Experimenteller Teil

Synthese der Chinolinium-Verbindungen **1–3**

0,1 mol **4** wurden mit 0,1 mol Chinolin oder seinen Derivaten, die über dem Molekularsieb Typ 4A aufbewahrt wurden, 2 h bei 353K unter intensivem mechanischen Mischen erhitzt. Das Produkt wurde durch Hexenextraktion im Soxhlet gereinigt.

Flüssigkeits-Chromatographie

Chromatograph Liquochrom 307 (Ungarn). Analysengang: Säulenlänge: 0,2 m, Füllung der Säule: Lichrosorb RP-8 (5 µm), mobile Phase: Methanol, Druck: 100 at., UV-Detektion bei 258 nm.

Prüfung der antibakteriellen Wirkung

Die Untersuchungen wurden bereits in früheren Publikationen[1)] beschrieben.

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[Ph 906]

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Studies on Potential Antiviral Compounds, XXIV***

New 1-Substituted Isatin β-thiosemicarbazones**

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Some new 1-substituted isatin β-thiosemicarbazones were examined for their ability to inhibit the growth of the wild-type strain of vaccinia virus and the isatin β-thiosemicarbazone (IBT) resistant mutant of this virus. Also investigated was the capability of the compounds to support the growth of the IBT dependent mutant of vaccinia virus. The most active derivative is 1-(3-hydroxypropyl)isatin thiosemicarbazone **20**, which was shown to be more effective than IBT.

Untersuchungen über potentiell antivirale Verbindungen, 24. Mitt.: Neue 1-substituierte Isatin-β-thiosemicarbazone

Einige neue 1-substituierte Isatin-β-thiosemicarbazone wurden untersucht auf ihre Fähigkeit wilde Stämme und die IBT-resistente Mutante von Vaccinia Virus zu hemmen und auf ihr Vermögen, das Wachstum von IBT-abhängigen Mutanten zu fördern. Das aktivste Derivat der Reihe war 1-(3-Hydroxypropyl)isatinthiosemicarbazon **20**, das sich als wirksamer als das zum Vergleich verwendete Isatin-β-thiosemicarbazon (IBT) erwies.

In extension of our research on the antiviral activity of heterocyclic carbonyl thiosemicarbazones, particularly of the indole series, we applied previously obtained results to the isatin series for two reasons: a) the use of a heterocyclic, the thiosemicarbazone of which has monopolized the interest of investigators because of its activity against pox viruses[1]; and b) we wished to confirm the effectiveness of substituents such as the meta-substituted benzoyl group, which is very effective in the indole series[2-4]. A comparison of the results obtained from the two series could lead to useful conclusions regarding the biological equivalence of the two groups of heterocyclic compounds.

Discussion

Meta substitution in the benzoyl group seems to be an essential requirement for the inhibition of the growth of vaccinia virus, a member of the pox viruses group, caused by 1-benzoylisatin and 1-benzoyl-2-chloro-3-formylindole thiosemicarbazones. Table 2 shows that only the isatin derivatives **15** and **18** display anti-vaccinia virus properties, unlike the corresponding ortho – and para – substituted compounds; the effectiveness of this substitution appears to be lower than in the indole series. Moreover, the finding that 1-(3-chlorobenzoyl)isatin thiosemicarbazone (**12**, Table 2), unlike the analogous indole **22**, is devoid of antiviral activity suggests that the nature of the substituent plays a very important role in the isatin series.

It appeared to us that it would be interesting to complete this study by evaluating the activity of 1-(3-hydroxypropyl)isatin thiosemicarbazone (**20**; Table 2) in comparison with that of the analogous indole[5] and IBT. The results were very satisfactory in that **20** showed greater inhibition of vaccinia virus growth than the reference compound IBT.

Thiosemicarbazones **15, 18, 20, 21** and **22** (Table 2) did not effect formation of plaques of the IBT-resistant mutant and were also able to support the growth of the IBT-dependent mutant of vaccinia virus. Compounds **11, 12, 13, 14, 16, 17** and **19** failed to inhibit the plaque formation of the wild-type strain of vaccinia virus even et a concentration of 50 μg/ml, the highest concentration used, which did not exhibit visible toxicity to the cells. The latter seven compounds also failed to support the growth of the IBT-dependent mutant.

The thiosemicarbazones described in the present study show that the inhibition of the growth of the wild-type strain, resistance of the IBT-resistant mutant and supporting of the growth of the IBT-dependent mutant, are three characteristics which are connected with one other. In this respect they behave similarly to the thiosemicarbazones we had previously characterized[6-8]. Until now we had not found a single thiosemicarbazone compound which would inhibit the wild-type strain of vaccinia virus and to which the IBT-resistant mutant shows resistance that would not supoort the growth of the IBT dependent mutant.

The remarkable agreement between structural features and biological properties, which has also been ascertained for 1-alkylderivatives[9], suggests a similar mechanism of antiviral activity exhibited by the two series of thiosemicarbazones.

Experimental Part

Elementary analyses: Laboratory for Microanalysis of the Faculty of Pharmacy of the University of Pisa, Italy. *Mp's:* not corr. (Electrothermal MP Apparatus). *TLC:* Baker-Flex Silica Gel IB2-F, ethyl acetate/petroleum ether 3 : 7 v/v and acetone/petroleum ether 1 : 1 v/v (for the thiosemicarbazones). *IR spectra:* Perkin-Elmer 177 (nujol mull technique). *^{1}H-NMR spectra:* Varian EM-390 NMR. Spectra agreed with the proposed structures.

Virology. The thiosemicarbazones listed in Table 2 were examined for their ability to inhibit the growth of the wild-type strain of vaccinia virus and the IBT-resistant mutant[10] of this virus, as well as for their ability to support the growth of the IBT-dependent mutant[10] of vaccinia virus, BGM, an african green monkey kidney cell-line[11], was grown in RPMI 1640 medium, supplemented with 5 % inactivated calf serum in 50 mm diameter plastic petri dishes. The cultures were incubated at 37°C in a humidified atmosphere supplied with 5 % CO_2. Cell monolayers were infected with 0.3 ml of virus suspension containing approximately 300 plaque-forming units. After 1 h at 37°C, the cells were overlaid with Eagle's medium containing 0.7 % Agar Noble, 5 % inactivated calf serum and the examined compound. The cultures were further incubated for 5 d at 37°. The cells were then fixed with 20 % formalin in buffered saline, stained with crystal-violet (0.1 % in 0.1 M-citric acid) and plaques were observed.

1-(Substituted-benzoyl)isatins **1–9** (Table 1)

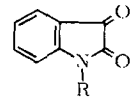
6.7 mmol of the appropriate benzoyl halide was added dropwise to a suspension of 1 g (6.7 mmol) isatin in 8 ml pyridine cooled to 0°C. After stirring at 0°C for 1 h the mixture was poured onto ice and water and filtered. Crystallization solvents and mp of the compounds are listed in Table 1. 1-(3-methoxybenzoyl)isatin **(8)** was better obtained from the corresponding benzoyl halide in 1,2-dimethoxyethane by the NaH method[13].

1-(3-Hydroxypropyl)isatin **(10)** (Table 1)

A suspension of 1 g (6.7 mmol) isatin and 0.326 g (6.8 mmol) NaH (50 % dispersion in mineral oil) in 5 ml anhydrous DMF at room temp. was stirred for 30 min. 1.27 ml (13.6 mmol) 3-bromo-1-propanol was then added. After heating at 150° for 15 min the mixture was poured onto ice and water then extracted with 3 × 20 ml ether. The combined extract, when washed with water, dried (Na_2SO_4) and evaporated, yielded a semisolid residue (0.9 g; 64 %), which was used directly in the subsequent reaction.

Thiosemicarbazones. The thiosemicarbazones listed in Table 2 were prepared using a standard method[12].

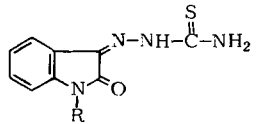
Table 1: *1-Substituted Isatins*



Comp.	R	Formula	Calc.	Found.	MP°	** crystn. solvent	Yield %
1	2Cl-C_6H_4CO	$C_{15}H_8ClNO_3$	C 63,1 H 2,82 N 4,9	63,2 2,69 4,8	158–160	A	67
2	3Cl-C_6H_4CO	$C_{15}H_8ClNO_3$	C 63,1 H 2,82 N 4,9	63,1 2,69 4,8	187–189	A	80
3	4Cl-C_6H_4CO	$C_{15}H_8ClNO_3$	C 63,1 H 2,82 N 4,9	63,1 2,56 4,7	180–182	B	44
4	2CH_3-C_6H_4CO	$C_{16}H_{11}NO_3$	C 72,4 H 4,18 N 5,3	72,6 3,97 5,1	158	B	53
5	3CH_3-C_6H_4CO	$C_{16}H_{11}NO_3$	C 72,4 H 4,18 N 5,3	72,5 4,1 5,2	145	B	47
6	4CH_3-C_6H_4CO	$C_{16}H_{11}NO_3$	C 72,4 H 4,18 N 5,3	72,5 4,02 5,2	162–164	B	51
7	2CH_3O-C_6H_4CO	$C_{16}H_{11}NO_4$	C 68,3 H 3,94 N 5,0	68,7 3,91 5,0	142	B	49
8	3CH_3O-C_6H_4CO	$C_{16}H_{11}NO_4$	C 68,3 H 3,94 N 5,0	68,0 3,92 4,9	157–159	B	38
9	4CH_3O-C_6H_4CO	$C_{16}H_{11}NO_4$	C 68,3 H 3,94 N 5,0	68,3 3,78 4,8	193–195	B	63
*10	$HOCH_2$-CH_2-CH_2	$C_{11}H_{11}NO_3$					

* Sticky product obtained according to a modified standard method[13] (see Exp. Part).
** A = ethyl acetate; B = ethyl acetate/petroleum ether.

Table 2: *Thiosemicarbazones*



Comp.	R	Formula	Calc.	Found	MP°	*** Recryst. solv.	Effect on the growth of vaccinia virus strains Wild-type $^{+}ED_{50}$ (µg/ml)	IBT-dependent $^{++}ED_{50}$(µg/ml)
11	2Cl–C_6H_4CO	$C_{16}H_{11}ClN_4O_2S$	C 55,5 H 3,09 N 15,6	55,1 2,94 15,3	158–160	A	---	---
12	3Cl–C_6H_4CO	$C_{16}H_{11}ClN_4O_2S$	C 55,5 H 3,09 N 15,6	55,3 2,84 15,2	275	A	---	---
13	4Cl–C_6H_4CO	$C_{16}H_{11}ClN_4O_2S$	C 55,5 H 3,09 N 15,6	55,1 2,78 15,4	260	A	---	---
14	2CH_3–C_6H_4CO	$C_{17}H_{14}N_4O_2S$	C 60,3 H 4,16 N 16,5	59,9 3,90 16,2	268	B	---	---
15	3CH_3–C_6H_4CO	$C_{17}H_{14}N_4O_2S$	C 60,3 H 4,16 N 16,5	60,1 4,03 16,3	137	C	6,25	6,25
16	4CH_3–C_6H_4CO	$C_{17}H_{14}N_4O_2S$	C 60,3 H 4,16 N 16,5	59,8 4,10 16,2	209	D	---	---

Fortsetzung **Tab. 2:**

Comp.	R	Formula	Calc.	Found	MP°	*** Recryst. solv.	Effect on the growth of vaccinia virus strains Wild-type $^{+}ED_{50}$ (μg/ml)	IBT-dependent $^{++}ED_{50}$(μg/ml)
17	$2CH_3O-C_6H_4CO$	$C_{17}H_{14}N_4O_3S$	C 57,6 H 3,98 N 15,8	57,3 3,62 15,5	190	E	---	---
18	$3CH_3O-C_6H_4CO$	$C_{17}H_{14}N_4O_3S$	C 57,6 H 3,98 N 15,8	57,2 3,52 15,4	182–183	E	12,50	12,50
19	$4CH_3O-C_6H_4CO$	$C_{17}H_{14}N_4O_3S$	C 57,6 H 3,98 N 15,8	57,5 3,71 15,7	205	E	---	---
20	$HO-CH_2-CH_2-CH_2$	$C_{12}H_{14}N_4O_2S$	C 51,7 H 5,06 N 20,1	51,5 4,94 19,9	168	E	0,20	0,39
21*							0,20	1,56
22**							1,56	1,56
IBT							0,78	1,56

* Thiosemicarbazone of 2-chloro-3-formyl-1-(3-hydroxypropyl)-1*H*-indole[5]

** Thiosemicarbazone of 2-chloro-1-(3-chlorobenzoyl)-3-formyl-1*H*-indole[2]

*** A = chloroform/petroleum ether; B = 95 % ethanol; C = DMSO/water; D = acetone/petroleum ether; E = ethyl acetate/petroleum ether

\+ determined by plaque reduction ++ lowest concentration supporting regular plaque size

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[Ph 907]

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Hydroxy-3-(hydroxyphenyl)indoles. Relationship between Structure and Estrogen Receptor Affinity

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Eingegangen am 1. Februar 1984

Syntheses and estrogen receptor affinities of the 1,2-dialkyl(hydroxy)-3-(hydroxyphenyl)indoles **11b–19b** are described. Derivatives **15b, 16b** with a hydroxy group at position 6 of the indole and an ethyl group at C-2 show a strong binding affinity with an RBA value of 0.72 (estradiol = 100).

**) Herrn Prof. Dr. *Helmut Schönenberger* zum 60. Geburtstag herzlichst gewidmet.

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