as modified by Richter et al.[21] (Biochrom, Berlin, FRG), supplemented with glutamine (0.3 g/L), gentamycin (60 mg/L), and 5% newborn calf serum (NCS) (Gibco) or charcoal-treated NCC (CCS). CCS was prepared by incubation of 500 mL of NCS with a dextran-coated charcoal pellet[22] for 4 h in a shaker at 0–4 °C. The procedure was repeated with a fresh pellet. After each incubation, the charcoal was removed by centrifugation. The serum was sterilized through a 0.20-μm filter (Sartorius, Göttingen, FRG) and stored at −20 °C. Cells were grown in a humified incubator in 5% CO_2 at 37 °C. Two weeks before start of the experiment, cells were switched from NCS to CCS and received two additional media changes before they were harvested with 0.05% trypsin–0.02% EDTA in 0.15 M NaCl. They were syringed gently to prevent clumbing, and approximately 2×10^4 cells in 2 mL were plated replicately in six-well dishes (Costar). One day later, cells were switched to a medium containing the substances and 0.1% ethanol in which the compounds had been dissolved. The medium of control wells contained an equal volume of ethanol. At the fourth day, media were changed. Three days later, cells were labeled with 1 μCi [^{3}H]thymidine/well for 2 h. Cells were washed with cold PBS and harvested in PBS containing 0.02% EDTA. After centrifugation, the cell pellet was resuspended in 1 mL of PBS and divided in two equal parts. One part was counted in a Z I Coulter Counter and the other one was sonicated. After addition of 4 mL of 10% trichloroacetic acid, the acid-insoluble fraction was collected on a 0.45-μm filter (Metricel, Gelman) and counted after addition of 10 mL of scintillation liquid (Quickszint 212, Zinsser) in a LS 8000 Beckman scintillation counter.

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Supplementary Material Available: ^{1}H NMR data of 11-alkyl-6,11-dihydromethoxy-5*H*-benzo[*a*]carbazoles (**12a–23a**), 11-alkyl-6,11-dihydrohydroxy-5*H*-benzo[*a*]carbazoles (**12b–23b**), acetoxy-11-alkyl-6,11-dihydro-5*H*-benzo[*a*]carbazoles (**12c–23c**), acetoxy-11-alkyl-11*H*-benzo[*a*]carbazoles (**24a–35a**), and 11-alkyl-hydroxy-11*H*-benzo[*a*]carbazoles (**24b–35b**) (12 pages). Ordering information is given on any current masthead page.

Imidazo[2,1-*b*]benzothiazoles. 2.[1] New Immunosuppressive Agents

Toshiyasu Mase, Hideki Arima, Kenichi Tomioka, Toshimitsu Yamada, and Kiyoshi Murase*

Central Research Laboratories, Yamanouchi Pharmaceutical Co. Ltd., No. 1-8, Azusawa 1-chome Itabashi-ku, Tokyo 174, Japan. *Received January 28, 1985*

A series of 2-phenylimidazo[2,1-*b*]benzothiazole derivatives was prepared and tested for immunological activities. Some of the compounds showed significant suppressive activity of delayed type hypersensitivity (DTH) without inhibition of humoral immunity in mice by oral administration. The most active compound was 2-(*m*-hydroxyphenyl)imidazo[2,1-*b*]benzothiazole (**20**).

Immunosuppressive agents have been used for the treatment of some autoimmune diseases, such as systemic lupus erythematosus, rheumatoid arthritis, and glomerulonephritis,[2] and in organ transplantation.[2] However, these drugs, for example, alkylating agents, such as cyclophosphamide, and antimetabolites, such as azathioprine, are cytotoxic agents, inhibit both cell-mediated and humoral immunity, and are associated with a number of side effects, such as bone marrow depression, infection, hepatic toxicity, and rash.[3] Therefore, they are not used widely. In recent years, several immunosuppressive agents, such as cyclosporin A,[4] niridazole,[5] and oxisuran[6] have been reported to suppress selectively cell-mediated immunity. Recently, cyclosporin A[7] has been widely used in organ transplantation. However, this agent also has side effects, such as hepatic and renal toxicities.[7] In these aspects, less toxic immunosuppressive agents are awaited.

In a search for new compounds that alter the immune

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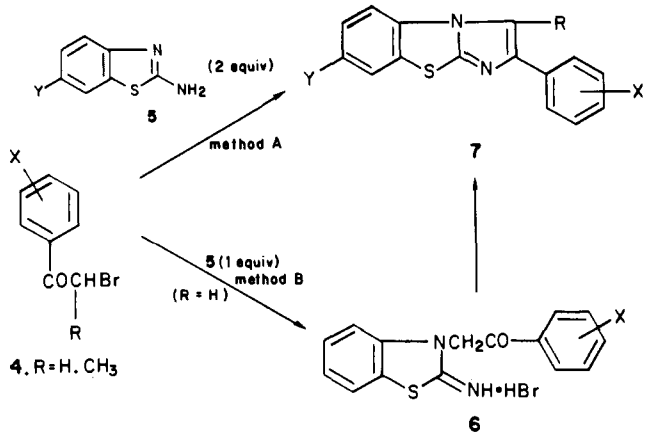
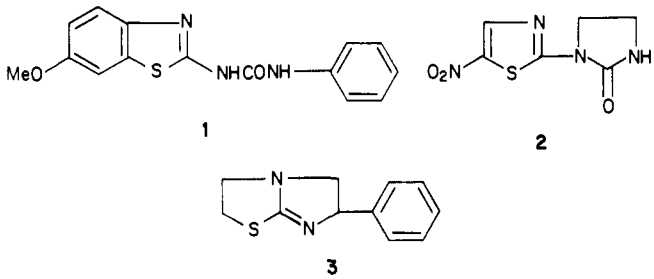
Scheme I



response, we removed alkylating agents, antimetabolites, and corticosteroids from consideration in order to avoid side effects.

Compounds that have the isothioureido moiety in their structure, e.g. frentizole (1),[8] niridazole (2),[5] and levamisole (3),[9] are known to alter the immune response. We were



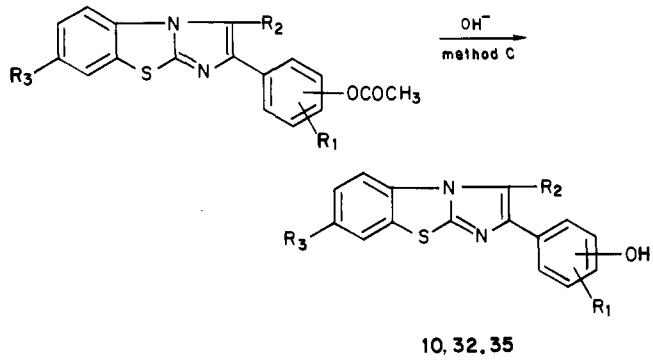
interested in the isothioureido moiety and synthesized a number of heterocyclic compounds having this moiety in their structure. We found that 2-phenylimidazo[2,1-*b*]-benzothiazole derivatives had interesting immunological activities. Several 2-phenylimidazo[2,1-*b*]benzothiazoles were known,[10] but immunological studies of these compounds have not been reported.

In this paper, we describe the synthesis and the immunological activity of 2-phenylimidazo[2,1-*b*]benzothiazoles and a few structurally related compounds.

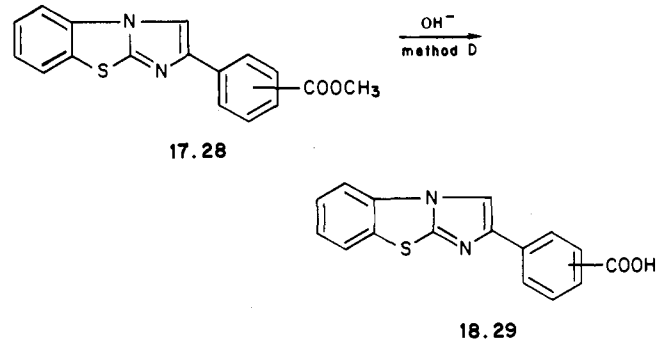
Chemistry. 2-Phenylimidazo[2,1-*b*]benzothiazoles **7** were prepared from the appropriately substituted phenacyl bromides **4** and the appropriately substituted 2-aminobenzothiazoles **5** as shown in Scheme I. Treatment of **4** with 2 equiv of **5** in 2-propanol or acetonitrile at refluxing temperature afforded **7** (method A). On the other hand, treatment of **4** with 1 equiv of **5** in methyl ethyl ketone or acetonitrile at refluxing temperature afforded the amino ketone hydrobromides **6**, which were cyclized by heating in refluxing 2-methoxyethanol to give **7** (method B).

Several derivatives were obtained from their parent compounds (Schemes II–IV).

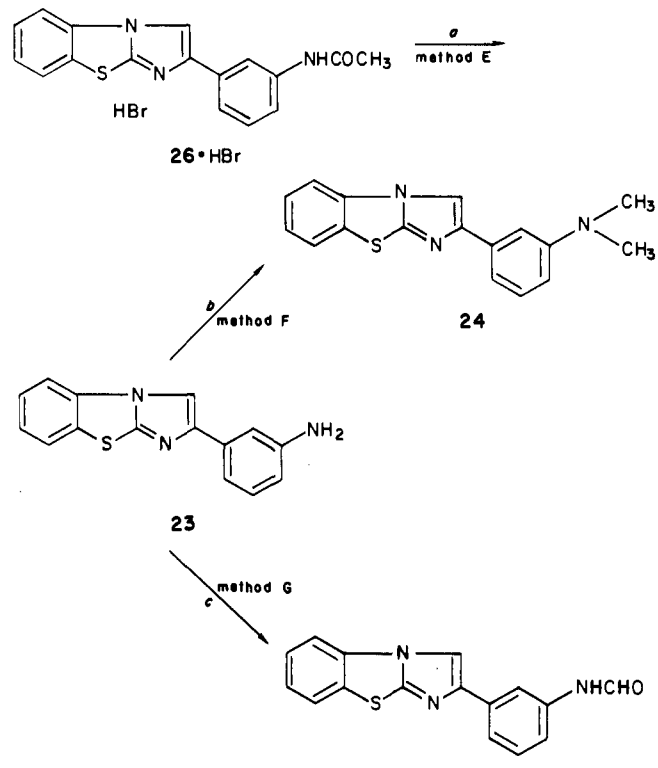
Scheme II



Scheme III



Scheme IV



[a] MeOH–2.2 N HCl refluxed. [b] 2 MeI, K_2CO_3 in methyl ethyl ketone, refluxed. [c] HCOOH–Ac_2O (3:5, v/v).

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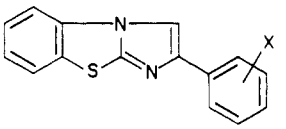
Table I. Chemical Characteristics and DTH Response of 2-Substituted Phenylimidazo[2,1-*b*]benzothiazoles



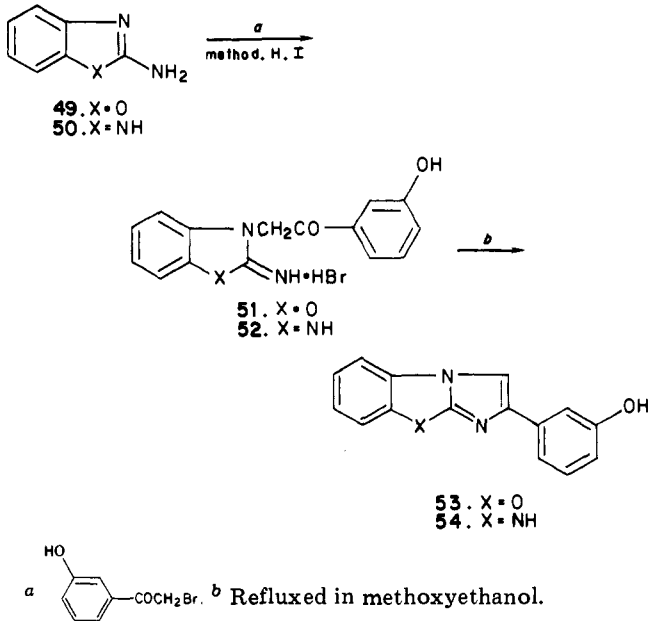
no.	X	mp, °C	method[a]	yield, %	formula	anal.	recrystn solv[b]	dose, mg/kg	increase in ear thickness, $^{1}/_{100}$ mm ± SE	% inhibn
8	H	106	B	30	$C_{15}H_{10}N_2S$[c]		A	control	5.8 ± 0.5	
								50	6.0 ± 1.0	-3.4
								400	5.7 ± 0.9	1.7
9	4-CH_3	124	B	39	$C_{16}H_{12}N_2S$[d]		B	control	5.2 ± 0.7	
								50	5.8 ± 0.7	-11.5
								400	5.3 ± 0.6	-1.9
10	4-OH	298	B	61	$C_{15}H_{10}N_2OS$	C, H, N, S	C	control	6.1 ± 0.7	
								50	3.4 ± 0.8	44.2[e]
								400	3.6 ± 0.4	40.9[e]
11	4-OCH_3	181	B	40	$C_{16}H_{12}N_2OS$[f]		D	control	5.2 ± 0.7	
								50	5.3 ± 0.8	-1.9
								400	4.6 ± 0.6	11.5
12	4-Cl	160	B	44	$C_{15}H_9ClN_2S$[g]		E	control	6.9 ± 0.8	
								50	4.8 ± 0.9	30.4
								400	5.9 ± 0.5	14.5
13	4-Br	164	B	16	$C_{15}H_9BrN_2S$[h]		F	control	5.2 ± 0.7	
								50	5.5 ± 1.1	-5.8
								400	4.9 ± 0.8	5.8
14	4-I	180	B	29	$C_{15}H_9N_2IS$	C, H, N, S	G	control	5.2 ± 0.7	
								50	6.4 ± 0.6	-23.1
								400	5.3 ± 1.8	-1.9
15	4-F	151	B	38	$C_{15}H_9FN_2S$[i]		B	control	5.2 ± 0.7	
								50	5.0 ± 1.0	-3.8
								400	5.9 ± 0.8	-13.5
16	4-$NHCOCH_3$	245	B	39	$C_{17}H_{13}N_3OS$	C, H, N, S	H	control	3.3 ± 1.4	
								25[j]	3.6 ± 0.7	-9.1
								200[j]	3.0 ± 0.6	9.1
17	4-$COOCH_3$	223	B	26	$C_{17}H_{12}N_2O_2S$	C, H, N, S	G	control	5.7 ± 0.5	
								50	6.0 ± 1.0	-5.3
								400	4.9 ± 0.9	14.0
18	4-COOH	>300	D	81	$C_{16}H_{10}N_2O_2S$	C, H, N, S	H	control	5.7 ± 0.5	
								50	3.8 ± 0.7	33.3[e]
								400	2.9 ± 0.6	49.1[e]
19	4-NO_2	284	B	50	$C_{15}H_9N_3O_2S$[k]		I	control	6.9 ± 0.8	
								50	5.4 ± 1.1	21.7
								400	5.0 ± 0.8	27.5
20	3-OH	248	A	70	$C_{15}H_{10}N_2OS$	C, H, N, S	J	control	5.6 ± 0.6	
								50	3.4 ± 0.7	39.3[e]
								400	0.9 ± 0.4	83.9[e]
21	3-OCH_3	156	B	39	$C_{16}H_{12}N_2OS$	C, H, N, S	J	control	3.2 ± 0.4	
								50	2.8 ± 0.5	12.5
								400	2.1 ± 0.5	34.4
22	3-Cl	175	B	31	$C_{15}H_9ClN_2S$	C, H, N, S	K	control	5.2 ± 0.7	
								50	5.0 ± 0.5	3.8
								400	5.8 ± 0.6	-11.5
23	3-NH_2	181	E	93	$C_{15}H_{11}N_3S$	C, H, N, S	L	control	5.3 ± 0.5	
								50	4.1 ± 1.5	22.6
								400	4.3 ± 0.5	18.9
24	3-$N(CH_3)_2$	220	F	55	$C_{17}H_{16}ClS \cdot 2HCl$	C, H, N, Cl	M	control	4.1 ± 0.5	
								12.5[j]	3.4 ± 0.9	17.0
								100[j]	3.2 ± 0.8	21.0
25	3-NHCHO	163	G	94	$C_{16}H_{11}N_3OS$	C, H, N, S	N	control	4.3 ± 0.7	
								25[j]	2.6 ± 0.7	39.5
								200[j]	2.2 ± 1.1	48.8
26	3-$NHCOCH_3$	232	B	37	$C_{17}H_{13}N_3OS$	C, H, N, S	J	control	5.3 ± 0.5	
								50	3.5 ± 0.6	34.0
								400	3.9 ± 0.8	26.0
27	3-$NHSO_2CH_3$	194	B	41	$C_{16}H_{13}N_3O_2S_2$	C, H, N, S	I	control	5.3 ± 0.5	
								50	3.8 ± 0.4	28.3
								400	3.6 ± 0.9	32.1
28	3-$COOCH_3$	146	A	53	$C_{17}H_{12}N_2O_2S$	C, H, N, S	O	control	5.3 ± 0.5	
								50	1.9 ± 0.7	64.2[e]
								400	1.9 ± 0.5	64.2[e]
29	3-COOH	>300	D	70	$C_{16}H_{10}N_2O_2S$	C, H, N, S	D	control	4.3 ± 0.7	
								50	2.8 ± 0.5	34.9
								400	3.2 ± 0.9	25.6
30	3-CN	234	B	21	$C_{16}H_9N_3S$	C, H, N, S	J	control	3.2 ± 0.4	

Table I (Continued)

no.	X	mp, °C	method[a]	yield, %	formula	anal.	recrystn solv[b]	dose, mg/kg	increase in ear thickness, $^1/_{100}$ mm ± SE	% inhibn
								50	3.0 ± 0.3	6.3
								400	4.7 ± 0.7	-46.9[e]
31	3-NO_2	232	B	34	$C_{15}H_9N_3O_2S$	C, H, N, S	J	control	5.3 ± 0.5	
								50	3.2 ± 0.4	39.6[e]
								400	2.1 ± 0.5	60.4[e]
32	2-OH	192	A/C	20	$C_{15}H_{10}N_2OS$	C, H, N, S	J	control	4.0 ± 0.5	
								50	3.5 ± 0.4	12.5
								400	1.4 ± 0.2	65.0[e]
33	2-OCH_3	185	A	13	$C_{16}H_{12}N_2OS$	C, H, N, S	A	control	5.7 ± 0.5	
								50	5.5 ± 0.7	3.5
								400	4.7 ± 1.0	17.5
34	2-Cl	179	B	26	$C_{15}H_9ClN_2S$	C, H, N, S	O	control	5.2 ± 0.7	
								50	5.0 ± 1.1	3.8
								400	6.3 ± 0.9	-21.2
frentizole								control	6.1 ± 0.7	
								50	3.5 ± 0.6	42.6[e]

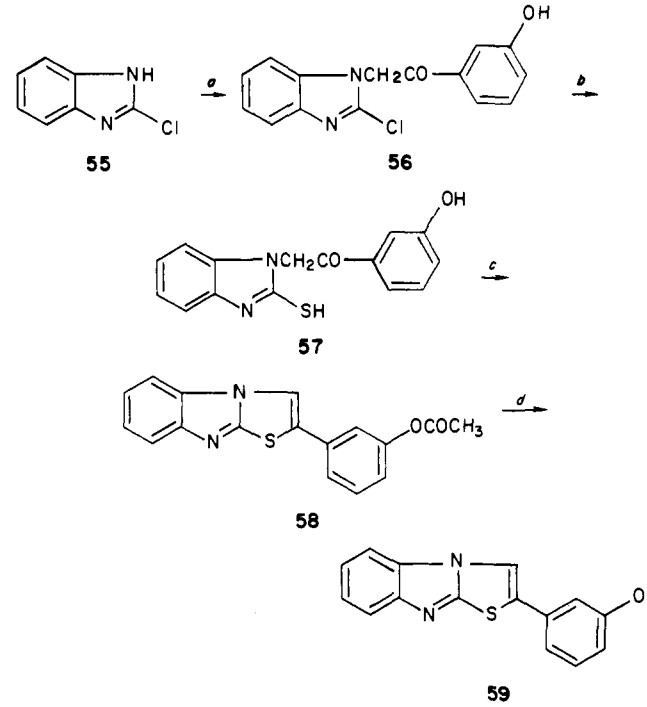
[a] See the Experimental Section. [b] A = toluene–*n*-hexane; B = washed with ether–*n*-hexane (1:1); C = purified by dissolving in dilute NaOH and reprecipitating with dilute HCl; D = washed with MeOH; E = washed with toluene–*n*-hexane (1:1); F = *n*-BuOH; G = toluene; H = washed with EtOH; I = washed with H_2O; J = $MeOCH_2CH_2OH$–H_2O; K = EtOH; L = AcOEt–ether; M = EtOH–ether; N = washed with ether; O = purified by column chromatography on silica gel with a mixture of toluene–AcOEt (10:1); P = *i*-PrOH. [c] See ref 10a (lit. mp 100 °C). [d] See ref 10g (lit. mp 140 °C). [e] Statistical significance of the data was estimated by using the Student's *t* test ($p < 0.05$). [f] See ref 10f (lit. mp 178 °C). [g] See ref 10b (lit. mp 159 °C). [h] See ref 10b (lit. mp 161 °C). [i] See ref 10b (lit. mp 148 °C). [j] Showed toxicity at high dose (1000 mg/kg, po). [k] See ref 10f (lit. mp 286 °C).

Scheme V



[a] HO, $COCH_2Br$. [b] Refluxed in methoxyethanol.

Scheme VI



[a] HO, $COCH_2Br$, MeONa in MeOH, refluxed. [b] Thiourea in MeOH, 50 °C. [c] Ac_2O, H_2SO_4, refluxed. [d] OH^-.

Hydroxy derivatives **10**, **32**, and **35** were obtained by alkaline hydrolysis of the acetoxy derivatives, which were prepared from **5** and acetoxyphenacyl bromides by method A or B (method C, Scheme II). Carboxylic acids **18** and **29** were obtained from methyl esters **17** and **28** by alkaline hydrolysis (method D, Scheme III). The *m*-amino derivatives were prepared according to Scheme IV. Acetamide **26** was converted to amine **23** by acidic hydrolysis (method E). Dimethylamino derivative **24** was obtained from **23** by treating with 2 equiv of methyl iodide in the presence of potassium carbonate in methyl ethyl ketone (method F). Formamide **25** was obtained from **23** by treating with a mixture of formic acid and acetic anhydride (3:5, v/v) (method G).

The structurally related analogues **53**, **54**, and **59** were prepared according to Schemes V and VI. 2-Aminobenzazoles **49** and **50** were treated with *m*-hydroxyphenacyl bromide in methyl ethyl ketone under reflux to give the amino ketone hydrobromides **51** and **52**, which were heated in 2-methoxyethanol to give 2-(*m*-hydroxyphenyl)-imidazo[2,1-*b*]benzoxazole (**53**) and 2-(*m*-hydroxyphenyl)-1*H*-imidazo[3,2-*a*]benzimidazole (**54**), respectively. 2-Chlorobenzimidazole (**55**)[11] was treated with *m*-

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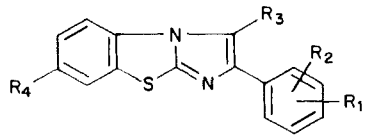
Table II. Chemical Characteristics and DTH Response of Disubstituted 2-Phenylimidazo[2,1-*b*]benzothiazoles



no.	R_1	R_2	R_3	R_4	mp, °C	method[a]	yield, %	formula	anal.	recrystn solv[b]	dose, mg/kg	increase in ear thickness, $^1/_{100}$ mm ± SE	% inhibn
35	3-OH	4-OH	H	H	235	C	28	$C_{15}H_{10}N_2O_2S\cdot HCl$	C, H, Cl, N, S	A	control	3.2 ± 0.4	
											25[c]	2.5 ± 0.6	21.9
											200[c]	3.1 ± 0.4	3.1
36	3-OH	5-OH	H	H	289 dec	A	32	$C_{15}H_{10}N_2O_2S$	C, H, N, S	B	control	4.6 ± 0.6	
											50	5.8 ± 0.3	−26.1
											400	3.6 ± 1.2	21.7
37	3-OH	4-CH_3	H	H	>300	B	64	$C_{16}H_{12}N_2OS$	C, H, N, S	C	control	5.3 ± 0.5	
											50	3.3 ± 1.3	37.7
											400	3.2 ± 1.0	39.6
38	3-OH	4-OCH_3	H	H	162	B	45	$C_{16}H_{12}N_2O_2S$	C, H, N, S	D	control	5.3 ± 0.5	
											50	4.9 ± 0.6	7.5
											400	4.6 ± 1.2	13.2
39	3-OH	4-Cl	H	H	>300	B	66	$C_{15}H_9ClN_2OS$	C, H, N, S	A	control	4.3 ± 0.7	
											50	2.4 ± 0.9	44.2
											400	3.7 ± 0.6	14.0
40	3-OH	H	CH_3	H	248	A	18	$C_{16}H_{12}N_2OS$	H, N, S: C[d]	E	control	4.3 ± 0.7	
											50	3.9 ± 1.0	9.3
											400	3.6 ± 0.5	16.3
41	3-OH	H	H	CH_3	>300	B	89	$C_{16}H_{12}N_2OS$	C, H, N, S	A	control	4.0 ± 0.5	
											50	4.5 ± 1.0	−12.5
											400	3.8 ± 1.0	5.0
42	3-OH	H	H	OH	180	B	17	$C_{15}H_{10}N_2O_2S\cdot{}^2/_3H_2O$	C, H, N, S	A	control	4.1 ± 0.5	
											50	2.3 ± 1.1	43.9
											400	2.4 ± 0.7	41.5
43	3-OH	H	H	OCH_3	295	A	62	$C_{16}H_{12}N_2O_2S$	C, H, N, S	A	control	4.0 ± 0.5	
											50	4.8 ± 1.0	−20.0
											400	4.9 ± 1.3	−22.5
44	3-$COOCH_3$	4-OH	H	H	226	B	30	$C_{17}H_{12}N_2O_3S$	C, H, N, S	F	control	4.0 ± 0.5	
											50	4.2 ± 0.5	−5.0
											400	2.3 ± 0.9	42.5
45	3-$COOCH_3$	H	H	OH	212	B	19	$C_{17}H_{12}N_2O_3S\cdot{}^1/_2H_2O$	C, H, N, S	A	control	4.1 ± 0.5	
											50	3.8 ± 0.6	7.3
											400	5.1 ± 0.9	−24.4
46	3-NO_2	4-OH	H	H	266	C	17	$C_{15}H_9N_3O_3S$	C, H, N	G	control	5.3 ± 0.5	
											50	4.1 ± 0.7	22.6
											400	4.2 ± 0.9	20.8
47	3-$SOCH_3$	4-CH_3	H	H	222	B	38	$C_{17}H_{14}N_2OS_2$	C, H, N, S	D	control	4.1 ± 0.5	
											25[c]	3.9 ± 0.5	4.9
											200[c]	1.9 ± 0.4	53.7[e]
48	3-SO_2CH_3	4-CH_3	H	H	263	B	65	$C_{17}H_{14}N_2O_2S_2$	C, H, N, S	C	control	4.1 ± 0.5	
											50	1.9 ± 0.6	53.7[e]
											400	3.9 ± 0.5	4.9

[a] See the Experimental Section. [b] A = $MeOCH_2CH_2OH$–H_2O; B = *i*-PrOH; C = $MeOCH_2CH_2OH$; D = EtOH; E = $MeOCH_2CH_2OH$–AcOEt; F = toluene–*n*-hexane; G = washed with EtOH. [c] Showed toxicity at high dose (1000 mg/kg). [d] Calcd: C, 68.55. Found: C, 67.54. [e] Statistical significance of the data was estimated by using the Student's *t* test

Table III. Chemical Characteristics and DTH Response of Structurally Related Compounds (53, 54, 59)

no.	mp, °C	method[a]	yield,[b] %	formula	anal.	recrystn solv	dose, mg/kg	increase in ear thickness, $^1/_{100}$ mm ± SE	% inhibn
53	233	H	18	$C_{15}H_{10}N_2O_2$	C, H, N	EtOH	control	3.2 ± 0.4	
							50	2.6 ± 0.6	18.8
							400	2.9 ± 0.9	9.4
54	264	I	11	$C_{15}H_{11}N_3O\cdot{}^1/_2H_2O$	C, H	c	d		
59	260	J	17	$C_{15}H_{10}N_2OS$	C, H, N, S	EtOH	control	4.1 ± 0.5	
							50	2.3 ± 0.5	43.9
							400	3.8 ± 0.4	7.3

[a] See the Experimental Section. [b] Overall yields are based on the starting heterocycles. [c] Purified by column chromatography on silica gel with a mixture of toluene–AcOEt (1:1). [d] See text for explanation.

hydroxyphenacyl bromide in the presence of sodium methoxide in methanol under refluxing to give amino ketone **56**, which was converted to thiol **57** by reaction with thiourea in methanol at 50 °C. Thiol **57** was cyclized by heating in a mixture of acetic anhydride and concentrated sulfuric acid to give **58**. Alkaline hydrolysis of **58** yielded 2-(*m*-hydroxyphenyl)thiazolo[3,2-*a*]benzimidazole (**59**).

Pharmacology and Structure Activities

The effects of new compounds on antibody formation (humoral immunity) in mice immunized with dinitrophenylated ovalbumin (DNP-OA) and on delayed-type hypersensitivity reaction (DTH) (cell-mediated immunity) induced by picryl chloride in mice were tested by oral administration at doses of 50 and 400 mg/kg and the results are summarized in Tables I–III.

As described before, immunological studies of 2-phenylimidazo[2,1-*b*]benzothiazoles have not been reported. Therefore, five compounds (**8–12**) were prepared to begin with and tested for their immunological activities.

Fortunately, **10** suppressed the DTH response without inhibition of antibody formation. This finding stimulated further work on the 2-phenylimidazo[2,1-*b*]benzothiazole derivatives and, initially, a series of derivatives having a single substituent in 2-phenyl ring was prepared and tested on DTH response (Table I). The hydroxy derivatives (**10**, **20**, **32**) showed suppressive activity and the meta derivative (**20**) tended to be more potent than its ortho (**32**) or para (**10**) isomers. However, O-methylation (**11**, **21**, **33**) resulted in loss of activity. Among the derivatives having electron-withdrawing groups, the *m*-carbomethoxy (**28**) and *m*-nitro (**31**) derivatives showed suppressive activity, but their para isomers (**17**, **19**) were inactive. In contrast to above results, the *p*-carboxy derivative (**18**) was active, but the meta isomer (**29**) was inactive and unexpectedly the *m*-cyano derivative (**30**) enhanced DTH response. The halogenated derivatives (**12–15**, **22**, **34**) and the free or substituted amino derivatives (**16**, **23–27**) were inactive.

Although consistent structure–activity relationships were not observed in the assay of DTH response (Table I), it seemed that the hydroxy (**20**) or electron-withdrawing group, such as the carbomethoxy (**28**) or nitro (**31**) group, at the meta position in the 2-phenyl ring played an important role in the activity.

With this in mind, a variety of disubstituted derivatives having a hydroxy or an electron-withdrawing group (NO_2, $COOCH_3$, SO_2CH_3, etc.) at the meta position in the 2-phenyl ring were prepared and tested on DTH response, but these disubstituted derivatives were inactive except for **47** and **48** (Table II). However, **47** was not as active as **20**, and **48** did not show dose-dependent effect on DTH response.

We turned our attention to some modification of the ring system and prepared the compounds replacing the sulfur atom of **20** with an oxygen atom (**53**) or a nitrogen atom (**54**) and the compound **59**, having thiazolo[3,2-*a*]benzimidazole ring system. However, **53** and **59** were inactive, and **54** was not tested because of its high toxicity (Table III).

Table IV. Effect of 20 on DTH

compd	dose, mg/kg, po	increase in ear thickness,[a] $^1/_{100}$ mm ± SE	inhibn, %
control		6.2 ± 0.8	
20	25	5.3 ± 0.8	14.5
	50	3.9 ± 0.5*	37.1
	100	2.4 ± 0.6**	61.3
	200	2.8 ± 0.9*	54.8
	400	1.3 ± 0.6**	79.0

[a] Statistical significance of the data was estimated by using the Student's *t* test (*, $p < 0.05$; **, $p < 0.01$).

Table V. Effect of 20 on PFC in BDF_1 Mice

compd	dose, mg/kg, po	*n*	no. of cells (× 10^6)	PFC/spleen (× 10^3)	PFC/10^6 cells
control		5	90 ± 9.7	188 ± 37.8	2090 ± 383
20	10	5	76 ± 7.5	177 ± 34.7	2370 ± 386
	30	5	80 ± 13.3	138 ± 15.7	1990 ± 421
	100	5	103 ± 20.2	198 ± 40.3	2350 ± 691
	300	5	109 ± 9.2	182 ± 28.6	1850 ± 520

None of the compounds, at any doses tested, showed significant effects on antibody formation. On the other hand, cyclophosphamide inhibited not only DTH response but also antibody formation in mice.[12]

On the basis of the above results, compound **20** has been selected as an orally active and selective immunosuppressive agent of cell-mediated immunity and examined for further biological studies as described below.

Compound **20** suppressed DTH response dose dependently (25–400 mg/kg, po, in mice, Table IV) and showed no effect on the plaque forming cell formation (10–300 mg/kg, po, in mice, Table V). Compound **20** showed no bone marrow suppression (in rats after 5 weeks treatment at doses up to 1000 mg/kg, po). Furthermore, **20** inhibits adjuvant arthritis and rejection of skin graft in mice or rats without inhibition of carrageenan-induced paw edema in rats.[12] These data suggested that **20** inhibits selectively cell-mediated immunity through the mechanism differed from those of classical immunosuppressive agents[2,3b] and nonsteroidal antiinflammatory drugs. However the

(12) Tomioka, K., unpublished data.

mechanism has not yet been elucidated.

In summary, several 2-phenylimidazo[2,1-*b*]benzothiazole derivatives were orally active in the suppression of DTH response and monosubstituted derivatives were more potent than disubstituted derivatives. Among four compounds (**20**, **53**, **54**, and **59**, which have different ring systems), only **20** having imidazo[2,1-*b*]benzothiazole ring was active. It indicates that this ring system seems to play an important role in altering the immune response. Among these compounds, 2-(*m*-hydroxyphenyl)imidazo[2,1-*b*]-benzothiazole (**20**) was found to be the most potent with respect to the suppressive activity of DTH response in the mice model. Compound **20** showed low acute toxicity (MLD of mice and rats were >2400 and >1600 mg/kg, po, respectively) and showed interesting results in some biological examinations, such as reverse passive Arthus reaction and immune complex-induced glomerulonephritis.[12]

Experimental Section

Chemistry. Melting points were taken on a Yanaco MP-3 melting point apparatus and are uncorrected. All compounds were characterized by IR, NMR, and elemental analyses (C, H, N, S, Cl), which were within ±0.4% of the theoretical values. In general, organic extract was dried over $MgSO_4$ and solvent was evaporated under reduced pressure.

2-Aminobenzothiazoles 5. 2-Aminobenzothiazole and 6-methyl- and 6-methoxy-2-aminobenzothiazoles were commercially obtained, and 2-amino-6-hydroxybenzothiazole was prepared by reported method.[13]

Acetophenones. Most acetophenones were commercially available. 3,4-Dihydroxy-,[14] 3-hydroxy-4-methyl-,[15] 3-hydroxy-4-methoxy-,[16] 4-chloro-3-hydroxy-,[15] 4-hydroxy-3-nitro,[17] 4-methyl-3-(methylsulfinyl)-,[18] and 4-methyl-3-(methylsulfonyl)-acetophenones[18] and 3-hydroxypropiophenone[15] were prepared as described.

Phenacyl Bromides 4. Phenacyl bromides were prepared by bromination of acetophenones or propiophenones.[19] 3-Carbomethoxy-, 4-carbomethoxy-, 4-fluoro-, 4-iodo-, and 3-cyanophenacyl bromides were prepared from benzoic acids by a known method.[20]

Method A. 2-(*m*-Hydroxyphenyl)imidazo[2,1-*b*]benzothiazole (20). A mixture of *m*-hydroxyphenacyl bromide (4.3 g, 20 mmol) and 2-aminobenzothiazole (6 g, 40 mmol) in *i*-PrOH (30 mL) was refluxed for 1–2 h. To the reaction mixture was added H_2O (3 mL) and the mixture was cooled. The deposited crystals were collected by filtration, washed with 90% *i*-PrOH, and recrystallized from 80% 2-methoxyethanol–H_2O to yield 3.7 g (70%), mp 248 °C. Anal. ($C_{15}H_{10}N_2OS$) C, H, N, S.

Method B. 2-(*m*-Chlorophenyl)imidazo[2,1-*b*]benzothiazole (22). A mixture of *m*-chlorophenacyl bromide (7 g, 30 mmol) and 2-aminobenzothiazole (4.5 g, 30 mmol) in methyl ethyl ketone (50 mL) was refluxed for 1–2 h. After cooling, the deposited crystals were collected by filtration, washed with methyl ethyl ketone and ether, and dried. A mixture of the obtained crystals in 2-methoxyethanol (50 mL) was refluxed for 1 h. The reaction mixture was cooled and made alkaline with diluted NH_4OH or 3% $NaHCO_3$. The deposited crystals were collected by filtration and recrystallized from EtOH to yield 2.2 g (31%), mp 175 °C. Anal. ($C_{15}H_9ClN_2S$) C, H, N, S.

Method C. 2-(*p*-Hydroxyphenyl)imidazo[2,1-*b*]benzothiazole (10). To a mixture of 2-(*p*-acetoxyphenyl)imidazo-[2,1-*b*]benzothiazole (2.2 g, 7.1 mmol), which was obtained from *p*-acetoxyphenacyl bromide and 2-aminobenzothiazole by method A or B, in MeOH (30 mL) was added 10% KOH (20 mL) and the mixture was stirred for 30 min at room temperature. The mixture was acidified with CH_3COOH, and the deposited crystals were collected by filtration and washed successively with water, MeOH, and ether and dried to yield 2.0 g (61%), mp 298 °C. Anal. ($C_{15}H_{10}N_2OS$) C, H, N, S.

Method D. 2-(*m*-Carboxyphenyl)imidazo[2,1-*b*]benzothiazole (29). To a mixture of **28** (2.7 g, 9.1 mmol) in MeOH (40 mL) was added a solution of KOH (3.5 g) in water (10 mL) and the mixture was refluxed for 10 min. The reaction mixture was cooled and acidified with 2 N HCl (30 mL). The deposited crystals were collected by filtration and washed successively with water, MeOH, and ether and dried to yield 1.8 g (70%), mp >300 °C. Anal. ($C_{16}H_{10}N_2O_2S$) C, H, N, S.

Method E. 2-(*m*-Aminophenyl)imidazo[2,1-*b*]benzothiazole (23). A mixture of **26**·HBr (5.5 g), 2.2 N HCl (30 mL), and MeOH (20 mL) was refluxed for 2 h. The reaction mixture was cooled and made alkaline with diluted NH_4OH and extracted with CH_3COOEt. The extract was washed with saturated NaCl aqueous solution, dried, and concentrated. The residue was recrystallized from CH_3COOEt–ether to yield 3.5 g (93%), mp 181 °C. Anal. ($C_{15}H_{11}n_3S$) C, H, N, S.

Method F. 2-[*m*-(*N*,*N*-Dimethylamino)phenyl]imidazo-[2,1-*b*]benzothiazole (24). A mixture of **23** (2.65 g, 10 mmol), K_2CO_3 (2.8 g, 20.3 mmol), and MeI (2.8 g, 20 mmol) in methyl ethyl ketone was refluxed for 2 h. The insoluble materials were removed by filtration. The filtrate was concentrated and to the residue was added 1 N HCl (50 mL). The aqueous solution was washed with CH_3COOEt (50 mL, two times) and made alkaline with 5% NH_4OH. The mixture was extracted with CH_3COOEt (50 mL). The extract was washed with water, dried, and concentrated. The oily residue was dissolved in 1 N HCl–EtOH (20 mL) and to the solution was added ether (20 mL). The deposited crystals were collected by filtration and dried to yield 2.0 g (55%), mp 220 °C. Anal. ($C_{17}H_{15}N_3S$·2HCl) C, H, N, Cl.

Method G. 2-(*m*-Formamidophenyl)imidazo[2,1-*b*]-benzothiazole (25). To a mixture of HCOOH–Ac_2O (3:5, v/v, 15 mL) was added **23** (1.2 g) at 3–15 °C and the mixture was stirred for 1 h at room temperature. To the reaction mixture was added H_2O (100 mL) and the mixture was extracted with CH_3COOEt (50 mL). The extract was washed with water and 5% $NaHCO_3$, dried, and concentrated. The residue was washed with ether and dried to yield 1.2 g (94%), mp 163 °C. Anal. ($C_{16}H_{11}N_3OS$) C, H, N, S.

Method H. 2-(*m*-Hydroxyphenyl)imidazo[2,1-*b*]benzoxazole (53).[21] A mixture of 2-aminobenzoxazole (**49**)[22] (1.34 g, 9.3 mmol), *m*-hydroxyphenacyl bromide (2.2 g, 10.2 mmol), and EtOH (16 mL) was refluxed for 1 h. After cooling, the deposited crystals were collected by filtration. A mixture of the obtained crystals and 2-methoxyethanol (20 mL) was refluxed for 5 h. After cooling, the reaction mixture was made alkaline with aqueous $NaHCO_3$ and added water (60 mL) and stirred for 3 h. The deposited crystals were collected by filtration and recrystallized from EtOH to yield 0.45 g (18%), mp 233 °C. Anal. ($C_{15}H_{10}N_2O_2$) C, H, N.

Method I. 2-(*m*-Hydroxyphenyl)-1*H*-imidazo[3,2-*a*]-benzimidazole (54).[23] A mixture of 2-aminobenzimidazole (**50**)[24] (2.66 g, 20 mmol), *m*-hydroxyphenacyl bromide (4.3 g, 20 mmol), and MeOH (20 mL) was stirred for 3 days at room temperature. The reaction mixture was cooled down to 0 °C and allowed to stand overnight at the same temperature. The crystals were removed by filtration, and the filtrate was concentrated to give crude crystals of 2-amino-1-(*m*-hydroxyphenacyl)benzimidazole hydrobromide (4.3 g) (**52**·HBr). Compound **52**·HBr (3.0 g) was

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dissolved in MeOH (20 mL) and the mixture was neutralized with aqueous $NaHCO_3$ and then H_2O (10 mL) was added. The deposited crystals were collected by filtration to give 2-amino-1-(*m*-hydroxyphenacyl)benzimidazole (**52**) (1.2 g). The mixture of **52** (1.2 g) and 2-methoxyethanol (12 mL) was refluxed overnight and the reaction mixture was concentrated. The residue was purified by column chromatography on silica gel (toluene/CH_3COOEt, 1:1) to yield 450 mg (11%), mp 264 °C. Anal. ($C_{15}H_{11}N_3O\cdot{}^1/_2H_2O$) C, H.

Method J. 2-(*m*-Hydroxyphenyl)thiazolo[3,2-*a*]benzimidazole (59).[25] A mixture of 2-chlorobenzimidazole (**55**)[11] (3.8 g, 25.1 mmol), *m*-hydroxyphenacyl bromide (5.4 g, 25.1 mmol), MeONa (1.4 g, 26 mmol), and MeOH (25 mL) was refluxed for 1.5 h. The reaction mixture was concentrated and the residue was extracted with CH_3COOEt. The extract was washed with water, dried, and concentrated. To the residue was added EtOH (120 mL) and the mixture was allowed to stand overnight at 0 °C. The insoluble materials were removed by filtration, and the filtrate was concentrated. The residue was triturated with a mixture of EtOH (10 mL) and ether (50 mL), and the crystals were collected by filtration and dried to give 2-chloro-1-[(*m*-hydroxybenzoyl)methyl]benzimidazole (**56**) (2.5 g, 35%).

A mixture of **56** (2.48 g, 8.7 mmol), thiourea (660 mg, 8.7 mmol), and MeOH (150 mL) was heated for 2.5 h at 50 °C. After cooling, the crystals were collected by filtration, washed successively with MeOH and ether, and dried to give 2-mercapto-1-[(*m*-hydroxybenzoyl)methyl]benzimidazole (**57**) (1.56 g, 63.5%).

A mixture of **57** (2.05 g) and Ac_2O (40 mL) was refluxed for 35 min, and to the reaction mixture 20 drops of concentrated H_2SO_4 was added and then the mixture was refluxed for 30 min and concentrated. To the residue was added H_2O–ice (50 g) and the mixture was extracted with CH_3COOEt. The extract was washed successively with aqueous $NaHCO_3$ and water, dried, and concenrated. The residue was recrystallized from EtOH to give 2-(*m*-acetoxyphenyl)thiazolo[3,2-*a*]benzimidazole (**58**) (1.8 g, 81%). A mixture of **58** (1.8 g), 1 N NaOH (12 mL) and MeOH (35 mL) was stirred for 30 min at room temperature. The reaction mixture was acidified with dilute CH_3COOH, and the deposited crystals were collected by filtration and washed successively with water and EtOH and dried to yield 1.5 g (97.2%), mp 260 °C. Anal. ($C_{15}H_{10}N_2OS$) C, H, N, S.

Pharmacology. Antibody Formation in Mice. Immunization was performed according to the method of Levine and Vaz.[26] Briefly, female BDF_1 mice weighing 17–19 g were intraperitoneally injected with 10 μg of DNP-OA and 1 mg of alum in 0.2 mL of saline and bled from the tail vein 7, 14 days later. Equal volumes of individual sera from the same group were pooled and used for antibody assay. The test drug was orally administered from day 0 to day 4 after immunization. For each dose level five mice were used.

The anti-DNP PHA antibodies (IgM and IgG) were determined by the method of Onkelinx.[27] Washed and packed sheep red blood cells (SRBC, 200 μL) were added to 10 mL of PBS together with 2.5 mL of 5 mg/mL DNP-BSA and 1 mL of 2.5% glutaraldehyde. The mixture was kept at room temperature for 2 h and then allowed to stand overnight at 4 °C. Thereafter, the cells were washed three times with PBS and made up to 2% with PBS containing 0.5% normal rabbit serum which had been heated at 56 °C for 2 h and absorbed with SRBC. Serial dilution of pooled antisera were mixed with 25 μL of a 2% sensitized SRBC suspension in V-bottomed microtiter plates. After 3 h, agglutination in the bottom of the plates was examined. The titers were expressed as the reciprocal of the maximum dilution giving a positive reaction; the difference greater than fourfold between titers was considered significant.

Delayed-Type Hypersensitivity Reaction Induced by Picryl Chloride in Mice. Male ICR mice (7 weeks old) were sensitized[28] by applying 0.1 mL of 7% picryl chloride solution in acetone to the shaved abdomen. After a 7-day sensitization period, the mice were challenged by painting the inside of each ear with 0.02 mL of 1% picryl chloride solution in olive oil. The ear thickness was measured with a dial thickness gauge before and 24 h after the challenge, and differences were obtained. Drug actions in the sensitization phase were examined by administering the drug from day 0 to day 2 after immunization.

Plaque Forming Cell (PFC) Formation in Mice. Seven-week-old male BDF_1 mice were immunized intravenously with 5×10^8 SRBC in 0.4 mL of saline. Four days after the immunization, the spleen was prepared by passing it through a stainless mesh (200 mesh) in 5 mL of Eagle's Minimum Essential Medium (Eagle's MEM) containing 5 units/mL of heparin. The suspension was diluted 10-fold with Eagle's MEM. Then 0.45 mL of 5×10^8 SRBC and 0.45 mL of 5% fresh guinea pig serum which had been absorbed with SRBC were added to the test tube containing 1.1 mL of the cell suspension. The mixture was implanted into Cunningham's chambers, sealed well with paraffin–vaseline melting mixture (1:1), and then incubated at 37 °C for 45 min. The reaction was stopped by cooling at 5 °C. The plaques appearing in the three chambers were counted and total PFC and PFC/10^6 splen cells were obtained. Test drugs were administered intraperitoneally from day 0 to day 3 after the immunization. The number of splen cells was counted by a Coutler Counter (Model ZB1, Coulter Electronics).

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Registry No. **5**, 136-95-8; **6** (X = H), 79889-86-4; **6** (X = 4-CH_3), 99582-70-4; **6** (X = 4-OH), 99582-71-5; **6** (X = OAc), 99582-72-6; **6** (X = 4-OCH_3), 99582-73-7; **6** (X = 4-Cl), 99582-74-8; **6** (X = 4-Br), 99582-75-9; **6** (X = 4-I), 99582-76-0; 6 (X = 4-F), 99582-77-1; **6** (X = $NHCOCH_3$), 99582-78-2; **6** (X = 4-$COOCH_3$), 99582-79-3; **6** (X = 4-NO_2), 99582-80-6; **6** (X = 3-OCH_3), 99582-81-7; **6** (X = 3-Cl), 79889-31-9; **6** (X = 3-$NHCOCH_3$), 99582-82-8; **6** (X = 3-$NHSO_2CH_3$), 99582-83-9; **6** (X = 3-CN), 99582-84-0; **6** (X = 3-NO_2), 99582-85-1; **6** (X = 2-Cl), 99582-86-2; **6** (X = 3-OH, 4-CH_3), 99582-87-3; **6** (X = 3-OH, 4-OCH_3), 99582-88-4; **6** (X = 3-OH, 4-Cl), 99582-89-5; **6** (X = 3-$COOCH_3$, 4-OH), 99582-90-8; **6** (X = 3-$SOCH_3$, 4-CH_3), 99593-32-5; **6** (X = 3-SO_2CH_3, 4-CH_3), 99582-91-9; **6** (X = 3-OH) 6-methylbenzothizole deriv., 99582-92-0; **6** (X = 3-OH) 6-hydroxybenzothiazole deriv., 99582-93-1; **6** (X = 3-COOMe) 6-hydroxybenzothiazole deriv., 99582-94-2; **7** (X = 4-OH), 79889-87-5; **7** (X = 2-OH), 79889-79-5; **7** (X = 3,4-OAc), 99582-95-3; **7** (X = 3-NO_2, 4-OH), 79889-53-5; **8**, 17833-07-7; **9**, 38956-27-3; **10**, 79889-87-5; 11, 26921-83-5; **12**, 7025-32-3; **13**, 7025-33-4; **14**, 79889-33-1; **15**, 7067-87-0; **16**, 79890-39-4; **17**, 79889-40-0; **18**, 79889-93-3; **19**, 17833-15-7; **20**, 79889-39-7; **21**, 79889-64-8; **22**, 79889-32-0; **23**, 79890-07-6; **24**, 79890-38-3; **25**, 79890-06-5; **26**, 79889-60-4; **26**·HBr, 99582-96-4; **27**, 79889-61-5; **28**, 79889-62-6; **29**, 79889-97-7; **30**, 79889-65-9; **31**, 79889-59-1; **32**, 79889-79-5; **33**, 79889-77-3; **34**, 79889-34-2; **35**, 79889-83-1; **36**, 79889-91-1; **37**, 79889-56-8; **38**, 79889-57-9; **39**, 79889-58-0; **40**, 79889-71-7; **41**, 79889-44-4; **42**, 79890-34-9; **43**, 79890-43-0; **44**, 79890-41-8; **45**, 79890-33-8; **46**, 79889-53-5; **47**, 79890-31-6; **48**, 79890-32-7; **49**, 4570-41-6; **50**, 934-32-7; **51**, 99582-97-5; **52**, 99582-98-6; **53**, 99582-99-7; **54**, 99583-00-3; **55**, 4857-06-1; **56**, 99583-01-4; **57**, 99583-02-5; **58**, 99583-03-6; **59**, 99583-04-7; phenacyl bromide, 70-11-1; *p*-methylphenacyl bromide, 536-38-9; *p*-hydroxyphenacyl bromide, 2491-38-5; *p*-acetoxyphenacyl bromide, 41104-10-3; *p*-methoxyphenacyl bromide, 2632-13-5; *p*-chlorophenacyl bromide, 536-38-9; *p*-bromophenacyl bromide, 99-73-0; *p*-iodophenacyl bromide, 31827-94-8; *p*-fluorophenacyl bromide, 403-29-2; *p*-(acetylamino)phenacyl bromide, 21675-02-5; *p*-(methoxycarbonyl)phenacyl bromide, 56893-25-5; *p*-nitrophenacyl bromide, 99-81-0; *m*-hydroxyphenacyl bromide, 2491-37-4; *m*-methoxyphenacyl bromide, 5000-65-7; *m*-chlorophenacyl bromide,

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New Structure–Activity Relationships of the Quinolone Antibacterials Using the Target Enzyme. The Development and Application of a DNA Gyrase Assay

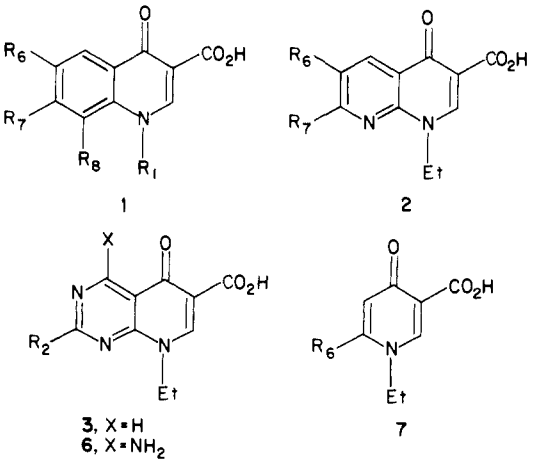
John M. Domagala,* Lori Doyle Hanna, Carl L. Heifetz, Marland P. Hutt, Thomas F. Mich, Joseph P. Sanchez, and Marjorie Solomon

Warner-Lambert/Parke-Davis Pharmaceutical Research, Ann Arbor, Michigan 48105. *Received April 25, 1985*

A series of 60 newly synthesized and known quinolone antibacterials, including quinoline- and 1,8-naphthyridine-3-carboxylic acids, pyrido[2,3-*d*]pyrimidine-6-carboxylic acids, and some monocyclic 4-pyridone-3-carboxylic acids, were tested and compared in a newly established, easy to perform, DNA gyrase assay. The results were correlated with minimum inhibitory concentrations (MICs) against a variety of organisms. Among the known quinolones were 14 clinically significant drugs (oxolinic acid, norfloxacin, ciprofloxacin, enoxacin, etc.) which were used as standards and compared side-by-side. The study focused on the changes in DNA gyrase inhibition brought about by certain features of the molecules, namely, the C_6-fluorine or the nature of the C_7 substituent. The intrinsic gyrase inhibition of the fused parent rings, quinoline vs. naphthyridine vs. pyrido[2,3-*d*]pyrimidine, was also explored. In all cases, loss of enzyme inhibition produced poor MICs, but some compounds with good DNA gyrase inhibition did not correspondingly inhibit bacterial growth. Possible explanations for this phenomena and the benefits of a DNA gyrase–MIC strategy for developing future structure–activity relationships are discussed.

During the last 25 years, the quinolone class of orally active antibacterials (which generically has come to include most of the 4-pyridone-3-carboxylic acid antibacterials) has been intensively studied and evaluated for use in antiinfective chemotherapy.[1] Many of the initial agents, such as oxolinic (**1a**) and nalidixic (**2a**) acids (shown in Figure 1), lacked substantial Gram-positive activity and had blood levels below their minimum inhibitory concentrations (MICs). After the discovery of norfloxacin (**1c**) and enoxacin (**2b**), which were the first members of this class of antibacterials to possess broad-spectrum activity with oral efficacy, an explosion of many new and useful agents such as ciprofloxacin (**1f**) and AM833 (**1h**) has occurred.[2] Several other significant quinolone antibacterials are shown in Figure 1.

Midway through the chemical development of this area, the mechanism of action of the quinolones was elucidated.



These agents were shown to be specific inhibitors of the A subunit of the bacterial topoisomerase DNA gyrase.[12]

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