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Novel Potential Anticancer Agents Derived from Benzimidazole

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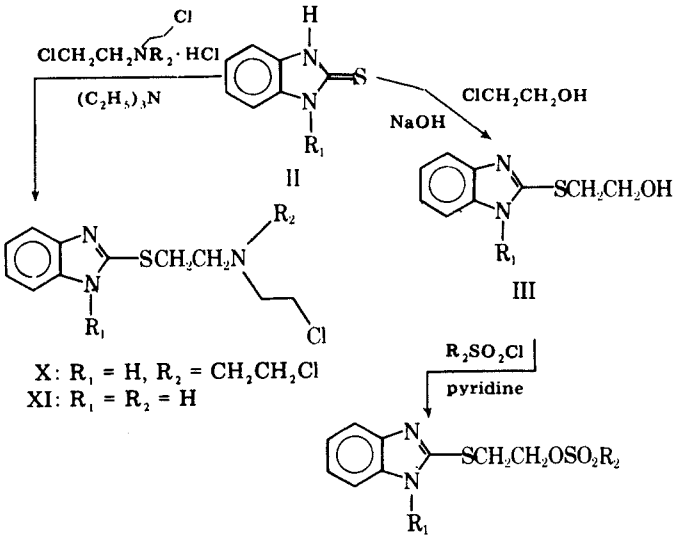
Received March 4, 1980, from the *Pharmaceutical Chemistry Department, Faculty of Pharmacy, University of Alexandria, Alexandria, Egypt.* Accepted for publication May 20, 1980.

Abstract □ Two novel series of benzimidazole derivatives bearing structural modifications of certain drugs were prepared for evaluation for potential anticancer activity. The first series was a group of alkylating agents, and the second series was a variety of 4-substituted-1-thioacetyl-3-thiosemicarbazides. The tests of some representative products for antileukemic activity against P-388 lymphocytic leukemia indicated no significant effects.

Keyphrases □ Antineoplastic agents, potential—benzimidazole sulfonic esters and nitrogen mustards, synthesis and evaluation for antileukemic activity □ Benzimidazole derivatives—sulfonic esters and nitrogen mustards, synthesis and evaluation for anticancer activity

Among the benzimidazole alkylating agents whose syntheses have been described (1–5), only 2-[bis(2-chloroethyl)aminoethyl]benzimidazole (benzimidazole mustard NSC 23891, I) has shown pronounced anticancer activity (6, 7). Interest in the effect of structural modification on the anticancer activity of such compounds prompted the synthesis of benzimidazole sulfonic esters (IV–IX) and nitrogen mustards (X and XI) (Scheme I), in which the thioethyl chain replaced the methylenic group in I. In addition, 4-substituted-1-(2-mercaptoacetylbenzimidazole)-3-thiosemicarbazides (XIV–XX) (Scheme II) were prepared to compare their anticancer properties with those reported for some heterocyclic α-formylthiosemicarbazone derivatives (8–11).



Scheme I

RESULTS AND DISCUSSION

Chemistry—The sulfonic esters (IV–IX) were prepared starting with *S*-alkylation of benzimidazole-2-thione or the 1-substituted derivatives (II) with ethylene chlorohydrin in the presence of potassium hydroxide, followed by treatment of the resulting alcohols (III) with methanesulfonyl chloride or *p*-toluenesulfonyl chloride and pyridine (12) (Scheme I). When the 1-position in the alcohols (III) was vacant, the reactions proceeded with the production of the disulfonated products (VIII and IX) (Table I). The bi- and monofunctional nitrogen mustards (X and XI) were synthesized by reacting benzimidazole-2-thione with a mixture of tris(2-chloroethyl)amine hydrochloride or bis(2-chloroethyl)amine hydrochloride and triethylamine in absolute ethanol.

Treatment of benzimidazole-2-thione (II) with ethyl bromoacetate gave

0022-3549/80/1100-1348$01.00/0
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Table I—1-Substituted-2-(alkyl or arylsulfonoxyethyl)thiobenzimidazoles

Compound	Melting Point	Yield, %	Molecular Formula	Analysis, % Calc.	Found
IV	73–75°	82	$C_{11}H_{14}N_2O_3S_2$	C 46.01	45.90
				H 4.90	4.80
				N 9.70	10.10
				S 22.30	22.70
V	87°	85	$C_{17}H_{18}N_2O_3S_2$	C 56.30	56.10
				H 5.00	5.00
				N 7.70	7.20
				S 17.69	17.80
VI	87–88°	78	$C_{17}H_{18}N_2O_3S_2$	C 56.30	56.50
				H 5.00	4.90
				N 7.70	7.70
				S 17.69	17.20
VII	100–101°	81	$C_{23}H_{22}N_2O_3S_2$	C 62.90	62.73
				H 5.00	5.14
				N 6.39	6.30
				S 14.60	14.70
VIII	197–199°	85	$C_{11}H_{14}N_2O_5S_3$	C 37.70	38.00
				H 4.02	4.10
				N 8.00	8.30
				S 27.42	27.30
IX	127–128°	90	$C_{23}H_{22}N_2O_5S_3$	C 54.90	54.50
				H 4.40	4.40
				N 5.57	5.90
				S 19.10	19.20

the corresponding *S*-alkyl ester (XII) (13), which, on reaction with hydrazine hydrate in ice, yielded benzimidazole-2-mercaptoacetohydrazide (XIII) (13). Heating this acid hydrazide with the equivalent amount of alkyl-, aryl-, or aralkylisothiocyanates in refluxing ethanol (14) produced the required 4-substituted-1-(2-mercaptoacetylbenzimidazole)-3-thiosemicarbazides (XIV–XX) (Scheme II and Table II). The new products were identified by microanalysis and IR spectra and, for some representative compounds, by PMR and mass spectra.

Anticancer Screening—Compounds IV–VIII, X, XI, XIV–XVI, and XX were evaluated against P-388 lymphocytic leukemia in mice[1]. The activities were measured as the ratio of the mean survival time of the test animals to that of the control animals expressed as a percentage (% T/C). All compounds tested were inactive since their % T/C values were <125.

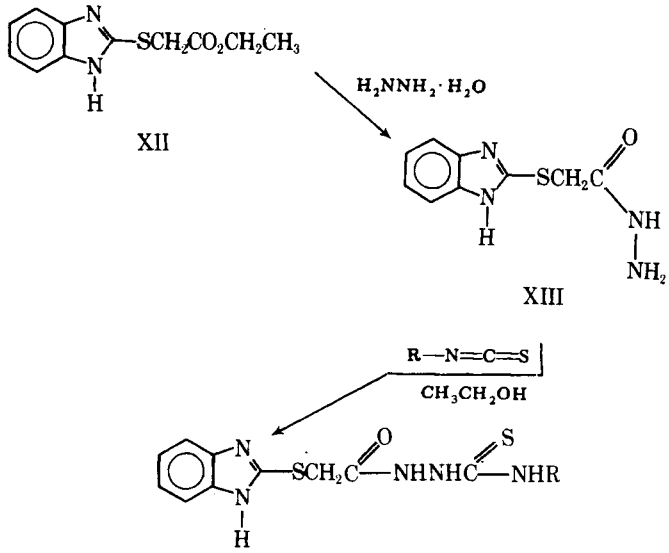
The inactivity of the synthesized alkylating agents was assumed to be due to the presence of sulfur in the thioethyl chain separating the alkylating function from the benzimidazole ring. This assumption is supported by the fact that no loss in carcinostatic activity or therapeutic index was observed when the methyl group in methylbis(2-chloroethyl)amine (HN_2 NSC 762) was replaced by a longer or more complex substituent (7). On the other hand, the lack of anticancer activity of the thiosemicarbazides (XIV–XX) was attributed to their inability to form the tridentate ligands (15) with the iron required by ribonucleoside diphosphate reductase; as a result, they cannot block DNA synthesis.

EXPERIMENTAL[2]

2-(2-Hydroxyethyl)thiobenzimidazole and Corresponding 1-Methyl- and 1-Benzyl Derivatives (III)—Ethylene chlorohydrin (7 g, 0.1 mole) was added dropwise to an ice-cold solution of benzimidazole-2-thione (II) (0.1 mole) in 12% KOH (40 ml). The mixture was stirred for 3 hr and set aside overnight at room temperature. The separated products were crystallized from ethanol or aqueous ethanol, identified by their IR spectra, and used for the preparation of the sulfonic esters; IR (mineral oil): 3175 (associated OH and NH), 1620 (C=N), 1510 (δ NH), 1265 (OH, out of plane bending), and 1065 (C–O) cm^{-1}.

1-Substituted-2-(alkyl or arylsulfonoxyethyl)thiobenzimidazoles (IV–IX)—Methanesulfonyl chloride or *p*-toluenesulfonyl chloride (0.3 mole) was added to a well-stirred and cooled (ice–salt bath) solution of the 2-(2-hydroxyethyl)thiobenzimidazoles (III) in dry pyridine (10 ml). Stirring was continued for 3 hr, and the mixture was left in the refrigerator overnight. Sufficient ice-cold water was added with vigorous stirring. The separated products were filtered, washed well with water, and crystallized from a benzene–light petroleum mixture. The yields and physical constants of the products are given in Table I; IR (KBr): 1355 and 1170 (asymmetric and symmetric SO_2) cm^{-1}; PMR for V (deuterochloroform): δ 2.29 (s, 3H, aromatic CH_3), 3.89 (s, 3H, *N*-CH_3), 4.38–4.62 (m, distorted, 2H, *S*-CH_2), 4.80–5.25 (m, distorted, 2H, CH_2OSO_2), and 6.89–7.81 (m, 8H, aromatic H) ppm.

2-[2-[Bis(2-chloroethyl)amino]ethyl]thiobenzimidazole Hydrochloride (X)—A mixture of benzimidazole-2-thione (0.45 g, 0.003 mole), tris(2-chloroethyl)amine hydrochloride (0.72 g, 0.003 mole), and triethylamine (0.3 g) in absolute ethanol (20 ml) was heated under reflux for 6 hr. The separated product was filtered and crystallized from absolute ethanol as white crystals, 0.55 g (72%), mp 223–225°; TLC: R_f 0.53



XIV: R = *n*-C_4H_9
XV: R = C_6H_5
XVI: R = 2-$CH_3C_6H_4$
XVII: R = 3-$CH_3C_6H_4$
XVIII: R = 4-$CH_3C_6H_4$
XIX: R = 2-$CH_3OC_6H_4$
XX: R = $C_6H_5CH_2$

Scheme II

[1] Assays were performed in accordance with the protocol of the Drug Evaluation Branch, National Cancer Institute, Bethesda, MD 20014. The compounds, dissolved or suspended in saline with Tween 80, were given as intraperitoneal injections 24 hr after tumor implantation in BDF_1 male and female mice. With a multiple-dose assay, the antileukemic activity of the products was evaluated on Day 30 after tumor implantation.

[2] All melting points are uncorrected. IR spectra were measured on a Beckman 4210 IR spectrophotometer. PMR and mass spectra were measured at Janssen Pharmaceutica Laboratories, Belgium.

Table II—4-Substituted-1-(2-mercaptoacetylbenzimidazole)-3-thiosemicarbazides

Compound	Melting Point	Yield, %	Molecular Formula	Analysis, %	Calc.	Found
XIV	172–174°	83	$C_{14}H_{19}N_5OS_2$	C	49.85	50.10
				H	5.60	5.70
				N	20.77	21.00
				S	18.99	18.60
XV	186–188°	85	$C_{16}H_{15}N_5OS_2$	C	53.60	53.70
				H	4.20	4.37
				N	19.56	19.00
				S	17.80	17.60
XVI	176–178°	85	$C_{17}H_{17}N_5OS_2$	C	54.98	54.89
				H	4.58	4.09
				N	18.80	18.50
				S	17.20	17.00
XVII	173–174°	86	$C_{17}H_{17}N_5OS_2$	C	54.98	54.89
				H	4.58	4.82
				N	18.80	19.20
				S	17.20	17.30
XVIII	168–169°	88	$C_{17}H_{17}N_5OS_2$	C	54.98	55.06
				H	4.58	4.67
				N	18.80	19.10
				S	17.20	17.60
XIX	130–132°	86	$C_{17}H_{17}N_5O_2S_2$	C	52.71	52.34
				H	4.39	4.73
				N	18.00	17.50
				S	16.49	16.60
XX	181–182°	93	$C_{17}H_{17}N_5OS_2$	C	54.98	55.27
				H	4.58	4.67
				N	18.80	18.70
				S	17.20	17.00

(chloroform–benzene–ethanol 2.5:3:0.5); IR (KBr): 2600 (broad, N^+H) cm^{-1}.

Anal.—Calc. for $C_{13}H_{18}Cl_3N_3S$: C, 44.10; H, 5.05; Cl, 30.04; N, 11.84; S, 9.02. Found: C, 44.00; H, 5.10; Cl, 29.60; N, 12.00; S, 8.60.

The PMR spectrum of the free base in deuterochloroform showed: δ 2.99 and 3.09 [2t, overlapping, 6H, J = 5 Hz, $CH_2N(CH_2)CH_2$], 3.42 and 3.53 [2t, overlapping, 6H, J = 5 Hz, SCH_2 and $(CH_2Cl)_2$], 7.11–7.32 (m, 2H, aromatic H), and 7.4–7.61 (m, 2H, aromatic H of benzimidazole) ppm; mass spectrum: *m/e* (relative abundance, %) (M^+ absent) 281 and 283 (2), 264 (3), 232 (26), 176 (44), 161 (14), 150 (100), 118 (16), 91 (8), 65 (11), 36 (16), and 28 (26).

2-[2-[(2-Chloroethyl)amino]ethyl]thiobenzimidazole (XI)—Compound XI was prepared as described for X except that bis(2-chloroethyl)amine hydrochloride was used instead of tris(2-chloroethyl)amine hydrochloride; TLC: R_f 0.61.

Anal.—Calc. for $C_{11}H_{14}ClN_3S$: C, 51.60; H, 5.50; Cl, 13.80; N, 16.40; S, 12.52. Found: C, 51.50; H, 5.50; Cl, 13.40; N, 16.00; S, 12.70.

1-(2-Mercaptoacetylbenzimidazole)-4-alkyl-, aryl-, and aralkyl-3-thiosemicarbazides (XIV–XX)—A solution of equimolar amounts of an alkyl-, aryl-, or aralkylisothiocyanate and benzimidazole-2-mercaptoacetohydrazide (XIII) (13) in ethanol was left overnight at room temperature to deposit the products. They were filtered and crystallized from ethanol or aqueous ethanol. The yields and physical constants of the products are summarized in Table II; IR (mineral oil): 3270–3140 (NH), 1670 (C=O, amide I band), 1350 (amide II band and, in part, N+C=S amide band), 1250 (amide III band), and 1565–1530, 1345–1325, 1075–1050, and 870–830 (N+C=S I, II, III, and IV amide bands, respectively) cm^{-1} (14).

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ACKNOWLEDGMENTS

The authors thank the members of Drug Research and Development, Division of Cancer Research, National Cancer Institute, Bethesda, MD 20014, for screening the compounds. They also thank the members of the Microanalytical Unit, Faculty of Science, Cairo University, Cairo, Egypt, for the microanalytical data.