CONNECTION BETWEEN THE PHYSICOCHEMICAL PROPERTIES OF BENZIMIDAZOLES AND THEIR EFFICACY AGAINST *Nippostrongyliasis* IN MICE

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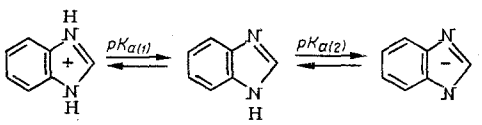
Benzimidazole derivatives are interesting biologically active compounds. They include a large number of herbicides [1], insecticides [2] and anthelmintic agents [3], as well as one of the strongest oxidative phosphorylation decouplers, viz., tetrachlorotrifluoromethylbenzimidazole (TTFB). The anthelmintic activity of the benzimidazoles is probably connected with their ability to change the transmembrane potential of cell membranes, as has been postulated for TTFB. It is known that the therapeutic activity of anthelmintic agents intended for the treatment of animals with helminthiasis localized in the gastrointestinal tract of the host depends on a number of factors, viz., the chemical nature of the substance, the amount used, and the physiological conditions in the digestive tract [4-6]. The chemical nature of the substance determines its behavior in the different sections of the gastrointestinal tract. It would be of interest, therefore, to compare some of the physicochemical constants (pK_a and R_f) characterizing the behavior of these compounds at different pH values, and their resorbability, with their activity against the intestinal nematode *Nippostrongyliasis brasiliensis*.

We investigated a series of benzimidazoles (I-XXIII), which we synthesized by the known method [7] of condensing o-phenylenediamine with the corresponding acids in pyrophosphoric acid (see Table 1). The ionization constants of the compounds under investigation were determined in 70% aqueous alcohol by titrimetry or, in the case of the 2-trifluoromethylbenzimidazoles, by spectrophotometry (Figs. 1 and 2). It should be noted that we were unable to find literature values for the $pK_{a(1)}$ of the trifluoromethylbenzimidazoles. The $pK_{a(2)}$ values are usually quoted without any indication as to the ionization stage to which the observed values relate [1, 8, 9].



The $pK_{a(1)}$ values obtained by us (see Table 1) are not true values but can be used for correlation within a given series of compounds. The lipophilicity of the test substances was characterized by their mobility (R_f) during thin-layer chromatography on Silufol UV-254 plates with acetone/carbon tetrachloride.

The compounds were tested against *Nippostrongyliasis* in white mice at doses of 0.1 and 0.5 g/kg by the method described in [10].

According to current ideas about the permeability of the cell walls in the gastrointestinal tract, nonionized drug molecules can pass through them. At stomach pH values of 1.0-3.0, the compounds that are absorbed best should be those with low $pK_{a(1)}$ values and high lipophilicity (i.e., high R_f values). Such compounds should pass rapidly into the blood and accumulate in the bile. For compounds active against fascioliasis, this is a contributory factor and, as we have shown in the case of bisphenols [4, 11], the connection between activity and basicity is quite marked.

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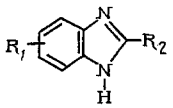
TABLE 1. Physicochemical Constants and Biological Activity of Benzimidazoles against *Nippostrongyliasis*



Compound	R_1	R_2	Melting point (in deg)	$pK_{a(1)}$	R_f	Effectiveness of dose (in %)		% mortality after administration of 0.5 g/kg to mice
						0.1 g/kg	0.5 g/kg	
I	H	H	170	5,40	0,16	18	54	40
II	H	CH_3	175	5,80	0,14	30	46	60
III	H	CF_3	205	0,30	0,81	0	64	80
IV	H	C_6H_5	293	4,20	0,65	0	40	—
V	H	*o*-C_6H_4OH	236	3,80	0,75	0	65	—
VI	H	*o*-C_6H_4Cl	235	3,30	0,67	0	25	—
VII	H	m-C_6H_4Cl	195	3,05	0,85	0	—	—
VIII	H	*n*-C_6H_4Cl	294	2,85	0,83	24	—	—
IX	H	*o*, *n*-$C_6H_3Cl_2$	225	2,52	0,85	20	—	—
X	H	*o*-C_6H_4Br	250	3,00	0,67	0	50	—
XI	H	*o*-$C_6H_4NH_2$	208	3,37	0,82	0	—	—
XII	H	m-$C_6H_4NH_2$	252	4,80	0,55	12	—	—
XIII	H	*n*-$C_6H_4NH_2$	237	5,77	0,33	60	—	—
XIV	H	2-C_5H_4N	216	3,75	0,20	42	—	—
XV	H	3-C_5H_4N	243	3,20	0,52	0	—	—
XVI	H	4-C_5H_4N	215	3,60	0,48	34	—	—
XVII	H	4-Thiazolyl	293	3,21	0,21	100	—	—
XVIII	5,6-$(CH_3)_2$	H	196	5,60	0,14	23	57	—
XIX	5(6)-NO_2	H	205	2,15	0,26	70	—	100
XX	5(6)-NO_2	CH_3	220	2,95	0,30	13	—	100
XXI	4,7-Br_2	CH_3	239	3,35	0,73	50	56	60
XXII	5(6)-NO_2	CF_3	154	—0 64	0,81	13	—	100
XXIII	4(7)-Br	CF_3	160	—0 24	0,86	30	—	100

In the case of intestinal helminthiasis, rapid absorption in the stomach has the result that only a small amount of the compound reaches the parasites living in the lower sections of the gastrointenstinal tract. Consequently, at low doses (0.1 g/kg), the activity of the compounds will be displayed only if they are absorbed in small amounts in the stomach. This will be true only of compounds that are present in ionized form or that have low lipophilicity (low R_f values).

Indeed, the most active of the compounds investigated (XVII and XIX) have low $pK_{a(1)}$ and low R_f values.

Absorption is evidently more intense in the case of low $pK_{a(1)}$ and high R_f values, and at a dose of 0.1 g/kg the compounds prove to be either inactive or weakly active (for example, III-XII, XV, and XXII). When the dose is increased to 0.5 g/kg, their activity increases as would be expected, but this dose proves to be higher than the toxic dose in a number of cases.

In an earlier study of the trichinocidal activity of benzimidazoles, a hypothesis was advanced about the connection between their activity and the coplanarity of the benzimidazole part of the molecule [12] and the aromatic substituent R_2 (compounds VI, XIV, and XVII), this coplanarity arising as a result of hydrogen bonding between the nitrogen atom of the imidazole ring and the o-acceptor group of the substituent. A comparison of compounds VI and VIII, XI and XIII, and XIV and XVI shows that their activity against *Nippostrongyliasis* does not adhere to such a dependence, but is rather determined by the relative values of $pK_{a(1)}$ and R_f in each of these groups of compounds. Thus, the pK_a and R_f are important characteristics of the benzimidazoles, largely determining the activity of these compounds against *Nippostrongyliasis*. However, among the compounds investigated there are some (for example, XIII and XXI) whose properties are not in accord with their observed activity. This may evidently be explained by the fact that factors not taken into account are of greater importance in this case.

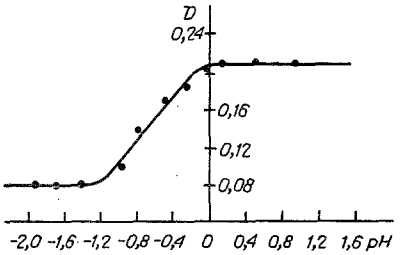


Fig. 1. Spectrophotometric measurement of the $pK_{a(1)}$ of compound XXII, $c = 10^{-4}$ mole/liter.

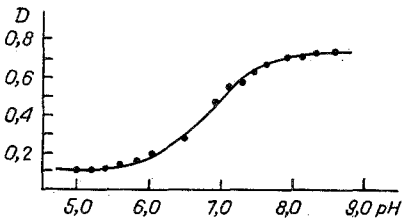


Fig. 2. Spectrophotometric measurement of the $pK_{a(2)}$ of compound XXII, $c = 10^{-4}$ mole/liter.

The data obtained indicate that any analysis of the relation between the physicochemical properties and anthelmintic activity of medicaments should take into account the location of the parasite in the organism.

EXPERIMENTAL METHOD

The benzimidazoles were synthesized by the known method [7] of condensing o-phenylenediamine with the corresponding acids in pyrophosphoric acid; 2-trifluoromethylbenzimidazole (III) was prepared by condensing o-phenylenediamine with trifluoroacetic acid in hydrochloric acid.

Compounds VII, VIII, IX, XI, XII, and XIII were kindly supplied by M. O. Kolosova, of the E. I. Martsinovskii Institute of Medical Parasitology and Tropical Medicine.

2-Methyl-4(7)-bromobenzimidazole. A solution of 15 g bromine in 20 ml acetic acid was added at 70° over 2 h to a solution of 10.8 g II in 20 ml acetic acid. The temperature was then raised to 115-120° and the reaction solution stirred for 5 h. When the reaction ceased, the mixture was cooled, and the precipitate filtered off, washed with a small amount of acetic acid and dried. The resulting hydrobromide was crystallized for analysis from acetic acid, mp 239-240°. Calculated, %: Br 54.76. $C_8H_7BrN_2HBr$. Found, %: Br 54.30.

The hydrobromide was dissolved in water and 40% sodium hydroxide solution added to precipitate an oily substance which crystallized on standing. The precipitate was filtered off, washed with water and dried, to give 10.5 g of product, mp 198.5-201°.

2-Methyl-4,7-dibromobenzimidazole (XXI). A solution of 3.6 g bromine in 10 ml acetic acid was added over 1 h to a solution of 10 g 2-methyl-4(7)-bromobenzimidazole in 20 ml acetic acid while heating at 70-80°. The temperature was then raised to 110-115° and the reaction mixture kept at this temperature for 5 h. The mixture was cooled, and the dark-red precipitate filtered off, washed with a small amount of acetic acid and dried. The dry hydrobromide was dissolved in water, neutralized to pH 7.0 with sodium hydroxide solution, and the product filtered off and washed with water and alcohol. Yield 54%.

For analysis, the product was crystallized from alcohol to give a substance with a mp of 240-241°. Calculated, %: C 32.1, H 2.06, N 9.66, Br 55.1, $C_8H_6Br_2N_2$. Found, %: C 33.49, H 2.40, N 10.20, Br 54.6.

R_f Determination. The R_f values were determined on Silufol UV-254 plates with acetone/carbon tetrachloride (60:40) in ultraviolet light.

Determination of Ionization Constant (pK_a). The pK_a values were determined graphically from the pH at the semineutralization point using a Radiometer TTTIs titrimeter with a glass electrode and a saturated calomel reference electrode. The measurements were made in 70% alcohol at 20°. The concentration of the substance was 0.1 or 0.05 mole/liter.

The reported ionization constant values are the averages of three determinations.

The $pK_{a(1)}$ values of compounds III, XXII, and XXIII were determined spectrophotometrically at a concentration of 10^{-4} mole/liter in sulfuric acid solutions with different concentrations.

Some of the substances were tested against *Nippostrongyliasis* in mice by A. I. Krotov and O. E. Kuznetsova at the E. I. Martsinovskii Institute of Medical Parasitology and Tropical Medicine.

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