Adamantylthiourea Derivatives as Antiviral Agents

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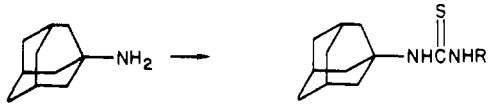
A series of nine 3-substituted 1-adamantylthioureas was prepared and tested for antiviral activity against influenza A2/Asian/J305 virus in vivo and in vitro. Protective dose$_{50}$ values were calculated for three of the compounds. One of these compounds, **7**, has antiviral activity which compares favorably with that of amantadine.

During the course of our work on derivatives of amantadine (**1**)[1] as potential antiviral agents, we observed that



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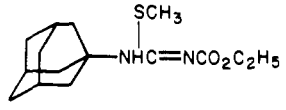
2, R = $CO_2C_2H_5$
3, R = $CO_2C_6H_5$
4, R = $PO(OC_2H_5)_2$
5, R = SO_2-C_6H_4-CH_3
6, R = SO_2-C_6H_4-$NHC(=O)CH_3$
7, R = SO_2-C_6H_4-NH_2
8, R = $CONH_2$
10, R = H



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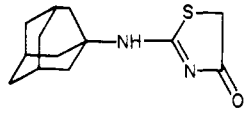

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the carbethoxythiourea **2**[2] afforded a statistically significant level of protection in mice infected with influenza A2/Asian/J305 virus. The lead prompted us to investigate the antiviral activity of a number of other adamantylthiourea derivatives of which the sulfonylthiourea **7** compared favorably with **1**.

The compounds **2**–**6** were prepared by the room-temperature condensation of **1** with the appropriately activated isothiocyanates in aprotic solvents. The thiobiuret **8** was obtained from the aminolysis of the phenyl carbamate **3**, and the amine **7** could be isolated by the hydrolysis of **6** when the reaction time was kept short. Methyl iodide treatment of **2** gave the isothiourea **9**, and the thiazole **11** was available from the reaction of the thiourea **10**[2] with ethyl chloroacetate. The structure assignment of **11** as the 4-oxo derivative rests on its IR spectrum, which displays C=N and carbonyl stretching bands at 1680 and 1675 cm^{-1}.

Royalhart mice weighing 9–12 g were lightly anesthetized with ether and infected intranasally with approximately 3 LD_{50} of influenza A2/Asian/J305 virus. Test substances were dissolved or suspended in H_2O, and 0.5 mL was administered intraperitoneally (ip) or orally (po) at the following times relative to virus infection at 0 h: immediately before virus infection and at 1, 5, 24, 30, 48, and 72 h after virus infection. The number of mice surviving at 21 days after infection was recorded and, where applicable, a protective dose$_{50}$ (PD_{50}) was calculated.

The results of intraperitoneal treatment with amantadine and thiourea derivatives of amantadine against influenza virus infection in mice are listed in Table I.

From the results shown in Table I, it can be seen that a PD_{50} value was obtained with amantadine and several of the thiourea derivatives (**4**, **7**, and **9**). Based on these results, amantadine (PD_{50} = 6 mg/kg ip) and **7** (PD_{50} = 16 mg/kg ip) were selected for additional antiviral testing. When administered orally, the PD_{50} of amantadine was 4 mg/kg and the PD_{50} of **7** was 35 mg/kg in mice infected with influenza A2/Asian/J305 virus.

The therapeutic effects of intraperitoneal administration of amantadine and **7** against influenza A2/Asian/J305 virus infection in mice are shown in Table II.

From the results of therapeutic studies (Table II) it can be seen that treatment with amantadine could be delayed until 24 h after mice were infected with influenza virus (77% corrected survival), but there was no protective effect (0% corrected survival) if treatment was initiated at 48 h after virus infection. A delay in the start of treatment with **7** until 24 h after virus infection resulted in a percent corrected survival of only 28, but this protective effect was statistically significant (p = 0.01). There was no protective effect noted when treatment with **7** was started 48 h after virus infection.

Intraperitoneal administration of amantadine or **7** was without protective effect in mice infected with herpes simplex, Semliki forest, or Sendai (parainfluenza type 1) viruses.

The in vitro antiviral effects of amantadine and **7** were tested against influenza viruses and rhinovirus 42 by the tube dilution assay. Monolayers of rhesus monkey kidney cells and WI-38 human lung fibroblasts were infected with serial tenfold dilutions of influenza viruses (A2/Asian/J305 and B/Lee/40) and rhinovirus 42, respectively. The mean tissue culture infective doses ($TCID_{50}$) of the virus-infected cultures in the presence and absence of noncytotoxic doses of the test substances were determined on the basis of hemadsorption (influenza viruses) after 4 days incubation at 37 °C or on the basis of cytopathogenic effect (rhinovirus 42) after 7 days incubation at 34 °C.

The minimum doses of the test substances which reduced the titer of influenza A2/Asian/J305 virus in rhesus monkey kidney cells by ≥ 2 logarithms were amantadine, 0.05 μg/mL; and **7**, 12.5 μg/mL. Neither amantadine nor **7** significantly inhibited in vitro growth of influenza B/Lee/40 or rhinovirus 42.

The acute toxicity and a CNS sympton profile of amantadine and **7** were determined in mice during a 24-h period following a single ip or po administration of the test substance. The LD_{50} of amantadine was 245 mg/kg ip and 900 mg/kg po. In the case of **7**, the LD_{50} values were 775 mg/kg ip and >1000 mg/kg po. It was further noted that amantadine produced numerous CNS-related side effects both ip (100 mg/kg) and po (300 mg/kg). No such side effects were observed after oral administration of **7** up to 1000 mg/kg, although mild symptoms (tremors and ataxia) were seen after 600 mg/kg ip.

Experimental Section

Melting points were determined on a Thomas-Hoover capilary melting point apparatus and are uncorrected. The IR, NMR, and mass spectral data were consistent with the assigned structures. Analyses for C, H, and N were with ±0.3% of the theoretical values.

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Table I. Adamantylthiourea Derivatives

compd	yield, %	mp, °C	crystn solv	formula[a]	PD_{50}, mg/kg ip
admantadine					6
2	57	108-109[b]	EtOH-H_2O	$C_{14}H_{22}N_2O_2S$	>400[c]
3[d]	82	171-175	EtOAc	$C_{18}H_{22}N_2O_2S$	>400
4[e]	80	133-135	EtOH-H_2O	$C_{15}H_{27}N_2O_3SP$	260
5[f]	76	174-176	EtOH-H_2O-DMF	$C_{18}H_{24}N_2O_2S_2$	>200
6	44	188-193	EtOH-H_2O-DMF	$C_{19}H_{25}N_3O_3S_2$	>200
7	83	176-179	MeOH-H_2O	$C_{17}H_{23}N_3O_2S_2$	16
8	78	202-205	EtOAc	$C_{12}H_{19}N_3OS$	>400
9	77	89-92	EtOAc-hexane	$C_{15}H_{24}N_2O_2S$	158
10	71	179-181[g]	EtOH	$C_{11}H_{18}N_2S$	>200
11	93	268-273	EtOH-H_2O-DMF	$C_{13}H_{18}N_2OS$	>200

[a] Analyses for C, H, and N were obtained for all compounds and were within ±0.3% of the theoretical values. [b] Lit.[2] mp 106-107 °C. [c] Greater than sign indicates that the results of antiviral testing did not permit calculation of a PD_{50}. [d] The starting material was prepared according to A. F. Dixon, *J. Chem. Soc.*, **89**, 892 (1906). [e] The starting material was prepared according to M Kulka, *Can. J. Chem.*, **37**, 525 (1959). [f] The starting material was prepared according to R. Gompper and W. Hagele, *Chem. Ber.*, **99**, 2885 (1966). [g] Lit.[2] mp 176-177 °C.

Table II. Therapeutic Effects of Amantadine and a Thoiurea Derivative against Influenza A2/Asian/J305 Virus Infection in Mice

compd[a]	time of initial treat., h[b]	no. of mice surviv/no. treated at 21 days: treated	control	% correct. surviv[c]	p[d]
aman-	0	40/48	5/48	72	<0.001
tadine	+5	37/48	5/48	67	<0.001
	+24	42/48	5/48	77	<0.001
	+48	1/31	1/32	0	>0.05
7	0	27/31	3/31	77	<0.001
	+5	14/30	3/31	36	0.001
	+24	12/32	3/31	28	0.01
	+48	0/24	0/24	0	>0.05

[a] Amantadine was tested at 100 mg/kg ip, except when treatment was initiated at 0 h (50 mg/kg). 7 was tested at 200 mg/kg ip. [b] Time of initial treatment is in relation to virus infection at 0 h. All mice received seven ip treatments. When treatment was initiated at 0 h, mice received three treatments on the day of virus infection, two treatments on the next day, and one treatment per day for the next 2 days. In the case of treatment initiated at +5 h, mice received a single treatment on the day of infection and two treatments per day for the next 3 days. In all other therapeutic regimens tested (+24, +48, +72 h) mice received treatment twice daily for 3 days, followed by a single treatment the following day. [c] Percentage survival in treated mice less percentage survival in control. [d] Fisher exact test.

1-Adamantyl-3-[(4-acetamidophenyl)sulfonyl]thiourea (6). To a solution of 53.5 g (0.24 mol) of N^4-acetylsulfanilamide in 200 mL of DMF was added simultaneously a solution of 33 g (0.50 mol) of potassium hydroxide in 50 mL of water and 16.6 mL (0.275 mol) of carbon disulfide over the course of 20 min while maintaining the reaction temperature below 20 °C. On completion of the addition, the mixture was allowed to stir for 1.5 h and was diluted carefully with 1.5 L of ethanol. The resulting precipitate was dried at 50 °C in vacuo and finally by suspension in 700 mL of benzene and azeotropic distillation to remove the ethanol of crystallization.

The dipotassium salt was suspended in 500 mL of methylene chloride in a 2-L flask, and 183 mL of a 12.5% solution of phosgene in benzene was added such that the internal temperature did not rise above 25 °C. The resulting suspension was allowed to warm to room temperature over 40 min, refluxed for 1.5 h, diluted with an additional 500 mL of methylene chloride, and filtered hot. A total of 500 mL of the resulting solution was distilled, and the residue was cooled as a solution of 20.42 g (0.135 mol) of 1 in 750 mL of methylene chloride was added. The resulting mixture was stirred overnight, filtered, and evaporated to 55.5 g of a gum. Trituration with 500 mL of ethanol then gave 24.06 g (44%) of 6, mp 176–178 °C. Recrystallization of a portion from DMF–water–ethanol gave the analytical sample, mp 188–193 °C. Anal. ($C_{19}H_{25}N_3O_3S_2$) C, H, N.

1-Adamantyl-3-[(4-aminophenyl)sulfonyl]thiourea (7). A suspension of 28.25 g (0.070 mol) of **6** and 28 g (0.70 mol) of sodium hydroxide in 500 mL of water was refluxed for 1 h. On cooling, the pH of the mixture was adjusted to 1 by the dropwise addition of 6 N HCl, and the resulting precipitate was recrystallized from methanol–water to give 20.8 g (83%) of **7**, mp 176–179 °C. Anal. ($C_{17}H_{23}N_3O_2S_2$) C, H, N.

1-Adamantyl-3-carbethoxy-2-methylisothiourea (9). A solution of 7.32 g (0.0259 mol) of **2** in 60 mL of iodomethane over 5.0 g of potassium carbonate was refluxed 48 h. Aqueous workup gave an oil, which was crystallized from ethanol–water to give 5.96 g (77%) of **9**, mp 84–90 °C, in two crops. A further crystallization gave the analytical sample, mp 89–92 °C. Anal. ($C_{15}H_{24}N_2O_2S$) C, H, N.

2-(1-Adamantylamino)-2-thiazolin-4(5*H*)-one (11). A solution of 42.61 g (0.202 mol) of **10**[2] and 21.3 mL (0.202 mol) of ethyl chloroacetate in 1 L of ethanol was refluxed overnight. The resulting precipitate was collected to give 46.97 g (93%) of 11, mp 260–266 °C dec. The analytical sample was obtained from aqueous ethanol–DMF, mp 268–273 °C dec. Anal. ($C_{13}H_{18}N_2OS$) C, H, N.

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References and Notes

(1) C. E. Hoffmann in "Selective Inhibitors of Viral Functions", W. A. Carten, Ed., Chemical Rubber Publishing Co., Cleveland, Ohio, 1973, p 199.
(2) Z. Gyorgydeak, D. Skwarski, and R. Bognar, *Acta Chim. Acad. Sci. Hung., Budapest*, **79**, 449 (1973).