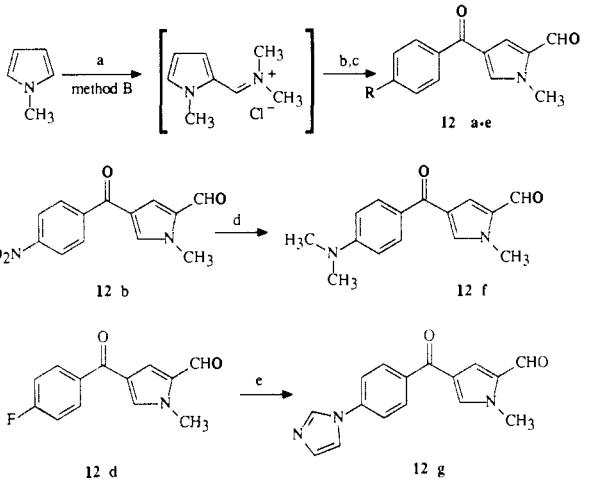


12 a-g 9 a-g

R= H (a), NO_2 (b), Cl (c), F(d), OCH_3 (e), $N(CH_3)_2$ (f), imidazole (g)

[a] (a) $(C_2H_5)_2P(O)CH_2COOC_2H_5$, K_2CO_3.

Scheme II[a]

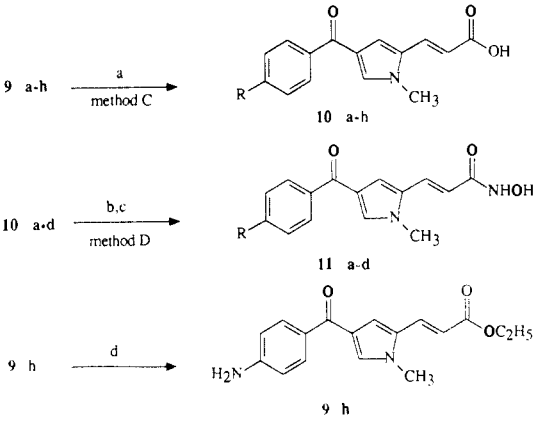


12 a-e 12 b 12 f 12 d 12 g

R = H (a), NO_2 (b), Cl (c), F (d), OCH_3 (e)

[a] (a) $(COCl)_2$, DMF; (b) aroyl chloride, $AlCl_3$; (c) 50% NaOH; (d) CH_2O, H_2 (Pd/C); (e) 80% NaH, imidazole.

Scheme III[a]



10 a-h 11 a-d 9 h

R = H (a), NO_2 (b), Cl (c), F(d), OCH_3 (e), NH_2 (f), $N(CH_3)_2$ (g), imidazole (h)

[a] (a) 2 N KOH; (b) $(COCl)_2$, DMF; (c) H_2NOH·HCl, $N(C_2H_5)_3$; (d) $SnCl_2·2H_2O$.

(13) Corelli, F.; Massa, S.; Stefancich, G.; Mai, A.; Artico, M.; Panico, S.; Simonetti, N. *Farmaco, Ed. Sci.* **1987**, *42*, 893.

Table I. Chemical and Physical Data for the Pyrrole-Containing Analogues of Trichostatin A

compd	R	R^1	formula	mp, °C	recrystn solvent[a]	% yield	anal.
9a	H	OC_2H_5	$C_{17}H_{17}NO_3$	88–90	A	84	C, H, N
9b	NO_2	OC_2H_5	$C_{17}H_{17}NO_3$	162–164	D	95	C, H, N
9c	Cl	OC_2H_5	$C_{17}H_{16}ClNO_3$	113–115	C	81	C, H, Cl, N
9d	F	OC_2H_5	$C_{17}H_{16}FNO_3$	80–82	E	97	C, H, F, N
9e	OCH_3	OC_2H_5	$C_{18}H_{19}NO_4$	100–102	A	80	C, H, N
9f	$N(CH_3)_2$	OC_2H_5	$C_{19}H_{22}N_2O_3$	135–137	A	87	C, H, N
9g	imidazole	OC_2H_5	$C_{20}H_{19}N_3O_3$	178–179	A	88	C, H, N
9h	NH_2	OC_2H_5	$C_{17}H_{18}N_2O_3$	oil		42	C, H, N
10a	H	OH	$C_{15}H_{13}NO_3 \cdot 0.25\ H_2O$	176–177	D	79	C, H, N
10b	NO_2	OH	$C_{15}H_{12}N_2O_5$	268–269 dec	F	50	C, H, N
10c	Cl	OH	$C_{15}H_{15}ClNO_3$	214–217	D	45	C, H, Cl, N
10d	F	OH	$C_{15}H_{12}FNO_3 \cdot H_2O$	198–200	F	70	C, H, F, N
10e	OCH_3	OH	$C_{16}H_{15}NO_4$	210–211	F	61	C, H, N
10f	$N(CH_3)_2$	OH	$C_{17}H_{18}N_2O_3 \cdot 0.3\ H_2O$	224–226	F	97	C, H, N
10g	imidazole	OH	$C_{18}H_{15}N_3O_3 \cdot H_2O$	272–275	F	90	C, H, N
10h	NH_2	OH	$C_{15}H_{14}N_2O_3 \cdot 0.25\ H_2O$	242–245	F	73	C, H, N
11a	H	NHOH	$C_{15}H_{14}N_2O_3$	181–182 dec	D	55	C, H, N
11b	NO_2	NHOH	$C_{15}H_{13}N_3O_5$	215–217	B	52	C, H, N
11c	Cl	NHOH	$C_{15}H_{13}ClN_2O_3$	189–190	D	49	C, H, Cl, N
11d	F	NHOH	$C_{15}H_{13}FN_2O_3$	192–194	D	76	C, H, F, N
12a	H		$C_{13}H_{11}NO_2$	107–109	A	40	C, H, N
12b	NO_2		$C_{13}H_{10}N_2O_4$	200–202	B	63	C, H, N
12c	Cl		$C_{13}H_{10}ClNO_2$	137–140	C	44	C, H, Cl, N
12d	F		$C_{13}H_{10}FNO_2$	120–122	C	60	C, H, F, N
12e	OCH_3		$C_{14}H_{13}NO_3$	133–135	D	52	C, H, N
12f	$N(CH_3)_2$		$C_{15}H_{16}N_2O_2$	159–160	D	55	C, H, N
12g	imidazole		$C_{16}H_{13}N_3O_2$	186–188	D	72	C, H, N

[a] Recrystallization solvents: A, benzene; B, *N,N*-dimethylformamide; C, benzene–cyclohexane; D, ethanol; E, diethyl ether-petroleum ether; F, *N,N*-dimethylformamide–water.

Table II. Cytotoxic and Antiviral Activities of Pyrrole-Containing Analogues of Trichostatin A

compd	R	R^1	MNTD,[a] μg/mL	TD_{50},[b] μg/mL	ED_{50},[c] μg/mL HSV-1	Vaccinia	ASFV	Adeno	VSV
8			30	125	100	100	8	60	>125
9a	H	OC_2H_5	2	4	30	30	30	30	30
9b	NO_2	OC_2H_5	2	8	125	60	60	ND[d]	ND[d]
9c	Cl	OC_2H_5	250	500	350	500	>500	30	>1000
9d	F	OC_2H_5	4	15	10	10	10	50	50
9e	OCH_3	OC_2H_5	8	15	15	20	8	4	15
9f	$N(CH_3)_2$	OC_2H_5	2	4	15	15	8	15	8
9g	imidazole	OC_2H_5	15	30	500	>500	30	15	500
10a	H	OH	250	>250	>500	>500	250	250	500
10b	NO_2	OH	15	60	50	15	50	50	>100
10c	Cl	OH	30	125	500	100	>250	>500	250
10d	F	OH	15	60	60	100	30	50	>50
10e	OCH_3	OH	500	>500	>500	500	350	250	1000
10f	$N(CH_3)_2$	OH	125	>250	>250	>250	>250	125	>500
10g	imidazole	OH	125	>250	>250	>250	250	250	250
10h	NH_2	OH	>1000	>1000	>1000	1000	250	1000	>1000
11a	H	NHOH	15	30	>30	>30	>30	>30	>30
11b	NO_2	NHOH	15	30	15	15	25	25	>50
11c	Cl	NHOH	15	30	30	30	20	15	>30
11d	F	NHOH	8	15	10	15	12	>50	>50
ACG			40	240	0.02	>110	40	ND[d]	>40
ara-A			6	10	10	16	40	ND[d]	>10

[a] Maximum nontoxic dose (compound concentration that allowed exponential cell growth for three cell cycles). [b] Toxic dose 50 (compound concentration that reduced by 50% the number of cells with respect to untreated controls). [c] Effective dose 50 (compound concentration that reduced by 50% the number of plaques with respect to untreated, virus-infected controls). [d] Not determined.

the type of R^1 substituent was compound **9c**, in which the presence of a chlorine atom in R abated the cytotoxicity related to the presence of $R^1 = OC_2H_5$.

Among compounds with R^1 = OH, the degree of toxicity was highly variable, depending on the substituent R. The most toxic compounds were those with $R = NO_2$ (TD_{50} = 60 μg/mL); the least toxic were those with $R = NH_2$ (TD_{50} = >1000 μg/mL). It is perhaps worthy of note that compound **9d**, the ester having a fluorine atom in R, was considerably more toxic than compound **9c**. The rule that the chloro derivative is less cytotoxic than the corresponding fluoro derivative was also true for the compounds having R^1 = NHOH (**11c** and **11d**) and R^1 = OH (**10c** and **10d**). In both cases, however, the decrease in toxicity was less dramatic than that obtained in the case of compound **9c**.

As far as the antiviral activity was concerned, several compounds were effective in inhibiting one or more of the DNA viruses tested at concentrations that were nontoxic for uninfected cells, but none was active against VSV, the reference RNA virus. Most compounds had a selectivity index (ratio $MNTD/ED_{50}$) equal to or only slightly higher than 1 (i.e. **9e**, **9g**, **10a**, **10b**, **10e**, **10f**, **11b**, **11c**). However, compounds 8 and **10h** showed a selectivity index greater

than 4 against ASFV, while compound **9c** was the most selective against the adenovirus (si > 8).

No activity whatsoever could be seen when the compounds were tested in antimicrobial assays, at least at concentrations equal to the TD_{50} for Vero cells (results not shown).

Quantitative structure–activity relationship studies (QSAR) of the above compounds were not the object of the present work. However, qualitatively speaking, a good correlation between toxicity and electronic effect of the substituents linked at the benzene ring was observed. In fact, an increase in the electron-donating properties of the substituents leads to less toxic compounds.

Experimental Section

Chemistry. Melting points (Büchi 530 melting point apparatus) are uncorrected. IR spectra (Nujol mulls) were recorded on a Perkin-Elmer 297 instrument. ^1H NMR spectra were recorded at 90 MHz on a Varian EM-390 spectrometer. Me_4Si was used as an internal reference standard. All compounds were routinely checked by TLC and ^1H NMR. NMR data were consistent with the indicated structures. TLC was performed with C. Erba silica gel Stratocrom SIF-254 precoated plates. Developed plates were visualized by UV light. Solvents were reagent grade and, when necessary, were purified and dried by standard methods. Concentration of solutions after reactions and extractions involved the use of a rotary evaporator operating at a reduced pressure of approximately 20 Torr. Organic solutions were dried over anhydrous sodium sulfate. Microanalyses (within ±0.4% of the theoretical values) were performed by the Microanalytical Laboratory of Prof. A. Pietrogrande, University of Padova, Italy.

Syntheses. Specific examples presented below illustrate general synthetic methods A–D. In general, samples prepared for physical and biological studies were dried in high vacuum over P_2O_5 for 20 h at temperatures ranging from 25 to 110 °C, depending on the sample melting point. Despite these measures, some compounds remained hydrated.

Method A Example. Ethyl *trans*-3-[1-Methyl-4-(4-nitrobenzoyl)-1*H*-pyrrol-2-yl]propenoate (9b). A suspension of **12b** (1.5 g, 5.8 mmol) in absolute EtOH (20 mL) was added in one portion to a mixture of triethyl phosphonoacetate (1.6 g, 7 mmol) and anhydrous potassium carbonate (2.4 g, 17.4 mmol). After stirring at 70 °C for 2 h, the reaction mixture was cooled to room temperature and diluted with water (50 mL). The precipitate which formed was filtered and washed to give **9b** (1.87 g) that was homogeneous by TLC analysis (SiO_2/ethyl acetate): ^1H NMR (DMSO-d_6) δ 1.22 (t, 3 H, $COOCH_2CH_3$), 3.78 (s, 3 H, CH_3), 4.15 (q, 2 H, $COOCH_2CH_3$), 6.46 (d, 1 H, J = 15.8 Hz, =CHCOOEt), 7.28 (m, 1 H, pyrrole β-proton), 7.51 (d, 1 H, J = 15.8 Hz, CH=CHCOOEt), 7.69 (m, 1 H, pyrrole α-proton), 7.95 (d, 2 H, J = 8.8 Hz, benzene H-2,6), 8.32 (d, 2 H, J = 8.8 Hz, benzene H-3,5).

Method B Example. 4-(4-Fluorobenzoyl)-1-methyl-1*H*-pyrrole-2-carboxaldehyde (12d). To a cooled (0–5 °C) solution of DMF (0.78 mL, 10 mmol) in 1,2-dichloroethane (20 mL) was added a 20-mL 1,2-dichloroethane solution of oxalyl chloride (1.27 g, 10 mmol) over a period of 5–10 min. After stirring at room temperature for 15 min, the suspension was cooled (0–5 °C) again and treated with a solution of 1-methylpyrrole (0.81 g, 10 mmol) in 1,2-dichloroethane (20 mL). The mixture was stirred at room temperature for 15 min and then treated with aluminum trichloride (2.92 g, 22 mmol) and 4-fluorobenzoyl chloride (1.59 g, 10 mmol). After 3 h, the reaction mixture was poured onto crushed ice (100 g) containing 50% NaOH (10 mL) and stirred for 10 min. The pH of the solution was adjusted to 4 with 37% HCl, the organic layer was separated, and the aqueous one was extracted with chloroform (2 × 20 mL). The combined organic solution was washed with water, dried, and evaporated to dryness. Recrystallization of the residue from cyclohexane/benzene gave **12d** (1.38 g): ^1H NMR ($CDCl_3$) δ 4.07 (s, 3 H, CH_3), 7.0–7.6 (m, 4 H, pyrrole protons and benzene H-3,5), 7.8–8.0 (m, 2 H, benzene H-2,6), 9.68 (s, 1 H, CHO).

Method C Example. *trans*-3-[4-[4-(Dimethylamino)benzoyl]-1-methyl-1*H*-pyrrol-2-yl]propenoic Acid (10f). A mixture of **9f** (0.9 g, 2.8 mmol), 2 N KOH (5.6 mL, 11 mmol), and EtOH (15 mL) was heated at 70 °C for 3 **h** while being stirred. The solution was poured into water (50 mL) and extracted with ethyl acetate. HCl (2 N) was added to the aqueous layer until the pH was 5 and the precipitate was filtered and recrystallized to give pure **10f** (0.8 g): ^1H NMR (DMSO-d_6) δ 3.0 (s, 6 H, $N(CH_3)_2$), 3.76 (s, 3 H, CH_3), 6.30 (d, 1 H, J = 15.8 Hz, =CHCOOH), 6.74 (d, 2 H, J = 9 Hz, benzene H-3,5), 7.13 (m, 1 H, pyrrole β-proton), 7.48 (d, 1 H, J = 15.8 Hz, CH=CHCOOH), 7.57 (m, 1 H, pyrrole α-proton), 7.73 (d, 2 H, J = 9 Hz, benzene H-2,6) 12.2 (br s, 1 H, COOH).

Method D Example. *trans*-*N*-Hydroxy-3-[1-methyl-4-(4-nitrobenzoyl)-1*H*-pyrrol-2-yl]propenamide (11b). All hydroxamates described in this paper were prepared by using the method reported by Summer et al.[14] Oxalyl chloride (1.1 mL, 12.8 mmol) was added slowly to a cooled (0 °C) solution of **10b** (1.7 g, 5.7 mmol) and DMF (0.44 mL, 5.7 mmol) in CH_2Cl_2 (100 mL). Gas evolution was noted during this addition. After being stirred for 40 min, this solution was added to a solution of hydroxylamine hydrochloride (1.58 g, 22.8 mmol) and triethylamine (4.8 mL, 34.2 mmol) in THF (30 mL)/water (15 mL). After being stirred for an additional 30 min, the mixture was poured into 2 N HCl and extracted with CH_2Cl_2. The organic phase was dried and evaporated to afford a solid residue, which was recrystallized to give pure **11b** (1.5 g): ^1H NMR (DMSO-d_6) δ 3.75 (s, 3 H, CH_3), 6.31 (d, 1 H, J = 15.7 Hz, =CHCO), 6.94 (s, 1 H, pyrrole β-proton), 7.34 (d, 1 H, J = 15.7 Hz, CH=CHCO), 7.63 (s, 1 H, pyrrole α-proton), 7.95 (d, 2 H, J = 8.4 Hz, benzene H-2,6), 8.33 (d, 2 H, J = 8.4 Hz, benzene H-3,5), 9.02 (s, 1 H, NH), 10.67 (s, 1 H, OH).

4-[4-(Dimethylamino)benzoyl]-1-methyl-1*H*-pyrrole-2-carboxaldehyde (12f). A mixture of **12b** (1.3 g, 5.0 mmol), 40% aqueous formaldehyde (1 mL), 10% Pd/C (200 mg), and acetic acid (60 mL) was hydrogenated in a Parr apparatus at 50 psi and 50 °C for 5 h. The catalyst was filtered off and the solution was poured onto crushed ice (200 g). After neutralization with 6 N NaOH, the oily precipitate was taken up in ethyl acetate. The organic solution was dried and evaporated to give a solid residue, which was chromatographed on an alumina column eluting with $CHCl_3$. Evaporation of central eluates furnished a solid containing minor impurities. Recrystallization from EtOH gave pure **12f** (0.7 g).

4-[4-(1*H*-Imidazol-1-yl)benzoyl]-1-methyl-1*H*-pyrrole-2-carboxaldehyde (12g). A solution of **12d** (0.6 g, 2.6 mmol) and imidazole (0.27 g, 3.9 mmol) in DMSO (10 mL) was added over a period of 45 min to a suspension of 80% NaH (70 mg, 3.1 mmol) in 3 mL of the same solvent.

The mixture was heated at 120 °C overnight and then cooled to room temperature and diluted with water (200 mL). The product was taken up into ethyl acetate (2 × 30 mL). The aqueous layer was made basic again with 6 N NaOH and the product which separated was extracted into ethyl acetate. After drying and evaporating, pure **12g** was obtained (0.5 g).

Ethyl *trans*-3-[4-(4-Aminobenzoyl)-1-methyl-1*H*-pyrrol-2-yl]propenoate (9h). A solution of **9b** (4.0 g, 12.2 mmol) in EtOH (30 mL) was added dropwise to a solution of $SnCl_2 \cdot 2H_2O$ (9.74 g, 43.2 mmol) in 37% HCl (10 mL). After heating at 90 °C for 30 min, the reaction was diluted with water (100 mL) and made basic with 6 N NaOH. The product was extracted into $CHCl_3$ and the organic solution was washed with brine (3 × 30 mL), dried, and evaporated to dryness. The oily residue was chromatographed on alumina eluting with ethyl acetate. The first fractions were discarded and the central ones were evaporated to give **9h** (1.6 g) as a light yellow oil.

Cytotoxicity Evaluation. The effect of drugs on cell multiplication was determined as described previously.[15] Briefly, exponentially growing Vero cells were treated with various concentrations of each compound. After incubation at 37 °C in 5% CO_2 for three cell cycles (3 days), viable cells were counted in a hemocytometer and both maximum nontoxic dose (MNTD) and 50% toxic dose (TD_{50}) of each compound were determined.

(14) Summer, Y. B.; Gum, B. P.; Mazdiyasni, H.; Goetze, A. M.; Young, P. R.; Bouska, J. B.; Dyer, R. D.; Brooks, D. W.; Carter, G. W. *J. Med. Chem.* **1987**, *30*, 2121.

(15) La Colla, P.; Gelli, G.; Mura, L.; Landini, M. P.; Corrias, M. V.; Pani, A.; Marongiu, M. E.; *Antivir. Res.* **1985**, *Suppl. 1*, 29.

Variation between duplicate samples was less than 10% (Table II).

Antiviral Activity Determination. The effect of drugs on viral replication was evaluated in plaque-reduction assays in Vero cells according to the procedure of Collins and Bauer.[16] Dose-response lines were drawn by linear-regression technique from which the 50% effective doses (ED_{50}) were calculated (Table II).

Antimycotic and Antibacterial Assays. Antifungal and antibacterial activities were evaluated on recent clinical isolates of *C. albicans, Staphylococcus aureus, Escherichia coli,* and group D *Streptococcus.* Tests were carried out in Sabouraud-dextrose broth, pH 5.6, in the case of fungi, and nutrient broth containing 5% NaCl, pH 7.2, in the case of bacteria. As for the above assays, compounds were solubilized in DMSO. The initial inoculum of bacteria was 10^3 cells; that of fungi was 10^4 cells. Minimum inhibitory concentrations (MIC) were determined after 18 (bacteria) or 24 h (fungi) of incubation at 37 °C in the presence of different concentrations of the compounds.

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(16) Collins, P.; Bauer, D. J. *N.Y. Acad. Sci.* **1977**, *284*, 49.

Synthesis and Antitumor Activity of 2-β-D-Ribofuranosyloxazole-4-carboxamide (Oxazofurin)[1]

Palmarisa Franchetti,† Gloria Cristalli,† Mario Grifantini,*,† Loredana Cappellacci,† Sauro Vittori,† and Giuseppe Nocentini

Dipartimento di Scienze Chimiche, Università di Camerino, 62032 Camerino, Italy, and Dipartimento di Medicina Sperimentale e Scienze Biochimiche, Sez. Farmacologia Speciale, Università di Perugia, 06100 Perugia, Italy.
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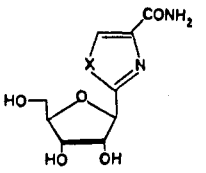
Condensation of 3,4,6-tri-*O*-benzoyl-2,5-anhydro-D-allonyl chloride (**4**) with ethyl 2-amino-2-cyanoacetate (**5**) provided 2-[(3′,4′,6′-tri-*O*-benzoyl-2′,5′-anhydroallonyl)amino]-2-cyanoacetate (**6**). Compound **6** was treated with hydrogen chloride gas to give ethyl 5-amino-2-(2′,3′,5′-tri-*O*-benzoyl-β-D-ribofuranosyl)oxazole-4-carboxylate (**8**). Reductive dediazotization of blocked nucleoside **8** provided ethyl 2-(2′,3′,5′-tri-*O*-benzoyl-β-D-ribofuranosyl)oxazole-4-carboxylate (**10**), which after deblocking with sodium methoxide and ammonolysis was converted to 2-β-D-ribofuranosyloxazole-4-carboxamide (oxazofurin, **3**), an analogue of the antitumor and antiviral C-nucleoside tiazofurin (**1**). Oxazofurin (**3**) was found to be cytotoxic toward B16 murine melanoma cells in culture but inactive against murine leukemia P388 and L1210.

C-glycosyl nucleosides which are analogues of nicotinamide nucleoside are expected to be converted to the analogues of NAD coenzyme and to inhibit NAD-dependent inosine monophosphate dehydrogenase (IMPD).[2] The inhibition of this enzyme produces the accumulation of IMP and the depletion of guanine nucleotides, which appeared to be linked to DNA synthesis inhibition. So, 2-β-D-ribofuranosylthiazole-4-carboxamide (tiazofurin, **1**)[3]



1 X= S, tiazofurin
2 X= Se, selenazofurin

and 2-β-D-ribofuranosylselenazole-4-carboxamide (selenazofurin, **2**),[4] which are metabolized to analogues of NAD, and thiazole-4-carboxamide adenine dinucleotide (TAD) and selenazole-4-carboxamide adenine dinucleotide (SAD), two strong inhibitors of IMPD, have pronounced antitumor activity in animals and broad-spectrum antiviral activity.[2]

Both tiazofurin and selenazofurin are highly active against Lewis lung carcinoma and P388 and L1210 leukemias in mice. Tiazofurin is also active in vivo against *ara*-C and cytoxan resistant lines of P388 leukemia and Glasgow osteogenic sarcoma. Selenazofurin is about 10 times more active than tiazofurin with a similar spectrum of antitumor activity.

A number of specific modifications of the parent tiazofurin structure have been reported.[5]

Structure-activity relationship studies revealed that the presence of a ring nitrogen adjacent to the carboxamide function and the β-ribofuranosyl moiety as the glycosyl component are the features necessary for biological activity. Substitution of the tiazole ring with isosteric ring systems such as selenazole and 1,2,4-oxadiazole[5] are allowed.

The present report describes the synthesis and biological evaluation of 2-β-D-ribofuranosyloxazole-4-carboxamide (oxazofurin, **3**), an analogue of tiazofurin in which the sulfur atom has been replaced with an oxygen.

Chemistry

The synthesis of oxazofurin (**3**) is outlined in Scheme I. Condensation of 3,4,6-tri-*O*-benzoyl-2,5-anhydro-D-allonyl chloride (**4**)[6a,b] with the ethyl 2-amino-2-cyanoacetate (**5**)[7] in anhydrous pyridine at room temperature gave ethyl 2-[(3′,4′,6′-tri-*O*-benzoyl-2′,5′-anhydroallonyl)-

† Università di Camerino.

(1) Presented in part at the 1er Congreso Conjunto Hispano-Italiano de Quimica Terapeutica, Granada, Spain, September 1989, Abstract P.B-023.
(2) Ahluwalia, G. S.; Jayaram, H. N.; Cooney, D. A. In *Concepts, Clinical Developments, and Therapeutic Advances in Cancer Chemotherapy*; Martinus Nijhoff Publishers: Boston, 1987; Chapter 3.
(3) Srivastava, P. C.; Pickering, M. V.; Allen, L. B.; Streeter, D. G.; Campbell, M. T.; Witkowski, J. T.; Sidwell, R. W.; Robins, R. K. *J. Med. Chem.* **1977**, *20*, 256.
(4) Srivastava, P. C.; Robins, R. K. *J. Med. Chem.* **1983**, *26*, 445.
(5) Avery, T. L.; Hermen, W. T.; Revankar, G. R.; Robins, R. K. in *New Avenues in Developmental Cancer Chemotherapy*; Harrap, K. R., Connors, T. A., Eds.; Academic Press, Inc.: Orlando, FL,1987; p 367.
(6) (a) Dan Cook, P.; Mc Namara, D. J. *J. Heterocycl. Chem.* **1986**, *23*, 155. (b) Bobek, M.; Farkas, *J. Collect. Czech. Chem. Commun.* **1969**, *34*, 247.
(7) Logemann, F. I.; Skaw, G. *Chem. Ind.* **1980**, *541*.

0022-2623/90/1833-2849$02.50/0