Method R. α-(*m*-Trifluoromethylanilino)phenylacetic Acid.—A solution of *m*-trifluoromethylaniline (12.0 g) and α-bromophenylacetic acid (6.5 g) in EtOH (100 ml) was heated at 100° for 5 hr, and the solvent was evaporated at 40°. The residue was equilibrated between H_2O (100 ml) and Et_2O (100 ml), and the organic layer was extracted with 10% NaOH (three 75-ml portions). The combined caustic extracts were extracted repeatedly with Et_2O and neutralized by the addition of HCl. A colorless oil separated which crystallized on standing as white crystals (6.0 g).

Method S. α-(*m*-Trifluoromethylanilino)-*p*-isopropylphenylacetic Acid.—Ethyl *p*-isopropylmandelate (9.0 g) and $SOCl_2$ (6.0 g) were mixed and heated at 100° for 1 hr after the initial vigorous reaction had subsided. Evaporation of the excess $SOCl_2$ at 50° yielded the fairly pure α-chloro compound (8.5 g).

This material (8.0 g) and *m*-trifluoromethylaniline (12.0 g) were stirred at 100° for 8 hr. EtOH (100 ml) was added and the heating was continued for 2 hr. The EtOH was evaporated and the residue dissolved in Et_2O (100 ml). The organic solution was washed successively with 2 *N* HCl and H_2O, dried (Na_2SO_4), and evaporated. This afforded ethyl α-(*m*-trifluoromethylanilino)-*p*-isopropylphenylacetate as a brown oil (9.7 g). This was dissolved in EtOH (10 ml) and added to a solution of KOH (2.8 g) in H_2O (10 ml). The mixture was stirred under gentle reflux for 3 hr, concentrated to half-volume and diluted with H_2O (50 ml). The clear aqueous solution was extracted with toluene (50 ml). The aqueous solution was neutralized by the addition of HCl and extracted with Et_2O. The dried (Na_2SO_4) Et_2O solution on evaporation yielded an oil (7.9 g). Trituration of the latter with petroleum ether (bp 40–60°) yielded a white solid (4.64 g) which was the pure expected amino acid.

Method T. 2-(α-Anilinotolyl)pentanoic Acid. Benzylidineaniline (36.2 g) and ethyl 2-bromopentanoate (42.0 g) gave 1,1-diphenyl-3-*n*-propyl-2-azetidinone, mp 90–92°, by the method of Gilman and Speeter.[13] The yield was 32 g (60%) after recrystallization from MeOH. *Anal.* ($C_{18}H_{19}NO$) C, H, N.

A solution of KOH (6.0 g) in H_2O (10 ml) was added to a solution of the azetidinone (10.0 g) in EtOH (100 ml). The mixture was stirred under reflux for 4 hr and evaporated. The residue was dissolved in H_2O and extracted twice with toluene. The aqueous solution was neutralized with HCl and the precipitate was filtered off. Recrystallization of this solid from aqueous MeOH, and then EtOH, yielded the expected acid (9.7 g, 90%), mp 118–120°.

Method U. α-(2,3-Dimethylphenoxy)phenylacetic Acid. Ethyl α-bromophenylacetate (12.15 g) and 2,3-dimethylphenol (20.0 g) gave ethyl α-(2,3-dimethylphenoxy)phenylacetate (5.3 g, 37%), bp 171–173° (1 mm), by the method of Guss.[14] *Anal.* ($C_{18}H_{20}O_3$) C, H, N. Hydrolysis of this ester yielded the corresponding acid.

Acknowledgment.—We wish to thank our colleagues in the Pharmacology Department of our Company for the biological results.

(13) H. Gilman and M. Speeter, *J. Am. Chem. Soc.*, **65**, 2255 (1943).
(14) C. O. Guss, *ibid.*, **71**, 3460 (1949).

Heterocyclic Substituted Ureas. I. Immunosuppression and Virus Inhibition by Benzimidazoleureas

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In an effort to define the structure–activity relationships (SAR) for immunosuppression and antiviral activity, a series of 1-(benzimidazol-2-yl)-3-substituted ureas and their close analogs have been synthesized. Many of these compounds display potent immunosuppression in the sheep erythrocyte test in mice, the most effective being approximately 2000 times as active as azathioprine. In addition, these compounds have potent *in vivo* antiviral activity in several viral diseases. We have defined the antiviral SAR using mice infected with Coxsackie A21 virus (Coe).

Our interest in immunosuppressive drugs has been stimulated by an increasing number of reports of the use of azathioprine and other immunosuppressive agents in the treatment of lupus erythematosus,[1] rheumatoid arthritis,[2] and glomerulonephritis.[3] These represent just a few of the many disease states which are considered to be of an autoimmune[4] nature and therefore amenable to immunosuppressant therapy.

In our laboratories we have searched for nontoxic compounds which alter an animal's immune responses and avoid the disadvantages of the drugs now in use. We wish to report a series of benzimidazoleureas which are potent immunosuppressives and which in addition are potent protective agents against several experimental virus infections in mice. This series was extended to determine more specifically the structural requirements for maximum specific activity in each biological system.

Immunosuppressive activity has not, to our knowledge, been reported for any benzimidazoles. Antiviral activity has been reported for urea derivatives[5] and for some benzimidazole derivatives.[5] However, most of the previous antiviral work was based on tissue culture systems, and the compounds were not protective or showed minimal activity in animal infections.

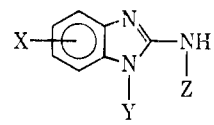
Chemistry.—The required 2-aminobenzimidazoles were either purchased or prepared from the appropriately substituted *o*-diamine and cyanogen bromide by the method of Leonard, *et al.*[6] The N-substituted 2-aminobenzimidazoles were prepared by autoclaving 2-chlorobenzimidazole and an appropriate primary amine.

These 2-aminobenzimidazoles were in turn treated with an appropriately substituted isocyanate or carbamoyl chloride in an inert solvent to give the de-

(1) (a) *Brit. Med. J.*, 1, 650 (1967); (b) N. E. Gary, J. F. Maher, and G. E. Schreiner, *Clin. Res.*, **14**, 490 (1966).
(2) (a) V. K. Philips, W. Bergen, and N. O. Rothermich, *Arthritis Rheumat.*, **10**, 305 (1967); (b) R. Guenther, *Wien. Med. Wochschr.*, **117**, 985 (1967); (c) M. F. Kahn and M. Bedoiseau, *Proc. Roy. Soc. Med.*, **60**, 130 (1967); (d) A. F. Michael, R. L. Vernier, K. N. Drummond, J. I. Levitt, R. C. Herdman, A. J. Fish, and R. A. Good, *New Engl. J. Med.*, **276**, 817 (1967); (e) D. A. Adams, A. Gordon, and M. H. Maxwell, *J. Am. Med. Assoc.*, **199**, 459 (1967).
(3) (a) Y. C. Tsao, *Brit. Med. J.*, II, 1057 (1966); (b) R. H. R. White, *Proc. Roy. Soc. Med.*, **60**, 1164 (1967); (c) *Can. Med. Assoc. J.*, **96**, 52 (1967).
(4) J. N. Humphrey and R. G. White, "Immunology for Students of Medicine," 2nd ed, F. A. Davis Co., Philadelphia, Pa., 1964.
(5) P. W. Sadler, *Pharmacol. Rev.*, **15**, 423 (1963).
(6) N. J. Leonard, B. Y. Curtin, and K. M. Beck, *J. Amer. Chem. Soc.*, **69**, 2459 (1947).

TABLE I

INTERMEDIATE 2-AMINOBENZIMIDAZOLES

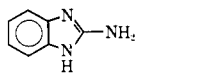
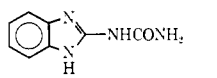
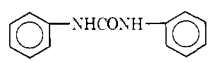
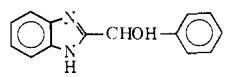


No.	X	Y	Z	Mp, °C	Formula	Recrystn solvent
1	5-Cl	H	H	158–161[a]	$C_7H_5ClN_3$	EtOAc–Skelly B
2	5-CF_3	H	H	152–155[b]	$C_8H_6F_3N_3$	Et_2O–Skelly B
3	5-CH_3	H	H	197–199[c]	$C_8H_9N_3$	Me_2CO–Skelly B
4	5-$COOC_2H_5$	H	H	194–197	$C_{10}H_{11}N_3O_2$	Me_2CO–Skelly B
5	5,6-CH_2O_2	H	H	235–239	$C_8H_7N_3O_2$	EtOAc–Skelly B
6	4-NO_2	H	H	271–274	$C_7H_5N_4O$	Me_2CO–Skelly B
7		H	H	297–299[d]	$C_{11}H_9N_3$	Me_2CO–Skelly B
8		H	H	218–221[e]	$C_{11}H_9N_3$	EtOAc–Skelly B
9	H	CH_3	H	196–198[f]	$C_8H_9N_3$	EtOAc–Skelly B
10	H	H	CH_3	176–178	$C_8H_9N_3$	Me_2CO–hexane
11	H	C_6H_5	H	149–153[g]	$C_{12}H_{11}N_3$	EtOAc–Skelly B

[a] N. J. Leonard, D. Y. Austin, and K. M. Beck [*J. Amer. Chem. Soc.*, **69**, 2459 (1947)] report mp 167–168°. [b] B. C. Bishop, A. S. Jones, and J. C. Tatlow [*J. Chem. Soc.*, 3076 (1964)] report mp 156–158°. [c] L. Joseph [*J. Med. Chem.*, **6**, 601 (1963)] reports 203–204°; N. P. Bednyagina and I. Ya Postovskii [*Zh. Obshch. Khim.*, **30**, 1431 (1960)] report mp 200–201°. [d] D. J. Brown [*J. Chem. Soc.*, 1974 (1958)] reports mp 301°. [e] G. B. Crippa and S. Maffei [*Gazz. Chim. Ital.*, **71**, 418 (1941); *Chem. Abstr.*, **37**, 120 (1943)] report mp 212.5°. [f] L. Joseph [*J. Med. Chem.*, **6**, 601 (1963)] reports mp 202–203°; N. P. Bednyagina and I. Ya Postovskii [*Zh. Obshch. Khim.*, **30**, 1431 (1960); *Chem. Abstr.*, **55**, 1596 (1961)] report mp 200–201°. [g] L. Joseph [*J. Med Chem.*, **6**, 601 (1963)] reports mp 154–155°; A. M. Simonov and A. F. Pozharskii [*Zh. Obshch. Khim.*, **33**, 2350 (1963); *Chem. Abstr.*, **59**, 1397 (1963)] report mp 151–152°.

TABLE II[a]

KNOWN IMMUNOSUPPRESSANTS AND POSSIBLE METABOLITES OF 2-AMINOBENZIMIDAZOLES

No.	Structure	Coe ED_{50}, mg/kg	Drug level (mg/kg × 3) for fourfold or greater immunosuppression
1	Azathioprine	>128	100
2	Cortisone		200
3		>128	>200
4		>128	400
5			>200
6		>512	
7		>128	>800

[a] All compounds obtained from commercial sources.

sired benzimidazoleureas. The activities and melting points are given in Tables I–IV.

Pharmacology. Evaluation of Antiviral Activity.—Compounds in this series have shown *in vivo* activity against a wide spectrum of viruses,[7] *e.g.*, polio, vaccinia, and influenza. The activity against Coxsackie A21 (Coe) infection in mice appeared to give the most quantitative measure of activity for structure–activity studies. Coe virus, isolated by Lennette, *et al.*,[8] from patients with mild respiratory illness, produces muscle degeneration and flaccid paralysis in addition to respiratory involvement in mice.[9] Activity was initially detected using multiple doses, but studies to determine the effect of dose schedules indicated that single doses were almost as effective. ED_{50} values reported in this paper are based on a single dose given intraperitoneally to mice 3 hr before intraperitoneal infection with about $1LD_{100}$ of virus. Mouse-adapted Coe virus carried in this laboratory by passage in mice was used in a dilution, usually 1:20, of the supernatant of a 10% gluteal muscle suspension. Dilution of virus suspension to give $1LD_{100}$ was determined by titration in mice. Compounds were administered as suspensions in 0.25 ml of sterile 3% "Emulphor" in water at levels of 16, 32, 64, and 128 mg/kg. Groups of 10 SPF white Swiss mice weighing 11–13 g were used for each drug level, and three to five groups of ten mice were used as controls. Control mice usually died on the 5th to 8th day postinfection. Animals alive on the 10th day were considered survivors if free from paralysis. The ED_{50} was calculated based on number of survivors by the method of Reed and Muench.[10]

Procedure for Determining Immunosuppression.—A modification of the procedure of Nathan, *et al.*,[11] was employed to determine immunosuppressive activity. Groups of five 20-g Swiss mice were injected intraperitoneally with 0.2 ml of 1:80 standardized suspensions of sheep red blood cells (*ca.* 5×10^7 RBC). At 72, 48, and 24 hr before the red cell injections, test compounds were injected by the same route. Eight

(7) D. C. DeLong, L. A. Baker, J. D. Nelson, and C. J. Paget, unpublished results.

(8) E. H. Lennette, V. L. Fox, N. J. Schmidt, and J. O. Culver, *Am. J. Hyg.*, **68**, 272 (1959).

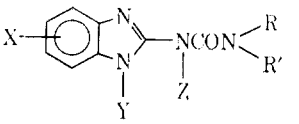
(9) G. E. Underwood, C. A. Wisner, S. D. Weed, and J. E. Gray, *ibid.*, **76**, 124 (1962).

(10) L. J. Reed and H. Muench, *ibid.*, **27**, 493 (1938).

(11) H. C. Nathan, S. Bieber, G. B. Elion, and G. H. Hitchings, *Proc. Soc. Exptl. Biol. Med.*, **107**, 796 (1961).

TABLE III

1-(BENZIMIDAZOL-2-YL)-3-SUBSTITUTED UREAS



No.	X	Y	Z	R	R′	Mp, °C	Formula	Cne virus ED_{50}, mg/kg	Drug level (mg/kg × 3) for fourfold or greater immunosuppression
1	H	H	H	H	C_6H_5	329–330	$C_{14}H_{12}N_4O$[a]	128	12.5
2	H	H	H	C_6H_5	C_6H_5	178–180	$C_{20}H_{16}N_4O$	85	100.0
3	H	H	H	H	1-$C_{10}H_7$	333	$C_{18}H_{14}N_4O$	23	3.1
4	H	H	H	H	4-ClC_6H_4	349–351	$C_{14}H_{11}ClN_4O$	47	12.5
5	H	H	H	H	3-ClC_6H_4	344–345	$C_{14}H_{11}ClN_4O$	69	3.1
6	H	H	H	H	2-ClC_6H_4	331–333	$C_{14}H_{11}ClN_4O$	32	12.5
7	H	H	H	H	4-BrC_6H_4	342–343	$C_{14}H_{11}BrN_4O$	70	100.0
8	H	H	H	H	2,5-$Cl_2C_6H_3$	348–349	$C_{14}H_{10}Cl_2N_4O$	32	0.8
9	H	H	H	H	3,4-$Cl_2C_6H_3$	337–338	$C_{14}H_{10}Cl_2N_4O$	46	0.8
10	H	H	H	H	4-FC_6H_4	338–339	$C_{14}H_{11}FN_4O$	26	6.2
11	H	H	H	H	2-FC_6H_4	347–348	$C_{14}H_{11}FN_4O$	38	1.6
12	H	H	H	H	4-$NO_2C_6H_4$	301–302	$C_{14}H_{11}N_5O_3$	78	>100.0
13	H	H	H	H	3-$NO_2C_6H_4$	317–318	$C_{14}H_{11}N_5O_3$	84	>100.0
14	H	H	H	H	2-$NO_2C_6H_4$	324–325	$C_{14}H_{11}N_5O_3$	32	>100.0
15	H	H	H	H	3-$NH_2C_6H_4$	330–331	$C_{14}H_{13}N_5O$	84	>100.0
16	H	H	H	H	3-$CF_3C_6H_4$	336–337	$C_{15}H_{11}F_3N_4O$	152	>100.0
17	H	H	H	H	4-$CH_3C_6H_4$	342–343	$C_{15}H_{14}N_4O$	32	3.2
18	H	H	H	H	2-$CH_3C_6H_4$	339–340	$C_{15}H_{14}N_4O$	100	25.0
19	H	H	H	H	CH_3	322–325	$C_9H_{10}N_4O$	239	100.0
20	H	H	H	H	$CH(CH_3)_2$	351–352	$C_{11}H_{14}N_4O$	60	12.5
21	H	H	H	CH_3	CH_3	174–175	$C_{10}H_{12}N_4O$	117	>400.0
22	H	H	H	H	$CH_2CH{=}CH_2$	335–336	$C_{11}H_{12}N_4O$	157	100.0
23	H	H	H	H	C_4H_9	343–344	$C_{12}H_{16}N_4O$	132	>100.0
24	H	H	H	H	$C(CH_3)_3$	349	$C_{12}H_{16}N_4O$	47	50.0
25	H	H	H	H	$(CH_2)_{11}CH_3$	300 dec	$C_{29}H_{52}N_4O$	71	100.0
26	H	H	H	H	$(CH_2)_{17}CH_3$	292–294	$C_{28}H_{44}N_4O$	45	>100.0
27	H	H	H	H	Cyclohexyl	335–336	$C_{14}H_{18}N_4O$	16	>100.0
28	H	H	H	H	Cycloheptyl	311–313	$C_{15}H_{20}N_4O$	130	>100.0
29	H	H	H	H	Cyclooctyl	322–324	$C_{16}H_{22}N_4O$	182	100.0
30	H	H	H	H	Adamantyl	327–328	$C_{18}H_{22}N_4O$	26	>100.0
31	5-Cl	H	H	H	C_6H_5	339–340	$C_{14}H_{11}ClN_4O$	72	25.0
32	5-Cl	H	H	H	1-$C_{10}H_7$	342–343	$C_{18}H_{13}ClN_4O$	67	12.5
33	5-Cl	H	H	H	3-ClC_6H_4	329–330	$C_{14}H_{10}Cl_2N_4O$	64	>100.0
34	5-Cl	H	H	H	2-ClC_6H_4	340	$C_{14}H_{10}Cl_2N_4O$	>128	100.0
35	5-Cl	H	H	H	4-FC_6H_4	348–350	$C_{14}H_{10}ClFN_4O$	<16	12.5
36	5-Cl	H	H	H	2-FC_6H_4	339–340	$C_{14}H_{10}ClFN_4O$	36	6.2
37	5-Cl	H	H	H	3-$NO_2C_6H_4$	294–296	$C_{14}H_{10}ClN_5O_3$	30	>100.0
38	5-Cl	H	H	H	2-$NO_2C_6H_4$	289–291	$C_{14}H_{10}ClN_5O_3$	>128	100.0
39	5-Cl	H	H	H	4-$CH_3C_6H_4$	319–320	$C_{15}H_{13}ClN_4O$	34	12.5
40	5-Cl	H	H	H	3-$CH_3C_6H_4$	315–316	$C_{15}H_{13}ClN_4O$	28	>100.0
41	5-Cl	H	H	H	C_6H_{11}	319–320	$C_{14}H_{17}ClN_4O$	>128	>100.0
42	5-$COOC_2H_5$	H	H	H	C_6H_5	327–328	$C_{17}H_{16}N_4O_3$	30	25.0
43	5-$COOC_2H_5$	H	H	H	1-$C_{10}H_7$	315–316	$C_{21}H_{18}N_4O_3$	27	6.2
44	5-$COOC_2H_5$	H	H	H	4-ClC_6H_4	309–311	$C_{17}H_{15}ClN_4O$	26	50.0
45	5-$COOC_2H_5$	H	H	H	3-ClC_6H_4	308–310	$C_{17}H_{15}ClN_4O_3$	22	50.0
46	5-$COOC_2H_5$	H	H	H	2-ClC_6H_4	308–309	$C_{17}H_{15}ClN_4O_3$	32	0.8
47	5-$COOC_2H_5$	H	H	H	4-FC_6H_4	310–311	$C_{17}H_{15}FN_4O_3$	46	25.0
48	5-$COOC_2H_5$	H	H	H	2-FC_6H_4	315–316	$C_{17}H_{15}FN_4O_3$	64	6.2
49	5-$COOC_2H_5$	H	H	H	4-$NO_2C_6H_4$	279–280	$C_{17}H_{15}N_5O_5$	16	3.1
50	5-$COOC_2H_5$	H	H	H	3-$NO_2C_6H_4$	309–310	$C_{17}H_{15}N_5O_5$	17	25.0
51	5-$COOC_2H_5$	H	H	H	2-$NO_2C_6H_4$	325–327 dec	$C_{17}H_{15}N_5O_5$	25	1.6
52	5-$COOC_2H_5$	H	H	H	3-$NH_2C_6H_4$	277–278	$C_{17}H_{17}N_5O_3$	81	50.0
53	5-$COOC_2H_5$	H	H	H	4-$CH_3C_6H_4$	305–307	$C_{18}H_{18}N_4O_3$	83	100.0
54	5-$COOC_2H_5$	H	H	H	3-$CH_3C_6H_4$	321–323	$C_{18}H_{18}N_4O_3$	32	12.5
55	5-$COOC_2H_5$	H	H	H	2-$CH_3C_6H_4$	304–305	$C_{18}H_{18}N_4O_3$	19	25.0
56	5-$COOC_2H_5$	H	H	H	C_6H_{11}	310–311	$C_{17}H_{22}N_4O_3$	30	100.0
57	5-CF_3	H	H	H	C_6H_5	309–310	$C_{15}H_{11}F_3N_4O$	128	12.5
58	5-CF_3	H	H	H	1-$C_{10}H_7$	307–308	$C_{19}H_{13}F_3N_4O$	21	50.0
59	5-CF_3	H	H	H	3-ClC_6H_4	320–322	$C_{15}H_{10}ClF_3N_4O$	30	>100.0
60	5-CF_3	H	H	H	C_6H_{11}	308–309	$C_{15}H_{17}F_3N_4O$	59	100.0
61	5,6-$(CH_3)_2$	H	H	H	C_6H_5	336–337	$C_{16}H_{16}N_4O$	30	12.5

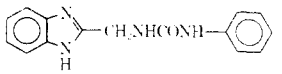
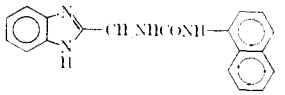
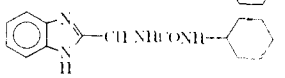
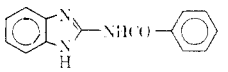
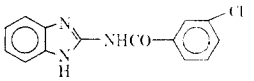
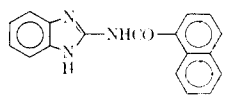
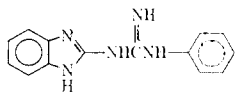
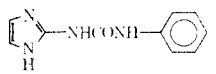
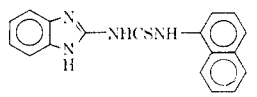
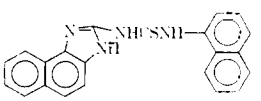
TABLE III (*Continued*)

No.	X	Y	Z	R	R'	Mp, °C	Formula	Coe virus ED_{50}, mg/kg	Drug level (mg/kg × 3) for fourfold or greater immunosuppression
62	5,6-$(CH_3)_2$	H	H	H	1-$C_{10}H_7$	298–300	$C_{20}H_{18}N_4O$	17	50.0
63	5,6-$(CH_3)_2$	H	H	H	4-ClC_6H_4	351–352	$C_{16}H_{15}ClN_4O$	26	12.5
64	5,6-$(CH_3)_2$	H	H	H	3-ClC_6H_4	342–343	$C_{16}H_{15}ClN_4O$	87	>100.0
65	5,6-$(CH_3)_2$	H	H	H	3,4-$Cl_2C_6H_3$	343–345	$C_{16}H_{14}Cl_2N_4O$	20	100.0
66	5,6-$(CH_3)_2$	H	H	H	2,5-$Cl_2C_6H_3$	336–337	$C_{16}H_{14}Cl_2N_4O$	>128	25.0
67	5,6-$(CH_3)_2$	H	H	H	4-FC_6H_4	227–228 dec	$C_{16}H_{15}FN_4O$	43	0.4
68	5,6-$(CH_3)_2$	H	H	H	2-FC_6H_4	321–322	$C_{16}H_{15}FN_4O$	44	1.6
69	5,6-$(CH_3)_2$	H	H	H	4-$NO_2C_6H_4$	297–299	$C_{16}H_{15}N_5O_3$	53	25.0
70	5,6-$(CH_3)_2$	H	H	H	3-$NO_2C_6H_4$	349–350	$C_{16}H_{15}N_5O_3$	22	>100.0
71	5,6-$(CH_3)_2$	H	H	H	2-$NO_2C_6H_4$	327–329	$C_{16}H_{15}N_5O_3$	74	100.0
72	5,6-$(CH_3)_2$	H	H	H	4-$CH_3C_6H_4$	344–345	$C_{17}H_{18}N_4O$	119	>100.0
73	5,6-$(CH_3)_2$	H	H	H	3-$CH_3C_6H_4$	329–330	$C_{17}H_{18}N_4O$	45	>100.0
74	5,6-$(CH_3)_2$	H	H	H	2-$CH_3C_6H_4$	342–345	$C_{17}H_{18}N_4O$	80	12.5
75	5,6-$(CH_3)_2$	H	H	H	C_6H_{11}	325–326	$C_{16}H_{22}N_4O$	90	50.0
76	5,6-Cl_2	H	H	H	C_6H_5	364–365	$C_{14}H_{10}Cl_2N_4O$	88	12.5
77	5,6-Cl_2	H	H	H	1-$C_{10}H_7$	339–340	$C_{18}H_{12}Cl_2N_4O$	>128	25.0
78	5,6-Cl_2	H	H	H	C_6H_{11}	363–364	$C_{14}H_{16}Cl_2N_4O$	>128	50.0
79	5-CH_3	H	H	H	C_6H_5	327–328	$C_{15}H_{14}N_4O$	>128	50.0
80	5-CH_2	H	H	H	1-$C_{10}H_7$	315–316	$C_{19}H_{16}N_4O$	<16	0.4
81	5-CH_3	H	H	H	C_6H_{11}	320–321	$C_{15}H_{20}N_4O$	174	>100.0
82	4-NO_2	H	H	H	C_6H_5	361–362	$C_{14}H_{11}N_5O_3$	101	25.0
83	4-NO_2	H	H	H	2-FC_6H_4	355–356	$C_{14}H_{10}FN_5O_3$	>128	3.1
84	4-NO_2	H	H	H	CH_3	340–341	$C_9H_9N_5O_3$	128	25.0
85	4-NO_2	H	H	H	$CH(CH_3)_2$	372–373	$C_{11}H_{13}N_5O_3$	30	0.8
86	4-NO_2	H	H	H	C_6H_{11}	335	$C_{14}H_{17}N_5O_3$	<16	6.2
87	5,6-CH_2O_2	H	H	H	C_6H_5	Chars at 350	$C_{15}H_{12}N_4O_3$	51	100.0
88	5,6-CH_2O_2	H	H	H	1-$C_{10}H_7$	300	$C_{19}H_{14}N_4O_3$	30	50.0
89	5,6-CH_2O_2	H	H	H	C_6H_{11}	Chars at 300	$C_{15}H_{18}N_4O_3$	55	>100.0
90	H	H	CH_3	H	C_6H_5	189–190	$C_{15}H_{14}N_4O$	>128	50.0
91	H	H	CH_3	C_6H_5	C_6H_5	174–175	$C_{21}H_{18}N_4O$	86	50.0
92	H	H	CH_3	H	3-ClC_6H_4	158–159	$C_{15}H_{13}ClN_4O$	52	>100.0
93	H	H	CH_3	H	4-ClC_6H_4	195–196	$C_{15}H_{13}ClN_4O$	78	50.0
94	H	H	CH_3	H	1-$C_{10}H_7$	205–206	$C_{19}H_{16}N_4O$	57	25.0
95	H	H	CH_3	H	C_6H_{11}	176–177	$C_{15}H_{20}N_4O$	>64, toxic 128	50.0
96	H	C_6H_5	H	H	C_6H_5	170–171	$C_{20}H_{16}N_4O$	41	50.0
97	H	C_6H_5	H	H	1-$C_{10}H_7$	166–167	$C_{24}H_{18}N_4O$	84	>50.0
98	H	C_6H_5	H	H	C_6H_{11}	166–167	$C_{20}H_{20}N_4O$	>128	12.5
99	H	C_6H_5	H	CH_3	CH_3	198–199	$C_{16}H_{16}N_4O$	>128	25.0
100	H	CH_3	H	H	C_6H_5	181–182	$C_{15}H_{14}N_4O$	104	3.1
101	H	CH_3	H	H	1-$C_{10}H_7$	238–239	$C_{19}H_{16}N_4O$	>128	100.0
102	H	CH_3	H	H	3-ClC_6H_4	190–191	$C_{15}H_{13}ClN_4O$	18	6.2
103	H	CH_3	H	H	4-ClC_6H_4	199–200	$C_{15}H_{13}ClN_4O$	64	100.0
104	H	CH_3	H	CH_3	CH_3	156–157	$C_{11}H_{14}N_4O$	>128	50.0
105		H	H	H	C_6H_5	362–364	$C_{18}H_{14}N_4O$	31	>100.0
106		H	H	H	1-$C_{10}H_7$	330 dec	$C_{22}H_{16}N_4O$	12	50.0
107		H	H	H	4-FC_6H_4	364–365	$C_{18}H_{13}FN_4O$	110	50.0
108		H	H	H	C_6H_{11}	252–254	$C_{18}H_{20}N_4O$	53	>100.0
109		H	H	H	C_6H_5	348–349	$C_{18}H_{14}N_4O$	108	50.0
110		H	H	H	1-$C_{10}H_7$	331–332	$C_{22}H_{16}N_4O$	76	12.5
111		H	H	H	3-ClC_6H_4	354–355	$C_{18}H_{13}ClN_4O$	22	0.05
112		H	H	H	4-FC_6H_4	349–350	$C_{18}H_{13}FN_4O$	95	25.0

[a] G. Pellizzari [*Gazz. Chim. Ital.*, **51**, I, 89 (1921)] reported the synthesis of this compound by another method; the melting point given was 250°.

TABLE IV

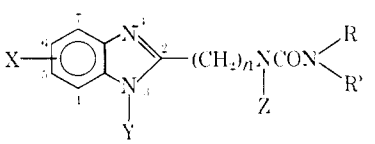
SOME CLOSE ANALOGS OF THE 1-(BENZIMIDAZOL-2-YL)-3-SUBSTITUTED UREAS

No.	Structure	Mp, °C	Formula	Cor virus ED_{50}, mg/kg	Drug level (mg/kg × 3) for fourfold or greater immunosuppression
1		205–206	$C_{15}H_{14}N_4O$	25	>100
2		246–247	$C_{17}H_{16}N_4O$	50	>100
3		248–249	$C_{15}H_{20}N_4O$	32	>100
4		234–235[a]	$C_{14}H_{11}N_3O$	87	100
5		246–247[a]	$C_{14}H_{10}ClN_3O$	128	100
6		278–280[a]	$C_{18}H_{13}N_3O$	80	100
7		161–162[b]	$C_{14}H_{13}N_5$	64, toxic 128	100
8		218–220	$C_{10}H_{10}N_4$	>128	>100
9		225–226	$C_{18}H_{14}N_4S$	<16	6.2
10		242–243	$C_{22}H_{16}N_4S$	<16	12.5

[a] All recrystallized from MeOH–Me_2CO. [b] N. Sato, S. Matsumoto, A. Tsunoda, and N. Ishida, *J. Antibiotics* (Tokyo), **19**, 172 (1966); *Chem. Abstr.*, **65**, 9363*g* (1966).

days after the red cell antigen injections, the mice were bled, and the sera from each five-mouse group were pooled. Antibody determinations were made on the serum pools by a hemagglutination pattern procedure, and comparisons were made between treated and control animals. Our results are reported as the minimum levels (mg/kg × 3) of drug necessary to suppress the hemagglutination titer by a factor of fourfold or greater from control titers. Generally, somewhat higher doses than these resulted in suppression of antibody to undetectable levels.

Immunosuppression Structure–Activity Relationships. Immunosuppressive activity in general was more sensitive to structural changes than the antiviral activity. In an effort to be sure that a hydrolysis product or simple metabolite was not the active species, we tested compounds **3**–**5** and **7** of Table II. These



compounds emphasize the fact that the major structural requirement of this series is an N,N′-substituted benzimidazoleurea. Azathioprine and cortisone (**1** and **2**) are shown in Table II for comparison as examples of a postantigen and preantigen optimally active drug. The following observations about structure–activity relationships can be made regarding the compounds reported herein.

When the benzimidazole ring is unsubstituted and R = H, then R′ must be aryl or substituted aryl to obtain activity (**1**, Table III). This activity decreases of R and R′ both are aryl (**2**).

Generally, when R and X are H, and R′ is an aliphatic group, the compounds are inactive at 100 mg (**23**, **26**, **27**, **30**). The two exceptions to this, the isopropyl and *t*-butyl compounds (**20**, **24**), are active at 12.5 and 50 mg, respectively. They both differ from the other aliphatic groups in being compact branched structures.

One X substituent, the 4-NO_2, yielded aliphatic ureas of outstanding activity when R′ = CH_3 (25 mg) or R′ = $CH(CH_3)_2$ (0.8 mg) (**84**, **85**). All examples when X was not 4-NO_2 gave less active aliphatic derivatives.

The N of the urea must be attached directly to the imidazole ring (n = 0), for when n = 1 the activity is decreased (**1**–**3**, Table IV).

Conversion of the urea to a thiourea decreases the activity by one-half (**9**, **10**, Table IV).

When Y = H (**1** and **3**–**5**, Table III) is changed to

Y = CH_3 (**100–103**), aryl substituents at R cause a decrease in activity in three out of four compounds tested. The one alkyl example (Y = H; R and R′ = CH_3) goes from inactive at 400 mg to active at 50 mg when Z = H (**21**) is changed to Z = CH_3 (**104**).

In the case where Y = phenyl, aryl substituents at R′ (**96**, **97**) decrease activity while alkyl substituents at R′ increase activity (**98**, **99**).

Removal of the benzene ring from the benzimidazole structure gives inactive compounds (8, Table IV).

Substitution of an amide for the urea function decreases the activity by roughly eightfold (**4–6**, Table IV). Conversion of the urea to a guanidine decreases activity and increases toxicity (**7**, Table IV).

Antiviral Structure–Activity Relationships.—The antiviral activity was less sensitive to structural change. This activity seemed to depend on the gross shape of the molecule and each compound's over-all physical properties, *e.g.*, solubility, rather than on fine adjustments of structure.

A few exceptions were noted, one being a definite preference for the cyclohexyl and adamantyl groups in the aliphatic series (**27**, **30**, Table III). Some other trends in structure–activity relationship could be noted. The activity was usually best when R was α-naphthyl, *p*-fluorophenyl, and *m*-nitrophenyl. Small substituents at Y, such as CH_3, increased activity, while large substituents at Y, such as Ph, decreased activity. When Z was changed from H to CH_3, only slight changes of activity were experienced. Changing *n* from 0 to 1 had no great effect. A definite increase in potency in 2,3-naphthimidazole over the 1,2 isomer was noted (**105**, **106** *vs.* **109**, **110**, Table III).

We have included hydroxybenzylbenzimidazole (**6**, Table II) in our test to show that the benzimidazole-ureas are a unique class of drugs and bear no relationship to the class of antiviral benzimidazoles reported by Eggers and Tamm.[12]

Discussion

When describing two types of activities for the same series of compounds, the question arises whether the two activities are due to the same process. *In vivo* antiviral activity due to suppression of the pathology caused by antigen–antibody reactions in the host might explain parallels between the two types of activities. However, further studies have indicated that antibody against Coe virus, as measured by plaque reduction, was not altered by treatment with different dose levels of these compounds,[13] in contrast to the results when sheep RBC are used as the antigen. Antibody levels with this type of infection are due to the initial inoculum since live or formalin-inactivated virus produced the same high antibody levels.[13] These studies would indicate that the antiviral activity is independent of the immunosuppresive activity. Similar results were noted by Muldoon and Jackson[14] who observed that 6-mercaptopurine did not lower antibody synthesis when avian influenza virus was used as the antigen but showed a striking immunosuppressive effect with bovine serum albumin. Whether or not this difference relates to the structural or chemical differences between the antigens involved remains to be elucidated. If so, it would be an exploitable difference in terms of chemotherapy in both antiviral and immunosuppressant therapy.

Experimental Section

Melting points were taken on a Mel-Temp apparatus and are uncorrected. Ir bands, nmr, and titrations were consistent for the proposed structures. All compounds were analyzed for C, H, N and gave results within ±0.4% of the theoretical value.

Generally, the starting amine was checked for solubility in THF, Me_2CO, C_6H_6, and toluene. The reaction was usually run in the solvent in which it was most soluble.

Examples are as follows.

1-(2-Benzimidazolyl)-3-naphthylurea.—To 5.32 g (0.04 mole) of 2-aminobenzimidazole in 150 ml of THF at 25° was added dropwise 6.76 g (0.04 mole) of naphthyl isocyanate in 100 ml of THF over a 30-min period with stirring. The mixture was then stirred, refluxed for 6 hr, and allowed to cool; the solid was filtered and washed twice with 100 ml of THF. The solid was checked for purity by tlc using silica gel plates in EtOAc. If one spot, the material was tested without further purification. If impure, recrystallization from Me_2CO or a THF–hexane mixture was used to obtain pure, one-spot material; yield dry 11.5 g (95%), mp 327–328°.

1-Methyl-2-aminobenzimidazole.—A mixture of 91 g (0.6 mole) of N-methyl-*o*-nitroaniline was hydrogenated at 3.5 kg/cm² in 900 ml of EtOH with 3.5 g of Pd–C. The catalyst was filtered, the EtOH was removed, and the oil was suspended in 1 l. of H_2O at 25° with vigorous stirring. The mixture was cooled to maintain a temperature of 20–30° while 63 g of CNBr was added portionwise. The mixture was stirred overnight and then extracted three times with 500 ml of Et_2O. The aqueous portion was basified to pH 11, and the precipitated solid was filtered. This solid crystallized from EtOAc–Skelly B after decolorization with carbon; mp 196–198°, yield 49.5 g (56%).

2-Aminomethylbenzimidazole.—A mixture of 0.2 mole of 2-chlorobenzimidazole and 1.2 moles of 30% aqueous CH_3NH_2 was autoclaved at 120° for 3 hr. The cooled reaction was freed of excess amine *in vacuo*, then acidified to pH 3 and extracted four times with 100 ml of Et_2O. The aqueous extract was basified to pH 8 and continuously extracted with Et_2O for 4 days. A white crystalline solid precipitated from the ether on cooling and was filtered and recrystallized from Me_2CO–hexane; mp 176–178°, yield 19.5 g (66%).

1-(2-Benzimidazolyl)-3,3-dimethylurea.—To a stirred solution of 13.3 g (0.1 mole) of 2-aminobenzimidazole in 150 ml of THF, containing 11 g (0.11 mole) of Et_3N, at 5° was added 10.7 g (0.1 mole) of dimethylcarbamoyl chloride in 100 ml of THF. The mixture was stirred 3 hr at 5° and then at 25° overnight. The THF was removed under vacuum, and 150 ml of H_2O was added. The oil solidified, was filtered, washed with H_2O, and recrystallized from Me_2CO–hexane; mp 176–177°, yield 6.4 g (31%).

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(12) H. T. Eggers and I. Tamm, *J. Exp. Med.*, **113**, 657 (1961).

(13) D. C. DeLong, L. A. Baker, J. D. Nelson, and C. J. Paget, *Bacteriol. Proc.* (Abstract V203), 179 (1968).

(14) R. L. Muldoon and G. G. Jackson, *Proc. Soc. Exp. Biol. Med.*, **126**, 26 (1967).