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Derivatives of Methyl *N*-[5(6)-(4-Aminophenylsulfanyl)benzimidazol-2-yl]carbamate and Phthalic Anhydrides. Synthesis and Biological Properties

V. S. Pilyugin, A. N. Mikhailyuk, V. M. Kosareva,
G. E. Chikireva, G. V. Kiseleva, and S. L. Kuznetsova

Research Technological Institute of Herbicides and Growth Regulators,
Academy of Sciences of Bashkortostan, Ufa, Bashkortostan, Russia

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Abstract—Products of the reactions methyl *N*-[5(6)-(4-aminophenylsulfanyl)benzimidazol-2-yl]carbamate with phthalic anhydrides, involving the aniline nitrogen atoms, were obtained. Biological properties (embryotoxicity and toxicity) of the products and their mixtures with phase-transfer catalysts were studied. On this basis, new antihelminthic agents were developed.

Helminthiases are common in cattle breeding and exert a pernicious economical effect [1]. For this reason, chemical means are widely applied in traditional cattle breeding technologies. However, chemical means have certain drawbacks, causing fast animal adaptation and side effects.

Derivatives of benzimidazole and methyl *N*-(benzimidazol-2-yl)carbamate represent an abundant group of antihelminthics. Benzimidazoles, combining a broad-spectrum antihelminthic activity and a low general toxicity, had initially been considered to hold great promise. However, subsequent investigations revealed embryotoxic, teratogenic, and cytogenetic activity in most benzimidazoles and thus imposed limitations on their practical use.

At present many derivatives of methyl *N*-(benzimidazol-2-yl)carbamate have been synthesized and shown to be potent antihelminthics [2–11]. At the same time, in view of the fact that certain of them have exhibited negative properties (embryotoxicity, general toxicity, etc.), search for new, nontoxic antihelminthic agents in this class of compounds still remains quite a topical problem.

It is known that methyl *N*-[5(6)-(4-aminophenylsulfanyl)benzimidazol-2-yl]carbamate (**I**) is an effective antihelminthic but possesses strong embryotoxicity and general toxicity. In view of the present notion of the mechanism of side effects of this compound [13], we can suggest that they can be overcome by introduction of substituents in the nucleophilic centers of ester **I**.

There are two nucleophilic centers in molecule **I**: aniline and benzimidazole nitrogens. This makes possible their reactions with phthalic anhydrides by one or both nucleophilic centers. However, it is known that *N*-acyl-substituted benzimidazoles readily transfer their acyl groups onto other nucleophiles, i.e. act as mild acylating agents. This property is of great biological importance, since *N*-acetylimidazoles are involved in enzymatic transacylation reactions [15].

On the other hand, the amino group in aniline is known to be more basic than in *o*-phenylenediamine [16]. Consequently, aminobenzimidazole **I** will react by its both nucleophilic centers only with excess anhydride. With equimolar reagent amounts, the reaction with involve exclusively the aniline nitrogen atom.

Judging from the results of investigations on antihelminthic agents derived from *N*-[5(6)-(4-aminophenylsulfanyl)benzimidazol-2-yl]carbamic acid [2–13], we can conclude that such substituents at the aniline nitrogen atom in ester **I** as carboxylic acid residues should not be rapidly cleaved in humans and animals to convert into the toxic starting ester **I**. This condition is met by aromatic acids whose both *ortho* positions are occupied by sufficiently bulky substituents coplanar to the anilide moiety and capable of preventing nucleophilic attack (for example, of hydroxyl) by the carbonyl carbon atom.

One of accessible compounds of this group is phthalic anhydride. However, its reaction with ester **I** gives a derivative bearing only one *ortho*-substituent. Consequently, this derivative will not be sufficiently resistant to hydrolysis in humans and embryos. The

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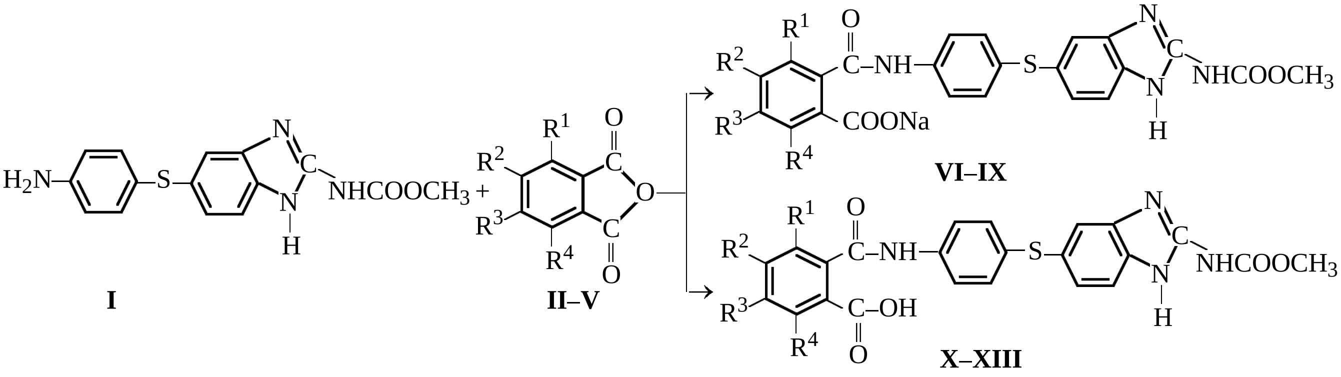
hydrolytic stability in phthalic acid derivatives having one more *ortho*-substituent with respect to the carbanilide moiety should be much enhanced. To check this assumption, we synthesized a series of substituted phthalic anhydrides by the procedures described in [17–22].

The most accessible of phthalic anhydrides are tetrachloro- and tetrabromophthalic anhydrides which are obtained in one stage in quantitative yields.

We reacted ester **I** with 3,6-dibromo-, tetrachloro-, tetrabromo-, and tetraiodophthalic anhydrides (compounds **II**–**V**, respectively) in acetic acid in the presence of sodium acetate at 40–45°C to obtain sodium salts of the corresponding phthalic acid derivatives in high yields. The same reactions in DMSO at 35–40°C gave, in high yields, too, the corresponding derivatives as free acids [Scheme (1)].

Scheme 1.

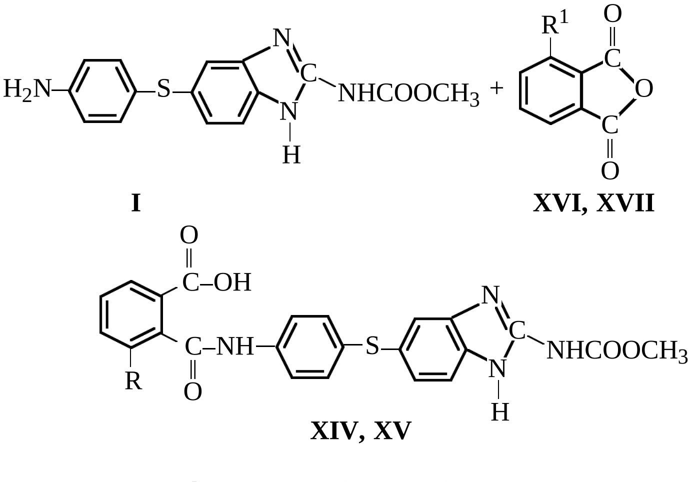


$R^1 = R^4 = Br$, $R^2 = R^3 = H$ (**II, VI, X**); $R^1 = R^2 = R^3 = R^4 = Cl$ (**III, VII, XI**), Br (**IV, VIII, XII**), I (**V, IX, XIII**).

Depending on reaction conditions, nucleophilic reagents can add to 3-substituted phthalic anhydrides either by the carbonyl group in position *1* and by the carbonyl group in position *2* [23]. The reaction in acidic media involves the 1-C=O group. In neutral and basic solvents, the reaction occurs in the required direction, involving the 2-C=O group.

With this in mind, we obtained in high yields derivatives **XIV** and **XV** of ester **I** with 3-chloro- and 3-nitrophthalic anhydrides (**XVI**, **XVII**), bearing substituents in both the *ortho* positions with respect to the carbanilide moiety, by performing the reactions in DMSO (40–50°C) [Scheme (2)].

Scheme 2.



R = Cl (**XIV, XVI**), NO_2 (**XV, XVII**).

Table 1. Biological properties of esters **X**–**XV** and **XVIII**

Comp. no.	Toxicity		Antihelminthic activity		
	dose, mg/kg	animal deaths, %	dose, mg/kg	number of nematodes per head	effectiveness, %
X	1000	0	100	85.6	0
XI	1000	0	100	78.4	5.0
XII	1000	0	100	76.7	7.0
XIII	1000	0	100	73.4	11.0
XIV	1000	0	100	81.5	0
XV	1000	0	100	88.7	0
XVIII	50	50	25	3.3	96.0
Control				82.5	–

The resulting compounds were tested for antihelminthic, toxic, and embryotropic activity. The resulting data are listed in Table 1.

As would be expected, derivatives **X**–**XV** of ester **I** with substituted phthalic anhydrides proved to be nontoxic but exhibited no antihelminthic activity, whereas phthalic anhydride derivative **XVIII** proved to be quite an effective antihelminthic but rather toxic. Compounds **X**–**XV** showed no embryotropic activity at a dose of 100 mg/kg in critical pregnancy days.

The antihelminthic, antimycotic, and other action of compounds belonging to the chemical group in hand is not underlaid by any common universal biological mechanism; in each case, a combination of effects takes place, one or another prevailing.

An essential factor that controls biotransformation and pharmacokinetic features of benzimidazole derivatives is substitution in position *5* of the benzene ring. As shown in [24–26], the adduct of methyl *N*-(benzimidazol-2-yl)carbamate and butyl isocyanate by N^1 (Benomil) metabolizes mostly via cleavage of the side chain and hydroxylation of the benzene ring in position *5*, followed by conjugate formation. Similar data were obtained in a biotransformational study of 2-(thiazol-4-yl)benzimidazole (Thiabendazole). Both Benomil and Thiabendazole undergo similar transformations in animals and are rather rapidly excreted. Their biotransformation products are inactive agaist helminthes, and, consequently, useful properties of these agents are limited by their rapid metabolic deactivation.

As shown in [27–30], the effectiveness of benzimidazole antihelminthics depends on their action time. Naturally, the rapidly absorbed, metabolized, and excreted Thiabendazole will be a less potent antihelminthic than benzimidazoles whose biodegradation is slow, depending on substitution in 2-aminobenzimidazole.

Benzimidazole antihelminthics strongly metabolize in animals upon oral administration. The parent compound rarely remains unchanged. In blood, liver, tissues, and urine, metabolites prevail over the parent compound. These free metabolites are formed by oxidation and hydrolysis. They most frequently are better soluble than the parent compounds and are readily excreted [31].

It is known that in drug synthesis phthalic acid derivatives are used for preparing drugs of prolonged action [32, 33]. The prolonged action of these drugs is explained by that most drugs are absorbed primarily in intestines where the medium is alkaline. In such media, phthalic acid derivatives will form salts that are insufficiently lypophilic to be absorbed in intestines until they hydrolyze by the amide bond and thus ensure steady and prolonged active substance supply for the organism. This explains why compounds **X**–**XV** proved ineffective antihelminthics: Forming in the intestines medium the same salts but possessing enhanced hydrolytic stability, they cannot cleave to form ester **I**.

Derivative **XVIII** of ester **I** with unsubstituted phthalic anhydride behaved in the organism like known drugs of prolonged action, exhibiting a high antihelminthic activity along with a high toxicity.

It is known that the lypophilicity of salts is strongly enhanced by phase-transfer catalysts [34]. We suggested that if we enhance the lipophilicity of salts **X**–**XV** then they, having preserved their positive properties (lack of toxicity and embryotropic activity), will get stronger antihelminthics.

The most common phase-transfer catalysts are crown ethers, polyethylene glycols, cryptands, and

Table 2. Antihelminthic activity of ester **XII** (1 g) in mixtures with various phase-transfer catalysts

No.	Phase-transfer catalyst and its quantity per 1 g of active substance						Antihelminthic activity of active substance		
	a	b	c	d	e	f	dose, mg/kg	number of nematodes per head	effectiveness, %
1	5	–	–	2	–	–	25	22.6	84
2	5	2	–	–	–	–	25	58.4	57
3	5	–	–	–	2	–	25	40.4	71
4	5	–	–	–	–	2	25	34.6	75
5	5	–	2	–	–	–	25	100.4	29
6	10	–	–	–	–	–	25	113.2	20
7	5	–	–	1	–	–	25	89.1	37
8	5	–	–	4	–	–	25	16.9	88
9	–	–	–	2	–	–	25	66.5	52
Control								141.5	–

[a] Polyethylene glycol (*M* 400, PEG 400). [b] Benzyltetraethylammonium chloride. [c] 2-Ethyldimethylphenoxyammonium chloride. [d] Alkylbenzyldimethylammonium chloride (Katamin AB) $[(CH_3)_2N^+(CH_2C_6H_5)C_{12-14}H_{23-29}]Cl^-$. [e] Triethylhexylammonum bromide. [f] Tetraethylammonium bromide.

Table 3. Effectiveness and toxicity of esters **X**–**XV** as medicines with a 1 : 5 : 2 benzimidazole : PEG 400 : Katamin AB ratio

Comp. no.	Toxicity		Antihelminthic activity		
	dose, mg/kg	animal deaths, %	dose, mg/kg	number of nematodes per head	effectiveness, %
X	500	0	25	31.2	77
XI	500	0	25	60.8	57
XII	500	0	25	22.6	84
XIII	500	0	25	41.5	71
XIV	500	0	25	50.8	63
XV	500	20	25	66.0	52
Control				141.5	–

quaternary alkylammonium salts [34]. Crown ethers and cryptands are expensive, whereas quaternary alkylammonium salts and polyethylene glycols are accessible and inexpensive. Polyethylene glycols are especially suitable, since they can be used as solvents for our benzimidazole derivatives. The resulting data are listed in Tables 2 and 3.

We lists antihelminthic activity data for ester **XII** which is applied as medical forms as a mixture with various quaternary alkylammonium salts and polyethylene glycol (PEG 400) in various ratios. PEG 400 with no additions of quaternary alkylammonium salts did not work here as a phase-transfer catalyst. Of all the four quaternary alkylammonium salts tested, dimethylalkylbenzylammonium chloride (Katamine AB) (**XIX**) proved the most effective. The medicine containing no PEG 400 (mixture no. 9) adversely affects the antihelminthic activity. Of importance is also benzimidazole:quaternary alkylammonium salt ratio; thus, at a 1 : 1 ratio, the effectiveness was as low as 37% (mixture no. 7), whereas at a 1 : 2 ratio it was 84% (mixture no. 1); further increase of the fraction of the ammonium salt only slightly enhanced the effectiveness of benzimidazole **XII** (mixture no. 8).

As seen from Table 3, ester **XII** proved the most effective. The medicine on the basis of derivative **XV** of 3-nitrophthalic anhydride was found to be the most toxic.

Table 4. Yields, melting points, and elemental analyses of sodium salts **VI–IX, XIX** and esters **XV, XVIII**

Comp. no.	mp, °C	Yield, %	Found, %			Formula	Calculated, %		
			Br	N	S		Br	N	S
VI	234–236	82	24.58	8.83	5.26	$C_{23}H_{15}Br_2N_4NaO_5S$	24.88	8.72	4.99
VII	>300	66	–	9.20	5.21	$C_{23}H_{13}Cl_4N_4NaO_5S$	–	9.00	5.15
VIII	230–232	98	39.48	7.15	–	$C_{23}H_{13}Br_4N_4NaO_5S$	39.95	7.00	–
IX	188–190	62	–	5.68	3.10	$C_{23}H_{13}N_4NaO_5SJ_4$	–	5.67	3.24
XV	141–143	91	–	13.92	6.35	$C_{23}H_{17}N_5O_5S$	–	13.80	6.32
XVIII	170–172	98	–	11.88	6.48	$C_{23}H_{18}N_4O_5S$	–	12.11	6.93
XIX[a]	222–224	82	–	10.72	–	$C_{13}H_{16}ClN_4NaO_5S$	–	10.80	–

[a] Compound **XIX** is sodium salt of methyl *N*-[5(6)-[4-(3-chlorophthaloylamino)phenylsulfanyl]benzimidazol-2-yl]carbamate. Found Cl, %: 6.56. Calculated Cl, %: 6.83.

The medicine listed in Table 3 were tested for embryotropic activity in critical days of pregnancy days at doses of 25, 50, and 100 mg/kg (per active substance). It was found that the compounds all exhibit no embryotropic activity at doses of 25 and 50 mg/kg. At a dose of 100 mg/kg, the medicine on the basis of compounds **XI** and **XV** exhibited slight embryotoxicity, while the other medicines were inactive. The medicine on the basis of ester **XII** was tested against tract strongylatosis in sheep at doses of 10 and 15 mg/kg and showed 100% effectiveness.

Thus, we synthesized a series of derivatives of methyl *N*-[5(6)-(4-aminophenylsulfanyl)benzimidazol-2-yl]carbamate and substituted phthalic anhydrides. The products showed a high antihelminthic activity comparable with that of methyl [5(6)-(4-phenylsulfanyl)benzimidazol-2-yl]carbamate (known preparations Fenbendazole and Panacur) which exerts no adverse effects.

EXPERIMENTAL

Sodium salt **VIII** was prepared by adding 0.024 mol of sodium acetate and 0.022 mol of tetrabromophthalic anhydride **IV** to 0.02 mol of ester **I** in 50 ml of acetic acid. The mixture was stirred at 40–45°C for 3 h. After cooling, the precipitate was filtered off, washed with acetic acid and water, and dried. Yield 31.4 g (98%).

Sodium salts **VII**, **IX**, and **VI** were prepared in a similar way using tetrachlorophthalic anhydride (**III**), tetraiodophthalic anhydride (**V**), and 3,6-dibromophthalic anhydride (**II**), respectively.

Sodium acetate, 0.024 mol, and 0.02 mol of 3-chlorophthalic anhydride was added to a solution of 0.02 mol of methyl ester **I** in 25 ml of DMSO. The mixture was heated for 2 h at 40–50°C and then cooled and poured into water. The precipitate was filtered off, washed with water, and dried to obtain 8.4 g (82%) of sodium salt of methyl *N*-[5(6)-[4-(3-chlorophthaloylamino)phenylsulfanyl]benzimidazol-2-yl]carbamate (**XIX**).

3-Nitrophthalic anhydride, 0.022 mol, was added to a solution of 0.02 mol of methyl ester I in 25 ml of DMSO. The reaction mixture was stirred for 2 h at 40–45°C and then cooled and poured into water. The precipitate was filtered off, washed with water, and dried to obtain 9.28 g (91%) of methyl *N*-[5(6)-[4-(3-chlorophthaloylamino)phenylsulfanyl]benzimidazol-2-yl]carbamate (**XV**). Esters **X**–**XIV** and **XVIII** were prepared in a similar way.

The yields, melting points, and elemental analyses of the resulting compounds are given in Table 4.

Toxicity tests were performed with mice 18–20 g in weight. Samples as suspensions were administered orally in one portion in doses of 100 to 1000 mg/kg. The animals were observed over the course of 5 days, keeping a record of deaths.

Nematocide activity tests were performed with rats 50–60 g in weight experimentally infected with nippostrongilus larvae (300–350 per head). Samples as suspensions were orally in doses of 10 do 100 mg/kg eight days after infection. For each dose, five infected and five control rats were used. After 5-day exposure the animals were killed. The effectiveness of the preparations was judged about by data of helminthological examination of thin intestines for tested and control groups.

Analysis of technical products for ester **I** and its reaction products with phthalic anhydrides was performed by HPLC on an Ultraspher-ODS column in the systems (vol% are given): 70% of acetonitrile and 30% of water plus 5% of DMF; and 67.5% of methanol, 29.0% of water, and 3.5% of DMF plus 4 ml/l of tetrabutylammonium phosphate (0.5 g per 20 ml of water).

The chromatograms were registered with a UV detector at λ 254 nm and treated by internal normalization of peak areas.

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