Dihydro-1,3-oxazine Derivatives and their Antitumor Activity

J. B. CHYLIŃSKA, T. URBAŃSKI,

Institute of Organic Chemistry, Polish Academy of Sciences, Warszawa 10, Poland

AND M. MORDARSKI

Institute of Immunology and Experimental Therapy, Polish Academy of Sciences, Wrocław, Poland

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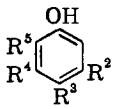
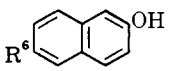
In continuation of earlier experiments[1a-d] on the carcinostatic activity of 1,3-oxazine derivatives, a number of dihydro-1,3-oxazine compounds condensed in the 5,6-positions with aromatic rings were prepared. They showed cytotoxic activity *in vitro* against amobarbital ascites sarcoma and Ehrlich ascites carcinoma. Some of the compounds show activity *in vivo* against the latter tumor.

Two recent papers[2,3] on dihydro-1,3-oxazines as antitumor agents have prompted us to report our own results on the subject, in continuation of previous work.[1a-d]

A number of phenols of the general formula I, β-naphthol derivatives (II), 2,7-dihydroxynaphthol, 4- and 5-hydroxyhydrindenes, and 2-hydroxyacridine have been condensed with formaldehyde and a number of



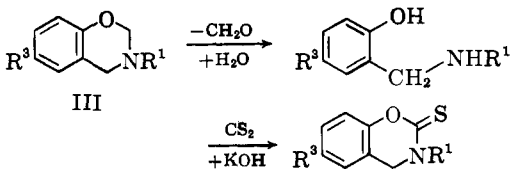
I



II

primary amines as described in the literature.[4] The formulas of dihydro-1,3-oxazine derivatives, with some experimental data such as reaction time, yields, melting points, and analyses of the products are given, in part, in Tables I–V.

Some 3,4-dihydro-1,3-benzoxazines (III) derived from certain phenols were used for preparing 2-thiono derivatives (IV).



III IV

The properties of these compounds with some experimental details are collected in Table V.

Experimental

Biological Methods.—Cytotoxic activity *in vitro* was estimated by a modification of Miyamura's test.[5] Two types of cells were employed in the experiments: Ehrlich's ascites carcinoma and amobarbital ascites sarcoma. Cells from the peritoneal exudate in mice, 10–12 days after transplanation, were used. The peritoneal exudate (with 5 μg./ml. of heparin added) was centrifuged and the separated cells were washed with .067 *M* phosphate buffer of pH 7.2 and then resuspended in a volume of Tyrode's solution sufficient to give suspensions containing 2×10^7 cells per ml. The cell suspensions were added to the medium (pH 7.2) containing 0.5% glucose, 0.25% NaCl, 0.3% K_2HPO_4, 1% peptone, and 15% heparinized sheep serum. The density of the tumor cell suspensions in the medium was *ca.* 2.4×10^6 cells per ml. The cell-containing medium was distributed in 1.5 ml. quantities in test tubes of 6 mm. diameter, and 0.5 ml. of the solution of the compound was added per tube; the same volume of physiological saline solution was added to the control tubes. The tubes were incubated for 6 hr. at 37°. The cells were then separated by centrifuging and washed 3 times with physiological saline. The washed cells were suspended in 2 ml. of the same medium, but with addition of 1% agar and methylene blue (0.85 ml. of a 0.5% solution of methylene blue per 100 ml. of medium), and again incubated for 3 hr. at 37°.

During incubation in the control tubes containing physiological saline instead of solutions of the compound, methylene blue is reduced by the tumor cells, only 2–3 mm. of the upper layer of the agar retaining the color of the dye due to secondary oxidation by atmospheric oxygen. In the tubes containing test compounds, the indicator dye remained unaltered (complete inhibition), or was only partly decolorized (partial or weak inhibition), depending on the concentration and activity of the compound. A tube of medium with methylene blue but not inoculated with cells constituted an additional control showing whether the medium alone was capable of reducing methylene blue. The results of these *in vitro* tests are collected in Table VI. Determination of the *in vivo* oncostatic activity of a few compounds was carried out with Ehrlich's ascites carcinoma model strain, in the form of subcutaneous tumor, instead of the usual exudative forms in the peritoneal cavity. The solid form of the tumors was produced by inoculating 10^6 exudate cells under the skin in male non-inbred albino mice (strain A or RIII) weighing 20–22 g.

The mice with implanted subcutaneous tumors were inspected after 2 weeks when the tumors were already palpable and their dimensions could be measured. Mice in which the tumor did not take, or with excessively large tumors, were rejected; however, with a dose of 10^6 exudate cells both these events were encountered but rarely. The animals qualifying for the experiments were divided into 2 groups, the controls comprising 12–15 mice, and the treated groups 10 mice each. The test compounds were administered subcutaneously to the abdomen in doses of 500 μg./mouse during 6 days per week for 2 weeks. The solvent used for preparing the solution of the compound in question was injected to the control groups. Forty-eight hours after the last injection of the compound, the mice were weighed, anesthetized with chloroform, the tumors were dissected and weighed separately. The experiments were repeated 4 times. Inhibition of growth of the tumors was expressed as percentage, according to the formula: % inhibition $= 100[(P - M)/P]$ where P is the average tumor weight in the control group, and M the average

(1) (a) T. Urbański, D. Gürne, Z. Eckstein, and S. Ślopek, *Bull. Acad. Polon. Sci., Cl.III*, **3**, 395 (1955); (b) T. Urbański, Cz. Radzikowski, Z. Ledóchowski, and W. Czarnocki, *Nature*, **178**, 1351 (1956); (c) T. Urbański, D. Gürne, S. Ślopek, H. Mordarska, and M. Mordarski, *Nature*, **187**, 426 (1960); (d) J. B. Chylińska and T. Urbański, *Bull. Acad. Polon. Sci., Ser. Chim.*, **7**, 635 (1959); (e) T. Urbański, D. Gürne, S. Ślopek, H. Mordarska, and M. Mordarski, *Nature*, **187**, 426 (1960); (f) S. Ślopek, H. Mordarska, M. Mordarski, T. Urbański, and D. Gürne, *Bull. Acad. Polon. Sci., Ser. Sci. Chim., Geol. Geograph.*, **6**, 361 (1958); (g) Z. Eckstein, P. Gluzinski, E. Grochowski, M. Mordarski, and T. Urbański, *Bull. Acad. Polon. Sci., Ser. Chim.*, **10**, 331 (1962); (h) T. Urbański, *Nature*, **168**, 562 (1951).

(2) M. E. Kuehne and E. A. Konopka, *J. Med. Pharm. Chem.*, **5**, 257 (1962).

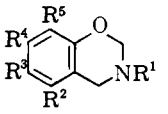
(3) M. E. Kuehne, E. A. Konopka, and B. F. Lambert, *ibid.*, **5**, 281 (1962).

(4) W. J. Burke, *J. Am. Chem. Soc.*, **71**, 609 (1949).

(5) S. Miyamura, *Antibiot. Chemother.*, **6**, 280 (1956).

TABLE I

PHYSICAL PROPERTIES OF DIHYDROBENZOXAZINE DERIVATIVES

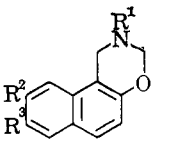


No.[a]	R^1	R^2	R^3	R^4	R^5	Reaction Time, hr. at b.p.	Yield, %	M.p., °C.	Formula	Calcd. C	Calcd. H	Calcd. N	Found C	Found H	Found N	Ref.
1	CH_3	H	Br	H	H	1	50	87-89.5	$C_9H_{10}BrNO$	47.4	4.4	6.1	47.6	4.6	6.2	
1c								185-187	$C_9H_{11}BrClNO$			5.5			5.3	
2	CH_3	H	Br	H	Br	1.5	35	77-79.5	$C_9H_9Br_2NO$	35.2	3.0	4.6	35.4	3.3	4.5	
2c								169-172	$C_9H_{10}Br_2ClNO$							
3	C_2H_5	H	Br	H	Br	1.5	30	57-60	$C_{10}H_{11}Br_2NO$	37.4	3.5	4.4	37.6	3.8	4.6	
3c								171-174	$C_{10}H_{12}Br_2ClNO$							
4	C_6H_{11}	H	CH_3	H	H	1.5	25	30-32	$C_{15}H_{21}NO$							1a
5c	C_6H_{11}	H	CH_3	H	CH_3			192-195	$C_{16}H_{24}ClNO$			4.6			4.9	4
6	C_6H_{11}	H	Br	H	H	1	40	89-92	$C_{14}H_{18}BrNO$							4, 1a
6c								240-243	$C_{14}H_{19}BrClNO$							4, 1a
7	C_6H_{11}	H	Cl	H	H	1	35	97-98	$C_{14}H_{18}ClNO$			5.6			5.7	6
7c								148-151	$C_{14}H_{19}Cl_2NO$			4.9			5.1	
8	C_6H_{11}	H	C_6H_5	H	H	1	55	70-72	$C_{20}H_{23}NO$			4.8			5.0	4
8c								135-137	$C_{20}H_{24}ClNO$							
9	$CH_2C_6H_5$	H	CH_3	CH_3	H	1	50	86-88	$C_{17}H_{19}NO$			5.5			5.6	1d
10	$CH_2C_6H_5$	H	H	CH_3	CH_3	1	60	71-73	$C_{17}H_{19}NO$							1d
10c								150-153	$C_{17}H_{20}ClNO$	70.5	6.7	4.8	70.7	7.0	5.1	1d
11	$CH_2C_6H_5$	CH_3	H	CH_3	H	1	90	94-97	$C_{17}H_{19}NO$			5.5			5.8	1s
12	$CH_2C_6H_5$	CH_3	H	C_2H_5	H	1	20	40-43	$C_{18}H_{21}NO$			5.2			5.5	1d
13	$CH_2C_6H_5$	H	C_6H_5	H	H	1	60	89-90	$C_{21}H_{19}NO$			4.7			4.8	
14	$CH_2C_6H_5$	H	Br	H	H	1	60	85-87	$C_{15}H_{14}BrNO$							1a
14c								182-184	$C_{15}H_{15}BrClNO$			4.1			4.2	1a
15	$CH_2C_6H_5$	H	Br	H	Br	1.5	30	74-76	$C_{15}H_{13}Br_2NO$	47.0	3.4	3.7	47.3	3.9	3.5	
15c								175-175.5	$C_{15}H_{14}Br_2ClNO$							
16	C_6H_4Br-*p*	H	CH_3	H	H	1	40	82-85	$C_{15}H_{14}BrNO$	59.2	4.6	4.6	59.4	4.8	4.8	7
17	C_6H_4Br-*p*	H	Br	H	H	1	65	109-111	$C_{14}H_{11}Br_2NO$	45.6	3.0	3.8	45.6	2.9	4.0	
18	C_6H_4Br-*p*	H	Br	H	Br	2	60	116-117.5	$C_{14}H_{10}Br_3NO$	37.5	2.2	3.1	38.0	2.3	3.2	
19	C_6H_4Br-*p*	H	Cl	H	H	1	45	87-89	$C_{14}H_{11}BrClNO$	51.7	3.4	4.3	51.7	3.4	4.4	7
20	C_6H_4Br-*p*	H	Cl	H	Cl	2	40	132-134	$C_{14}H_{10}BrCl_2NO$	46.8	2.8	3.9	47.2	2.8	3.9	

[a] Hydrochloride of the corresponding base denoted by c.

TABLE II

PHYSICAL PROPERTIES OF NAPHTHOXAZINE DERIVATIVES



No.[a]	R^1	R^2	R^3	Reaction Time, hr. room temp.	Yield, %	M.p., °C.	Formula	Calcd. C	Calcd. H	Calcd. N	Found C	Found H	Found N	Ref.
21				1[b]	80	67-68	$C_{13}H_{13}NO$			7.0			6.8	
21c	CH_3	H	H			185-187	$C_{13}H_{14}ClNO$							8,1b
22	CH_3	OH	H	24	98	210	$C_{13}H_{13}NO_2$	72.9	6.1	6.5	73.0	6.3	6.5	1d
23	C_2H_5	OH	H	24	87	190	$C_{14}H_{15}NO_2$			6.1			6.2	1d
24	$CH_2C_6H_5$	OH	H	24	96	139-140	$C_{19}H_{17}NO_2$	78.3	5.9	4.8	78.6	5.9	4.9	1d
25	$CH_2C_6H_5$	H	SO_3Na	24	75		$C_{19}H_{16}NNaO_4S$	60.5	4.3	3.7	60.7	4.9	4.0	1d
26	C_6H_4Br-*p*	H	H	24	90	118-119	$C_{17}H_{14}BrNO$							9
27	$C_6H_4CH_3$-*p*	H	H	24	85	86-88	$C_{19}H_{17}NO$			5.1			4.9	9

[a] Hydrochloride of corresponding base denoted by c. [b] At boiling point.

(6) R. H. Rigtering, U. S. Patents 2,806,031; 2,811,523 (1957).

(7) N. Noda, *J. Chem. Soc. Japan*, **62**, 743, 747 (1959); *Chem. Zentr.*, **131**, 7856, 7857 (1960).

(8) W. J. Burke, M. J. Kolbezen, and S. Wayne, *J. Am. Chem. Soc.*, **74**, 3601 (1952).

(9) W. J. Burke, K. C. Murdock and G. Ec, *ibid.*, **76**, 1677 (1954).

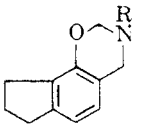
TABLE III

PHYSICAL DERIVATIVES OF 2,3,11,12-TETRAHYDRO-1*H*,10*H*-NAPHTHO[1,2-*e*:8,7-*e'*]BIS[1,3]-OXAZINE DERIVATIVES

No.	R	Reaction Time, hr. at b.p.	Yield, %	M.p., °C.	Formula	Calcd. C	Calcd. H	Calcd. N	Found C	Found H	Found N	Ref.
28	CH_3	1	45	171–173	$C_{16}H_{18}N_2O_2$			10.4			10.5	1d
29	C_2H_5	1	30	133–135	$C_{18}H_{22}N_2O_2$			9.4			9.7	1d
30	$CH_2C_6H_5$	1	40	123–124	$C_{28}H_{26}N_2O_2$	79.6	6.2	6.6	79.3	6.4	6.6	1d

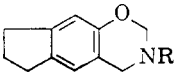
TABLE IV

PHYSICAL PROPERTIES OF 2,3,4,7,8,9-HEXAHYDROINDENO[5,4-*e*]-1,3-OXAZINES



No.	R	Reaction Time, hr. at b.p.	Yield, %	M.p., °C.	Formula	Calcd. C	Calcd. H	Calcd. N	Found C	Found H	Found N	Ref.
31	CH_3	1		97–99	$C_{12}H_{15}NO$			7.4			7.6	
32	$CH_2C_6H_5$	24[a]	45	77–78	$C_{18}H_{19}NO$	81.8	7.2	5.3	82.2	7.0	5.3	1d

2,3,4,6,7,8-HEXAHYDROINDENO[5,6-*e*]-1,3-OXAZINES

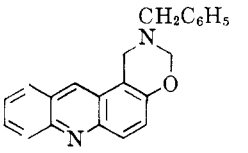


33	CH_3	1	26	89–92	$C_{12}H_{15}NO$	76.2	8.0	7.4	76.7	8.0	7.6	
34	C_2H_5	1	32	62–64	$C_{13}H_{17}NO$	76.8	8.4	6.9	77.2	8.3	6.7	
35	$CH_2C_6H_5$	24[a]	45	98–99	$C_{18}H_{19}NO$	81.8	7.2	5.3	81.6	7.1	5.6	1d

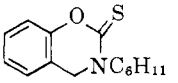
[a] At room temperature.

TABLE V

PHYSICAL PROPERTIES OF 2-BENZYL-2,3-DIHYDRO-1H[1,3]OXAZINO[5,6-*a*]ACRIDINE



No.	R	Reaction Time, hr. at b.p.	Yield, %	M.p., °C.	Formula	Calcd. C	Calcd. H	Calcd. N	Found C	Found H	Found N	Ref.
36	$CH_2C_6H_5$	24[a]	65	131–132	$C_{22}H_{18}N_2O$	81.0	5.6	8.6	81.1	6.0	8.8	1d



						C	H	N	Cl	C	H	N	Cl
37	CH_3	8	13	107–109	$C_{15}H_{19}NOS$	68.9	7.3	5.4		69.1	7.5	5.5	
38	Cl	10	8	169–170	$C_{14}H_{16}ClNOS$	59.7	5.7	5.0	12.6	59.7	6.0	5.2	12.7

[a] At room temperature.

tumor weight in the treated group. The results of the *in vivo* tests are collected in Table VII.

Results and Discussion

Out of 49 compounds examined *in vitro* (38 bases and 11 hydrochlorides) only 3 did not show any activity. Some of the active compounds, *e.g.*, No. 6c, 8c, 9, 13, 14c, and 36, showed very strong activity against both types of test tumor cells. Some of them were highly active against only one species, *e.g.*, No. 25 and 27. No general conclusion about the relation between the structure of the compounds and their cancerostatic activity could be drawn.

In vivo tests were carried out with compounds which showed strong or moderately strong *in vitro* activity and at the same time were not highly toxic and possessed relatively good solubility in water or propylene glycol. Toxicity data will be published later.

Compounds 6c and 10c show moderate (*ca.* 50%) inhibition of tumor growth. The strongest inhibition of 55 and 58% was obtained with compounds 14c and

TABLE VI

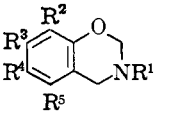
CYTOTOXIC ACTIVITY OF DIHYDROOXAZINE DERIVATIVES

No.[a]	Cytostatic concentration (μg./ml.) producing inhibition: Ehrlich ascites carcinoma, Complete	Ehrlich ascites carcinoma, Partial or weak	Amobarbital ascites sarcoma, Complete	Amobarbital ascites sarcoma, Partial or weak
1	1000		1000	
1c	1000		1000	
2	100		100	10
2c	1000	100	1000	
3	1000		1000	
3c	1000		1000	
4	1000		1000	100
5c	100		1000	100
6	1000		1000	
6c	100	10	100	10
7	100	10	1000	
7c	100	10	1000	100
8	1000		1000	
8c	100	10	100	10
9	100	10	100	10
10	100		100	
10c	100	10	100	
11	1000			10
12	100		100	
13	100	10	100	10
14c	100	10	100	10
15	1000		1000	
15c	100	10	100	10
16	100	10	100	
17	1000	10	1000	
18	1000	100	1000	100
19	100		100	
20	1000			
21	100		100	
21c				
22	100		0	
23	100		1000	100
24	1000	100		10
25	10		10	
26	1000		1000	10
27	10		1000	100
28	100		100	
29	1000	100	1000	
30	100		1000	
31	1000		1000	
32	100		100	
33				
34	1000		1000	
35	1000		1000	100
36	10		10	
37	1000	10	1000	10
38				

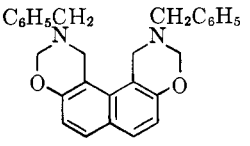
[a] Hydrochloride of corresponding base denoted by c.

TABLE VII

In Vivo ACTIVITY AGAINST EHRLICH ASCITES CARCINOMA



No.[a]	R[1]	R[2]	R[3]	R[4]	R[5]	Daily dose, mg./mouse	% inhibition	Weight reduction of mice, g.	t[b]	v[c]
14c	$CH_2C_6H_5$	H	H	Br	H	1.0	48	0	2.5	20
9	$CH_2C_6H_5$	H	CH_3	CH_3	H	1.0	0	0		
10	$CH_2C_6H_5$	H	H	CH_3	CH_3	1.0	0	0.6		
10c	$CH_2C_6H_5$	H	H	CH_3	CH_3	1.0	47	2.2	3.4	20
12	$CH_2C_6H_5$	H	C_2H_5	H	CH_3	0.5	19	1.2		
6c	C_6H_{11}	H	H	Br	H	1.0	55	0.7	4.0	20
7c	C_6H_{11}	H	H	Cl	H	1.0	0			



No.[a]	Daily dose, mg./mouse	% inhibition	Weight reduction of mice, g.	t[b]	v[c]
30	1.0	58	1.8	3.6	20

[a] Hydrochloride of corresponding base denoted by c. [b] Coefficient according to distribution of Student. [c] Number of degrees of freedom.

30, respectively. These substances will be used for a more detailed study.

The present experiments confirm our former results on the carcinostatic activity of benzhydro-1,3-oxazine derivatives[1b,c] and tetrahydro-1,3-oxazine derivatives.[1e,f,g] This seems to support a general conclusion that 1,3-oxazine derivatives are compounds exhibiting not only a definite biological activity[1h] but some of them are carcinostatic.