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Cytotoxic Activity of Imidazole Derivatives

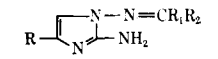
Keyphrases ☐ Imidazole derivatives—synthesis ☐ Cytotoxicity—imidazole derivatives

Sir:

In this communication, we wish to report the synthesis and cytotoxic activity of a number of imidazole derivatives (Compounds 1–8). The synthesis of these imidazole derivatives was accomplished by the reaction of α-haloketones with guanylhydrazones of aromatic aldehydes. The method of preparation of these compounds was very much similar to the one reported by Beyer *et al.* (1). The imidazole derivatives, Compounds 1–8, were characterized by IR and UV spectroscopy.

All these compounds were subjected to L-1210 *in vitro* assay for cytotoxic activity (2). In these screening experiments, the samples were weighed (about 5–10 mg.) into glass homogenizers (32-ml. size) and sterilized with 0.1 ml. of 70% ethanol and about 0.1 ml. of dimethylsulfoxide (DMSO) to help them solubilize. The sample was ground with sterile water to make a suspension containing L-1210 leukemic cells. The tubes were stoppered and incubated at 37° for 3 days; then cell counts were made on each tube by a Coulter counter. The percent inhibition and the ID_{50} and ID_{90} were calculated.

Table I—Screening Data



Compd.	R	R_1	R_2	ID_{50}	ID_{90}
1	Phenyl	H	Phenyl	0.022	0.038
2	Phenyl	Me	Phenyl	0.96	1.5
3	Phenyl	H	3,4-Methylenedioxyphenyl	0.29	0.47
4	3,4-Dihydroxyphenyl	H	3,4-Methylenedioxyphenyl	27	50
5	*m*-Nitrophenyl	Me	Phenyl	40	50
6	*p*-Nitrophenyl	H	*m*-Nitrophenyl	1.25	2.0
7	Methyl	H	*m*-Nitrophenyl	21	50
8	Methyl	H	*o*-Hydroxyphenyl	9.0	21

The assay values for the compounds are shown in Table I. Values of 1 or less for ID_{50} were considered potentially active.

Three compounds showed ID_{50} values less than 1. Based on these encouraging results, attempts are being made in our laboratories to synthesize a wide variety of these imidazole derivatives and to test them for L-1210 *in vitro* assay for possible cytotoxic activity. Obviously, more extensive testing will be required before any structure–activity correlation can be drawn.

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Antigenicity of a Polypeptide with a Known Sequence of Amino Acids

Keyphrases ☐ Polypeptides—known amino acid sequence ☐ Antigenicity—polypeptide

Sir:

Random copolymers containing varying amounts of the amino acid residues, alanine, glutamic acid, and

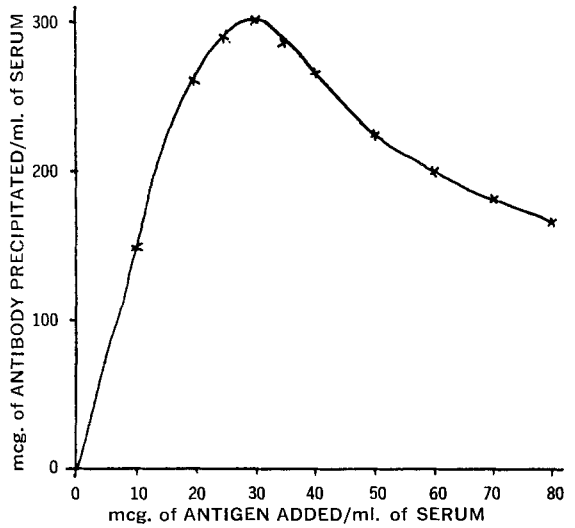

Figure 1—*The precipitin curve.*

tyrosine, have been shown to be antigenic (1–3). However, due to the unknown primary amino acid sequence of these random polymers, it is difficult to describe the locus of the active site of these antigenic polymers. To overcome this difficulty, the use of linear polypeptides with a known repeating sequence of amino acids has been suggested. For this purpose, poly-(L-tyrosyl-L-glutamyl-L-alanylglycyl)glycine-1-^{14}C ethyl ester was recently synthesized (4), and we wish to report the antigenic properties of this polymer.

After obtaining preimmunization sera, four rabbits were treated at weekly intervals with 500 mcg. of poly-(tyr-glu-ala-gly)gly-1-^{14}C ethyl ester. The first 2 weeks they were injected intradermally using complete Freunds adjuvant as suspension medium, and the 3rd week they were injected subcutaneously. The injection on the 4th week was done intravenously using buffered saline. Bleedings were conducted on the following week, and the serum from each animal was found to give a precipitin reaction with the polymer. The preimmunized sera under the same conditions gave a negative precipitin reaction. The quantitative determination of the antibody was obtained by the addition of dilutions of poly-(tyr-glu-ala-gly)gly-1-^{14}C ethyl ester to 1-ml. samples of the pooled rabbit sera. The precipitates were kept at 4° for 48 hr., washed twice with small volumes of buffered saline, and collected by centrifugation. The total amount of protein precipitated was estimated by analysis for nitrogen by a micro-Kjeldahl method (5). The amount of antigen contained in each precipitate was estimated by use of the Folin-Ciocalteu method. From these results the precipitin curve shown in Fig. 1 was obtained.

From these results it can be seen that this polypeptide is antigenic; further studies pertaining to the specificity of its antibodies are presently in progress.

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Quantitative Correlation of Absorption and *In Vitro* Dissolution Kinetics of Aspirin from Several Dosage Forms

Keyphrases ☐ Aspirin dosage forms—dissolution, absorption ☐ Absorption–dissolution, aspirin—correlation

Sir:

Several types of *in vivo–in vitro* correlation are described in the pharmaceutical literature (1). The most informative of these, but the most difficult to achieve, is the quantitative correlation between *in vitro* dissolution and *in vivo* absorption, particularly one involving several different dosage forms. An example is found in the report of Levy *et al.* (2) who were able to correlate the absorption of aspirin from three different dosage forms with a function of the dissolution rate, using the beaker method (3) at 50 r.p.m. A plot of the percent absorbed at time *T versus* the percent dissolved in (*T*-lag time)/2 gave a straight line with a slope of unity.

The present report introduces a new dissolution method, which has permitted an absolute quantitative correlation between the absorption and *in vitro* dissolution of aspirin from these three dosage forms. These findings extend the work of Levy *et al.* (2) because this type of 1:1 correlation was not attainable with the beaker method.

A schematic diagram of the rotating-flask apparatus used to determine dissolution is shown in Fig. 1. The apparatus consists of a spherical glass flask suspended in a constant-temperature bath. The globe is supported by glass rods, fused to its sides, which form the horizontal axis of the sphere. One support rod is coupled to a constant-speed motor, which provides rotation about the horizontal axis. A sampling port is molded into the sphere to permit introduction of the dosage form and periodic withdrawal of samples. The volume of the dissolution medium (in this case 400 ml.) and the position of the sampling port are such that fluid