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Studies on Antitumor Agents. I. Antitumor Activity of 5(4)-Amino-4(5)-imidazolecarboxamide Analogs

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Some of the derivatives of imidazole such as 5′-Phosphoribosyl-5-amino-4-imidazole carboxamide and 5-amino-4-imidazolecarboxamide have been shown to be the precursors of the natural purine ribonucleotides and are incorporated in liver and tumor RNA,[2] and 5-amino-4-imidazolecarboxamide and its riboside inhibit the usual metabolic pathway of nucleotide synthesis at α-N-formylglycinamide ribonucleotide formation.[3] Guanase is inhibited by 5-amino-4-imidazolecarboxamide.[4]

The mercaptopurine derivatives such as 6-mercaptopurine and its riboside have been reported as potent antitumor agents. Therefore, mercapto analogs of 5-amino-4-imidazolecarboxamide are interested as antitumor agents.

Of the derivatives of imidazole, 5-amino-4-imidazolethiocarboxamide was reported to be slightly active on Sarcoma 180 ascites cell *in vitro*,[5] 5-diazo-4-imidazolecarboxamide to be slightly active on solid type Ehrlich carcinoma,[6] and 2-aza-4-imidazolecarboxamide and 2-aza-5-amino-4-imidazolecarboxamide to be also slightly active on Sarcoma 180 *in vitro*[5] and on Ehrlich carcinoma *in vivo*.[6]

The present experiments deal with the antitumor activity on Nakahara-Fukuoka sarcoma[7] of the analogs of 5-amino-4-imidazolecarboxamide, and its related compounds, mainly mercapto derivatives.

Materials and Methods

Animals and Tumor System——Female mice of ddN strain weighing 20±2 g and Nakahara-Fukuoka sarcoma were used as reported previously.[8] The general procedure was to transplant the tumor to mice by the usual trocar method and to inject compounds to be tested intraperitoneally once daily for 5 days, starting 24 hours after transplantation at the dose level of 30 mg/kg/day. The tumor used was found to be especially sensitive to purine analogs such as 6-mercaptopurine, thioguanine and their ribonucleosides.[8]

Antitumor Activity and Toxicity——Formula of the sixteen compounds tested were listed up in Table I. ED_{50} and ED_{90} of the active compounds were calculated with tumor weight ratio (T/C) in several dosages, and LD_{50} and LD_{10} were calculated with daily injection for 5 days and survivors at the 14th day after first injection.[9]

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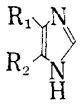
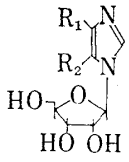
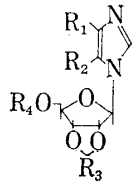
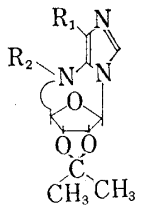
Table I. Test Compounds

Basic formula	Compound No.	R_1	R_2	R_3	R_4	Reference for preparation
	I	$-\overset{O}{\overset{\|}{C}}-NH_2$	$-NH_2$			10)
	II	$-\overset{O}{\overset{\|}{C}}-NH_2$	$-NH-\overset{O}{\overset{\|}{C}}-H$			11)
	III	$-\overset{S}{\overset{\|}{C}}-NH_2$	$-NH_2$			12)
	IV	$-\overset{S}{\overset{\|}{C}}-NH_2$	$-NH-\overset{O}{\overset{\|}{C}}-H$			12)
	V	$-\overset{S-\overset{O}{\overset{\|}{C}}-O-C_2H_5}{\overset{\|}{C}}=NH$	$-NH_2$			12)
	VI	$-\overset{O}{\overset{\|}{C}}-NH_2$	$-NH_2$			13)
	VII	$-\overset{O}{\overset{\|}{C}}-NH-CH_3$	$-NH_2$			*a*)
	VIII	$-CN$	$-NH_2$			14)
	IX	$-\overset{S}{\overset{\|}{C}}-NH_2$	$-NH_2$			12)
	X	$-\overset{S}{\overset{\|}{C}}-NH_2$	$-NH_2$	$=C\langle^{CH_3}_{CH_3}$	$-H$	12)
	XI	$-CN$	$-NH_2$	$=C\langle^{CH_3}_{CH_3}$	$-\overset{O}{\overset{\|}{C}}-CH_3$	14)
	XII	$-CN$	$-NH-SO_2CH_3$	$=C\langle^{CH_3}_{CH_3}$	$-H$	*a*)
	XIII	$-\overset{S}{\overset{\|}{C}}-NH_2$	$-H$			*a*)
	XIV	$-\overset{S}{\overset{\|}{C}}-NH_2$	$-SO_2-C_6H_5$			*a*)
	XV	$-\overset{S}{\overset{\|}{C}}-NH_2$	$-SO_2-C_6H_4-CH_3$			*a*)
	XVI	$-CN$	$-SO_2-CH_3$			*a*)

a) Prepared at Ajinomoto Co., Inc. (unpublished)

Results and Discussion

As shown in Table II, two out of sixteen test compounds were found to be active on Nakahara–Fukuoka sarcoma, namely, 5–formylamino–4–imidazolethiocarboxamide (IV) and

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1-β-D-ribofuranosyl-5-amino-4-imidazolethiocarboxamide (IX). 4-Cyano-, 2',3'-O-isopropylidene- and/or 2,5'-N-cyclo- derivatives of imidazole tested were inactive. The activity of the former two compounds was examined quantitatively and ED_{50} and ED_{90} were compared

Table II. Antitumor Activity of the Test Compounds

Compound	Dose (mg/kg/day)	Tumor weight ratio (T/C%)	Body weight change (T/C, g)	Activity[a]
I	30	115	1.2/2.4	−
II	30	111	2.9/2.4	−
III	30	105	3.6/2.9	−
IV	30	15	1.9/2.3	###
V	30	136	2.0/2.7	−
VI	30	127	2.5/1.6	−
VII	30	100	4.2/3.3	−
VIII	30	116	0.4/3.0	−
IX	30	30	1.9/3.9	##
X	30	129	4.4/2.9	−
XI	30	93	3.9/3.3	−
XII	30	100	3.2/3.3	−
XIII	30	88	3.5/3.3	−
XIV	30	79	2.5/3.3	−
XV	30	119	2.3/3.3	−
XVI	30	134	4.1/4.1	−

a) −: 71 or more in T/C% ($P>0.05$), +: 70—51 ($P\fallingdotseq0.05$), ##: 50—21 ($P\fallingdotseq0.01$), ###: 20—0 ($P\fallingdotseq0.001$). P was the value for the difference between treated and control groups for the indicated reduction in tumor growth.

Table III. Antitumor Activity of Compounds IV and IX

Compound	Dose (mg/kg/day)	Tumor weight ratio (T/C%)	Body weight change (T/C, g)
IV	2	72	3.4/3.3
	4	49	3.9/3.0
	8	40	0.5/1.0
	15	20	3.5/2.5
	30	15	1.9/2.3
	60	0	1.3/3.2
IX	15	67	3.0/2.9
	30	30	1.9/3.9
	60	12	1.6/2.3
	90	4	1.0/2.8
	120	2	0.7/3.0

Table IV. Toxicity and Therapeutic Evaluation of Compounds IV and IX

Compound	LD_{50}	LD_{10} (mg/kg/day)	ED_{50}	ED_{90}	Therapeutic index[a]
IV	180	110	4.8	35	3.1
IX	780	660	21	61	10.8
6-Mercaptopurine	130	100	3.6	13	7.7
6-Mercaptopurine ribonucleoside	490	370	5.1	15	24.7

a) LD_{10}/ED_{90}

with those of 6–mercaptopurine and its ribonucleoside (Table III and IV). IV was active almost equal to 6–mercaptopurine and to its ribonucleoside but IX was weaker than the latter two. Toxicity of IV was almost eual to that of 6–mercaptopurine. IX was less toxic than 6–mercaptopurine ribonucleoside.

5–Amino–4–imidazolethiocarboxamide was inactive on Nakahara–Fukuoka sarcoma instead weakly active *in vitro* on Sarcoma 180.[5] Formylation of aminogroup at position 5 of 5–amino–4–imidazolethiocarboxamide would not occur in animal body, unelss ribofuranosyl group conjugates with it at position 1.

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