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STUDIES ON THE ANTIVIRAL ACTIVITY OF GUANYLHYDRAZONES ESPECIALLY AGAINST ARBO- AND MYXO-VIRUSES

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ABSTRACT

Alkoxy benzalacetone amidinohydrazones and N', N' anhydrobis (2-hydroxyetyl) amidinohydrazones were newly synthesized and antiviral effectiveness of these derivatives against influenza virus was investigated.

Out of 39 compounds, 31 derivatives (79.5 % of total compounds tested) were found to possess the virucidal activity, and 13 compounds (33.3 %) were of antiviral effectiveness against the virus with the chemotherapeutic ratio of 2 or greater. Five derivatives (#283, #284, #286, #299, and #300) have highly virucidal effectiveness to the virus with the minimal concentration as low as 1.6 γ/ml or 0.8 γ/ml in final. Three (#283, #284, and #300) out of five derivatives have been found to be active with one-sixtyfourth less concentration of their cytotoxic doses.

By the inhibition test, seven compounds (#268, #274, #275, #276, #279, #280, and #282) have antiviral activity to the virus with the final concentration of 12.5 γ/ml or less. A final concentration of 6.3 γ/ml of #268, and 3.2 γ/ml of #275 could completely inhibit the virus replication in the membrane culture system, and those concentrations were found to be a half of their contact- inhibitory concentrations.

CONTENTS

1. INTRODUCTION

During recent years we have been investigating the antiviral activities of amidinohydrazones, phenols, azlactones, hydrazones, and miscellaneous compounds against arbo- and myxo-viruses, among which several amidinohydrazones and phenols have been found to possess the inhibitory activity against influenza virus (1, 2, 3). The activity of these amidinohydrazones prompted us to investigate the antiviral effectiveness of newly synthesized alkoxy benzalacetone amidinohydrazones.

During the past year, 39 derivatives were synthesized. Thirty-one derivatives were found to be contact inhibitory (3) and 13 compounds were inhibitory to influenza virus. This final report will describe the results in progressive experiments of antiviral effectiveness of amidinohydrazones against the virus.

2. MATERIALS AND METHODS

Virus; Adachi strain of influenza virus type A_2 was used throughout the experiments described herein. One-tenth ml of 10 MID_{100} (Minimal HA Inducing Dosis of 100 percent) was the inoculum per membrane culture.

Chemical compounds; The compounds tested were synthesized, during the past year, at the Department of Organic Chemistry, Kitasato University School of Hygienic Sciences. A total of 39 amidinohydrazones listed in Table I were quantitatively determined both for toxic and antiviral inhibitory concentrations with the system of Maitland type membrane culture and influenza virus.

Methods and materials for the chorio-allantoic membrane culture, influenza virus titration, and HA test were fully reported eleswhere (1, 2). For the determination method for cytotoxicity of compounds

was used the same dilution technique as described previously. Following two methods for determinating antiviral activity of each compound were employed; 1) Contact inhibition test (formerly named antiviral activity or inhibition in the previous reports) was performed with the same determination technique. The inoculum into membrane cultures was the mixture kept at room temperature for 30 minutes of same volumes of virus and appropriately diluted compound solution to be tested, and 2) Inhibition test is that one-tenth ml of virus and diluted test samples are simultaneously inoculated into membrane cultures instead of inoculating the mixture. The calculation of the antiviral inhibitory concentration of each derivatives was exactly identical to the contact inhibition test fully mentioned eleswhere (1. 2).

3. RESULTS

Effectiveness of 39 compounds; the hydrochloride was dissolved in 50 % aqueous glycerol and the free base was dissolved in distilled water by adding 1N hydrochloric acid and compound solutions were heated at 121°C for 15 minutes prior to use. The minimal concentrations of 100 % inhibitory and of 100 % cytotoxic activity were determined for each derivative by two-fold serial dilutions. Each concentration was calculated from results with 4 cultures. Appropriate controls were made in parallel with tests. The results are summarized in Table 2. The numbers shown in the column A, B, and C are the minimal concentrations of complete inhibition by the contact inhibition test and the inhibition test, and of 100 % toxicity. The numbers in the column D are indicating the cytotoxic/virucidal ratio of the number at the column A to the column C, and the column E presents the effective ratio of the number in the column B to the

number in the column C.

Compounds listed in Table 2 can be classified by their ratios into 5 groups as shown in Table 3 and in Table 4. As can be seen in Table 3, the numbers of derivatives showing the ratio 1 or less, 2, from 4 to 8, 16 to 32, and 64 or greater were 8(20.5 %), 9(23 %), 13(33.2 %), 5(12.7 %) and 4(10.2 %), respectively. The numbers of compounds in Table 4 giving the ratio tested by the inhibition test, 1 or less, 2, 4 to 8, and 16 or greater were 19(48.7 %), 8(20.5 %), 4(10.3 %) and 1(2.5 %) in order.

Out of total derivatives, 31 compounds (79.5 %) resulted from the contact inhibition test were found to possess virucidal action against influenza virus with ratio of 2 or greater, and only 13 compounds (33.3 %) determined by the inhibition test were completely antiviral inhibitory with the ratio of 2 or greater. One derivative, serial number 275, is effective to the virus with the high ratio of 16 or greater as demonstrated by both testing techniques.

Two minimal inhibitory concentrations of each compound resulted from the tests by two different procedures were compared. The comparative ratio of virucidal concentration in the column B to the antiviral inhibitory concentration in the column A is withdrawn. All derivatives tested are grouped with reference of the ratio and presented in Table 5. It is worth to note that two derivatives, serial number #268 and #275, inhibited the growth of influenza virus at a final concentration of 6.3 γ/ml, respectively, and were found to possess the two-fold higher antiviral inhibitory effectiveness than direct-contact action.

Further studies on the mode of action, chemotherapeutic effect of those fruitful antiviral derivatives in ovo, in vitro, and in

vivo systems to be infected with arbo- and other myxoviruses remain to be done.

4. SELECTED BIBLIOGRAPHY

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ble 1. List of Compounds

Serial#	Amidino hydrazone HCl
266*	P- Methoxy benzalacetone
267	O- Methoxy benzalacetone
268	P- Methoxy benzalacetone
269*	P- Propoxy benzalacetone
270	O- Methoxy -α- methyl benzalacetone
271*	O- Ethoxy benzalacetone
272*	O- Hydroxy benzalacetone
273	α- Methyl -P- hydroxy benzalacetone
274	α- Methyl -P- methoxy benzalacetone
275	P- Butoxy benzalacetone
276*	P- Hexyloxy benzalacetone
277	α- Methyl -P- hydroxy benzalacetone
278	α- Methyl -P- ethoxy benzalacetone
279	α- Methyl -P- propoxy benzalacetone
280	α- Methyl -P- amyloxy benzalacetone
281	α- Methyl -P- hexyloxy benzalacetone
282	α- Methyl -P- butoxy benzalacetone
283	α- Methyl -P- heptyloxy benzalacetone
284	α- Methyl -P- octyloxy benzalacetone
285	P- Pentoxy benzalacetone
286	P- Heptyloxy benzalacetone
	N', N'- Anhydrobis (2-hydroxyetyl) amidino hydrazone HCl
287	α- Methyl -P- ethoxy benzalacetone
288*	α- Methyl -P- amyloxy benzalacetone
289*	α- Methyl -P- hexyoxy benzalacetone
290*	α- Methyl -P- heptyloxy benzalacetone
291*	α- Methyl -P- octyloxy benzalacetone
292*	α- Methyl -P- decyloxy benzalacetone
293	P- Hxdroxy benzalacetone
294	P- Methoxy benzalacetone
295*	P- Ethoxy benzalacetone
296*	P- Propoxy benzalacetone
297	P- Butoxy benzalacetone
298*	P- Amyloxy benzalacetone
299*	P- Hexyloxy benzalacetone
300*	P- Hepthyloxy benzalacetone
301*	P- Octyloxy benzalacetone
302	α- Methyl -P- propoxy benzalacetone
303	α- Methyl -P- butoxy benzalacetone
304	α- Methyl -P- methoxy benzalacetone

* Free base

Table 2. Results of tests for inhibition and cytotoxicity of compounds

Serial No.	Inhibitory conc. determined by the test of		Toxic conc. (C)[2]	Ratios*		
	Contact (A)[1]	Inhib. (B)[1]		(D)[3] C/A	(E)[4] C/B	(F)[5] A/B
266	50	100	100	2	1	0.5
267	12.5	NT	25	2	-	-
268	12.5	6.3	25	2	4	2
269	25	50	25	1	0.5	0.5
270	3.2	NT	6.3	2	-	-
271	12.5	NT	50	4	-	-
272	100	NT	100	1	-	-
273	100	200	100	1	0.5	0.5
274	12.5	12.5	25	2	2	1
275	6.3	3.2	100	16	32	2
276	3.2	12.5	25	8	2	0.25
277	25	50	25	1	0.5	0.5
278	12.5	25	25	2	1	0.5
279	6.3	12.5	25	4	2	0.5
280	3.2	12.5	25	4	2	0.5
281	3.2	NT	50	8	-	-
282	3.2	6.3	12.5	4	2	0.5
283	0.8	50	50	64	1	0.02

284	0.8	50	50	64	1	0.02
285	25	-	50	2	-	-
286	0.8	-	25	32	-	-
287	50	50	100	2	2	1
288	100	100	50	0.5	0.5	-
289	12.5	25	25	2	1	0.5
290	12.5	200	50	4	0.25	0.06
291	25	200	25	1	0.13	0.04
292	12.5	200	400	32	2	0.08
293	50	400	400	8	1	0.02
294	25	100	400	16	4	0.04
295	6.3	50	400	64	8	0.16
296	12.5	100	100	8	1	0.08
297	50	100	50	1	0.5	0.5
298	12.5	200	100	8	0.5	0.08
299	1.6	50	50	32	1	0.64
300	1.6	200	100	64	0.5	0.64
301	25	400	200	8	0.5	0.04
302	25	50	100	4	2	0.5
303	25	200	25	1	0.25	0.04
304	50	50	200	4	4	1

Explanation for Table 2. [1] Minimal concentration (γ/ml) in final of 100 % inhibition for influenza virus replication. [2] Minimal concentration (γ/ml) in final of 100 % cytotoxicity. [3] Ratio for virucidal concentration in the column A to toxic conc. in the column C. [4] Ratio for antiviral effect in the column B to toxic effect in the column C. [5] Comparative ratio for antiviral activity in the column B to virucidal activity in the column A.

Table 3. Grouping of compounds with reference to the chemotherapeutic ratio (D)*

Ratio (D)	Compounds in serial number	Total**	percent
64 or greater	283, 284, 295, 300	4	10.2
16 - 32	275, 286, 292, 294, 299	5	12.5
4 - 8	271, 276, 279, 280, 281 282, 290, 293, 296, 298 301, 302, 304	13	33.2
2	266, 267, 268, 270, 274 278, 285, 287, 289	9	23
1 or less	269, 272, 273, 277, 288 291, 297, 303	8	20.5

* The chemotherapeutic ratio; 100 % minimal toxic concentration / 100 % minimal inhibitory concentration.

** Total numbers of compounds showing the inhibitory concentration.

Table 4. Grouping of compounds with reference to the chemotherapeutic ratio (E)[1].

Ratio (E)	Compounds in serial number	Total	percent
16 or greater	275	1	2.5
4 - 8	268, 294, 295, 304	4	10.3
2	274, 276, 279, 280, 282 287, 292, 302	8	20.5
1 or less	266, 269, 273, 277, 278 283, 284, 288, 289, 290 291, 293, 296, 297, 298 299, 300, 301, 303	19	48.7
Not determined	267, 270, 271, 272, 281 285, 286	7	17.9

[1] See the column E in Table 2.

Table 5. List of compounds with reference to the comparative ratio (F)*.

Comparative ratio (F)	Compounds in serial numbers	Total	Percent
1 or less	266, 268, 273, 274, 276 277, 278, 279, 280, 282 283, 284, 287, 288, 289 290, 291, 292, 293, 294 295, 296, 297, 298, 299 300, 301, 302, 303, 304	30	76.9 %
2	268, 275	2	5.3 %
ND**	267, 270, 271, 272, 281 285, 286	7	17.9 %

* Comparative ratio for antiviral concentration to virucidal concentration.

** Not determined.

Table 6. Classification of compounds with reference to the minimal inhibitory concentration

Inhibition with*	Compounds in serial numbers	Total**	Percent
100 - 50	266, 272, 273, 287, 288 293, 297, 304	8	20.5
25 - 12.5	267, 268, 269, 271, 274 277, 278, 285, 289, 290 291, 292, 294, 296, 298 301, 302, 303	18	46.2
6.3 - 3.2	270, 275, 276, 279, 280 281, 282, 295	5	20.5
1.6 or less	283, 284, 286, 299, 300	5	12.8

* Numbers indicate the 100 % minimal inhibitory concentration (γ/ml) in final.

** Total numbers of compounds showing the inhibitory concentration.

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13. ABSTRACT

Alkoxy benzalacetone amidinohydrazones and N', N' anhydrobis (2-hydroxyetyl) amidinohydrazones were newly synthesized and antiviral effectiveness of these derivatives against influenza virus was investigated. Out of 39 compounds, 31 derivatives (79.5% of total compounds tested) were found to possess the virucidal activity, and 13 compounds (33.3%) were of antiviral effectiveness against the virus with the chemotherapeutic ratio of 2 or greater. Five derivatives (#283, #284, #286, #299, and #300) have highly virucidal effectiveness to the virus with the minimal concentration as low as 1.6 /ml or 0.8 /ml in final. Three (#283, #284, and #300) out of five derivatives have been found to be active with one-sixtyfourth less concentration of their cytotoxic doses. By the inhibition test, seven compounds (#268, #274, #275, #276, #279, #280, and #282) have antiviral activity to the virus with the final concentration of 12.5 /ml or less. A final concentration of 6.3 /ml of #268, and 3.2 /ml of #275 could completely inhibit the virus replication in the membrane culture system, and those concentrations were found to be a half of their contract- inhibitory concentrations. (Author)

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