preparation of 5-(*p*-nitrophenoxy)propyl bromide from the corresponding phenol and 1,3-dibromopropane.[27]

Method F.—Compound **15** was prepared by the method of Heininger in the preparation of 3-(*p*-chlorophenoxy)propionitrile[14] except that the condensing agent used was Triton B instead of NaOMe.

Method G.—Compounds **37–41** were prepared as described by Knoevenagel for phenylaminoacetonitrile (**37**).[15] (Note: see also under method C for amides.)

Method H.—Compound **21** was prepared by the method of Gattermann and Hausknecht in the preparation of phenyl thiocyanate.[16]

Method I.—The method of Grebenyuk and Tsukervanik[17] was used to prepare compound **43**.

Tetrazoles (Table III). Method A.—Compounds **58** and **74** were prepared by a procedure reported by D'Orazio for DL-5-(α-phthalimido-β-phenylethyl)tetrazole.[19]

Method B.—Compounds **47–57**, **59–67**, **69–73**, **75**, and **78–87** were prepared by the procedure of Finnegan, *et al.*, for 5-propyltetrazole.[18]

Method C.—Compounds **53** and **68** were prepared by a novel method as described for 5-[β-(3-chlorophenylthio)ethyl]tetrazole (**53**). To a mixture of 3-chloropropionitrile (17.9 g, 0.2 mole) and NaN_3 (39 g, 0.6 mole) in 50 ml of dry THF was added a solution of anhydrous $AlCl_3$ (30 g, 0.22 mole) in 400 ml of THF. The resultant mixture was stirred and heated at reflux for 22 hr. The mixture was cooled in an ice bath, and about 200 ml of H_2O was added dropwise and with stirring. The THF was evaporated under reduced pressure, and the residual aqueous solution was acidified with concentrated HCl, saturated with NaCl, and extracted with three portions of Et_2O. The combined extracts were dried ($MgSO_4$) and evaporated, yielding 20.9 g (78.9%) of a yellow solid, mp 97–104°. Recrystallization from $CHCl_3$ gave white needles of 5-(β-chloroethyl)tetrazole, mp 103.5–106.5°.

To a solution of 3-chlorophenol (2.58 g, 0.02 mole) in 70 ml of dry DMF cooled in an ice bath were added a 55.8% NaH dispersion (3.08 g, equivalent to 0.04 mole of NaH) in mineral oil and 5-(β-chloroethyl)tetrazole (2.66 g, 0.02 mole). After the vigorous H_2 evolution had ceased the cooling was discontinued, and the mixture was stirred at room temperature until the H_2 evolution had completely ceased. After addition of about 0.2 g of NaI (catalyst), the mixture was stirred and heated at *ca.* 100° for 18 hr. About 30 ml of H_2O was added and the mixture was concentrated under reduced pressure on a steam bath to a small volume. The residual solution was acidified with concentrated HCl and extracted with Et_2O. The Et_2O extracts were combined and extracted with a saturated aqueous $NaHCO_3$ solution. Upon acidification of the $NaHCO_3$ extracts, 2.1 g of a white solid (46.7%) was obtained. Crystallization ($CHCl_3$–CCl_4) afforded **53**, mp 128–130°.

Method D.—Compound **76** was prepared from compound **61** by the procedure of Gilman and Beaber for the oxidation of *n*-butyl *p*-tolyl sulfide to the corresponding sulfone.[28]

Method E.—Compound **77** was prepared from 5-aminotetrazole monohydrate and 4-chlorobenzenesulfonyl chloride by a procedure described by Jensen and Pedersen for the preparation of 5-acetylsulfanilamidotetrazole.[20]

Method F.—5-(3-Chlorophenylthiomethyl)-2-methyltetrazole (**88**) and 5-(3-chlorophenylthiomethyl)-1-methyltetrazole (**89**) were obtained by treating 5-(3-chlorophenylthiomethyl)tetrazole (**62**) (9.07 g) with CH_2N_2 in Et_2O and separating the resulting mixture on an alumina column. The 2-methyl isomer **88** was eluted with C_6H_6 (7.63 g, 79.2%) and further purified by distillation through a Vigreux column: bp 130–133° (0.1 mm); nmr ($CDCl_3$) δ 4.30 (3 H, $CH_3N<$). The 1-methyl isomer **89** was eluted with $CHCl_3$ (2.00 g, 20.8%), and crystallization from *i*-PrOH gave white crystals, mp 65–67°, nmr ($CDCl_3$) δ 4.00 (3 H, $CH_3N<$).

2-Azido-2-(5-tetrazolyl)propane (VI).—To a mixture of 4-chlorophenoxyisobutyronitrile (5.0 g, 25.6 mmoles) and NaN_3 (5.0 g, 77 mmoles) was added a solution of anhydrous $AlCl_3$ (4.66 g, 35 mmoles) in 70 ml of dry THF. The mixture was stirred and heated at reflux for 48 hr. The THF was distilled from the reaction mixture while sufficient H_2O was added dropwise to maintain a constant volume. An oil separated from the aqueous solution. Ether and a 10% aqueous NaOH solution were added and the layers were separated. The alkaline extract was acidified with 6 *N* HCl and then extracted with Et_2O and $CHCl_3$. The combined organic extracts were dried ($MgSO_4$) and evaporated, affording a yellow oil. Crystallization from CCl_4 gave 0.87 g of a white solid (22%). Recrystallization from $CHCl_3$–CCl_4 gave white needles of VI: mp 87–90° dec; ir ν_{max} (KBr) at 3440, 3000, 2880, 2740, 2720, 2630, 2140, 1270, 1160, 1050 cm^{-1}; nmr peak ($CDCl_3$) at δ 1.9 (6 H, singlet, $C(CH_3)_2$).

Anal. Calcd for $C_4H_7N_7$: C, 31.36; H, 4.61; N, 64.03. Found: C, 31.53; H, 4.39; N, 64.20.

Acknowledgments.—The authors wish to express their gratitude to Dr. F. S. Caruso and his staff for the pharmacological data. We also thank Mr. R. B. Babel and Mr. M. Brown for technical assistance, and the analytical and spectroscopic departments for their services.

(27) J. D. Genzer, C. P. Horner, and H. C. van Wessem, *J. Amer. Chem. Soc.*, **73**, 3150 (1951).

(28) H. Gilman and N. J. Beaber, *ibid.*, **47**, 1449 (1925).

Some Substituted Phenylalkanoic Acids and N-Substituted Malonanilic, Succinanilic, and Anilinoalkanoic Acids as Potential Antiinflammatory Agents

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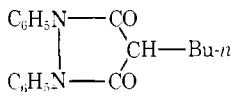
Thirty one of the title compounds were prepared and, together with some of the intermediate esters and nitriles, were screened for antiinflammatory activity. None of the compounds showed appreciable activity in the carrageenin-induced edema test, but thirteen of the acids were effective in stabilizing a bovine serum albumin fraction against heat coagulation.

Whitehouse and Skidmore[1] have suggested that acidic antiinflammatory drugs may exert their therapeutic effect by interaction with the essential lysyl ε-amino groups of the enzyme systems implicated in the inflammatory response of animal tissues. We consider that this reaction need not be restricted to the ionic type of interaction envisaged by these investigators and have examined the reaction between phenylbutazone **1** and certain aliphatic amines which would act as crude models for the lysyl ε-amino group.

We have found that phenylbutazone reacts smoothly with *n*-butylamine and *n*-pentylamine at 100° to give

(1) M. W. Whitehouse and I. F. Skidmore, *J. Pharm. Pharmacol.*, **17**, 688 (1965).

high yields of the open-chain hydrazide amides **2a** and **2b**. The related hydrazide acid **2c** bears a resemblance



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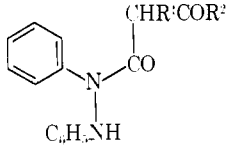
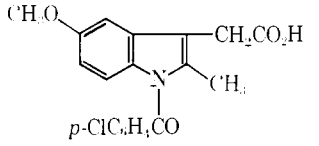


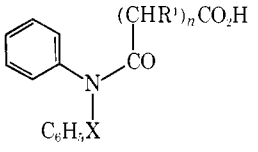
2a, $R^1 = n$-Bu; $R^2 = NH(CH_2)_3CH_3$
b, $R^1 = n$-Bu; $R^2 = NH(CH_2)_4CH_3$
c, $R^1 = n$-Bu; $R^2 = OH$



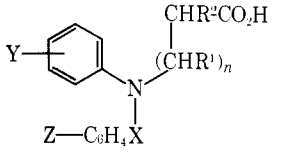
3

to indomethacin (**3**) and possesses structural features allowing a good fit with the hypothetical antiinflammatory receptor site which was proposed by Shen[2] and by Scherrer.[3] These factors made us consider that **2c** may be a hitherto unidentified active metabolite of phenylbutazone and that compounds related to this may incorporate the desirable properties of both **1** and **3**. J. R. Geigy S.A. have subsequently described the preparation of the acid **2c** and claim that it is active in the formalin-induced edema test in rats.[4] While this appears to lend weight to the idea that phenylbutazone acts *via* the postulated metabolite **2c**, the possibility also exists that **2c** is a precursor of **1** *in vivo*.

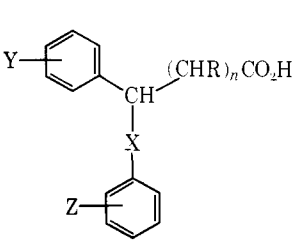
The above observations have been incorporated into our search for new antiinflammatory drugs and we have synthesized three series of compounds, represented by the structures **4**–**6**, which incorporate certain structural



4, X = NH, CH_2; R^1 = H, n-Bu; n = 1, 2



5, X = CH_2, CO, CS, SO_2; Y, Z = H, Cl, CF_3, Me; R^1, R^2 = H, Me, Et; n = 0, 1, 2



6, X = NH, O, CH_2; Y, Z = H, Me, i-Pr, CF_3; R = H, n-Pr; n = 0, 1

features of both acids **2c** and indomethacin **3**. These compounds, together with some of the intermediates, are enumerated in Tables I–III and the methods of synthesis are described in the Experimental Section. Some of the acids of type **5** were isolated as gums and were subsequently converted into their crystalline sodium or benzylamine salts for biological testing.

All of the compounds except for **2a**, **2b**, **13**, **15**, **23**, **24**, and **34** were screened for antiinflammatory activity in the carrageenin-induced edema test in rats[5] but none gave greater than 30% reduction of swelling compared with the controls when doses of 100 mg/kg were given 0.5 and 3 hr prior to the injection of carrageenin.

The compounds were also tested in a modified form[6] of the Mizushima test[7] which involves the measurement of the degree of stabilization of a bovine serum albumin fraction against heat coagulation in the presence of the test compound. Mizushima observed a good correlation between the degree of stabilization and the antirheumatic properties of a large number of compounds. Our results are given in Tables I–III. Compounds **7**, **11**, **12**, **16**, **17**, **19**, **22**, **27**, **28**, **43**, **45**, **47**, and **49** were very active and three of these, namely **11**, **12**, and **22**, were retested against carrageenin edema using the subcutaneous route of administration. However, no reduction of the edema was observed which indicates that the lack of oral activity may not be due to poor absorption from the gastrointestinal tract and that the positive results in the modified Mizushima test may be examples of the nonspecificity of the test.

Experimental Section[8]

All of the compounds are new except the ones noted otherwise. Examples of the reaction procedures are given below. Further details of the individual compounds are given in Tables I–III.

Method A. N-Anilino-2-*n*-butylmalonanilic Acid *n*-Butylamide.—Phenylbutazone (1.00 g) and *n*-butylamine (5.0 ml) were heated at 100° for 5 hr. The yellow solution was concentrated at 50° under reduced pressure and the residual gum was triturated with petroleum ether (bp 40–60°). The resulting white solid was filtered off and recrystallized from EtOH–petroleum ether (bp 60–80°) yielding the diamide as white rods (0.70 g). Amide I absorptions were at 1635 and 1655 cm^{-1}; corresponding absorptions in phenylbutazone are at 1705 and 1750 cm^{-1}.

Method B. N-Anilinosuccinanilic Acid.—A mixture of hydrazobenzene (3.6 g) and succinic anhydride (2.0 g) in cyclohexane (100 ml) was heated under reflux for 6 hr. The cooled mixture was filtered, and the solid was washed with cyclohexane and dried. The pure acid was thus obtained as white crystals (4.8 g).

Method C. N-Benzyl-N-phenylcyanoacetamide.—N-Benzylaniline (18.3 g), cyanoacetic acid (8.5 g), and dicyclohexylcarbodiimide (22.0 g) were dissolved in THF (100 ml). After 4 hr, the precipitate of dicyclohexylurea was filtered off and the filtrate was evaporated under reduced pressure. The residue was dissolved in EtOAc (250 ml), washed repeatedly with 2 *N* HCl, then with a 10% solution of Na_2CO_3, and finally with H_2O. Evaporation of the dried (Na_2SO_4) organic solution yielded yellow crystals (17.6 g) of the pure cyanoamide.

Method D. N-Benzyl-2-*n*-butylmalonanilic acid.—Ethyl N-benzyl-2-*n*-butylmalonanilate was prepared by method C from *n*-butylmalonic acid monoethyl ester (4.7 g) and N-benzylaniline (4.6 g). The undistilled ester (5.5 g) was dissolved in EtOH (15 ml) and added to a solution of NaOH (2.4 g) in H_2O (15 ml). After being stirred overnight at room temperature, the mixture was filtered and the filtrate was evaporated under reduced pressure. The residue was dissolved in H_2O (50 ml), extracted with Et_2O (two 50-ml portions), and acidified with HCl. The lib-

(2) T. Y. Shen, International Symposium on Non-steroidal Anti-inflammatory Drugs, Milan, 1964, Excerpta Medica Foundation, Amsterdam, 1964, p 13.

(3) R. A. Scherrer, "Annual Reports in Medicinal Chemistry," Academic Press, New York, N. Y., 1965, p 225.

(4) J. R. Geigy S. A., French Patent 3853M (1966).

(5) D. Evans, K. S. Hallwood, C. H. Cashin, and H. Jackson, *J. Med. Chem.*, **10**, 428 (1967).

(6) C. H. Cashin and M. E. Tarrant, British Pharmacology Society Winter Meeting, Nottingham, England, 1967.

(7) Y. Mizushima and H. Suzuki, *Arch. Intern. Pharmacodyn.*, **157**, 115 (1965).

(8) Melting points are uncorrected. Where analyses are indicated only by symbols of the elements, analytical results obtained for those elements were within ±0.4% of the theoretical values.

TABLE I

N-SUBSTITUTED MALONANILIC AND SUCCINANILIC ACIDS AND THEIR DERIVATIVES

$C_6H_5N(CO)(CHR^1)_nR^2$

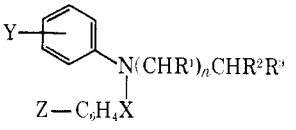
C_6H_5X

No.	X	n	R^1	R^2	Method	Mp, °C	Yield, %	Formula	Analyses	Heat coagulation test[a]
2a	NH	1	*n*-Bu	$CONH(CH_2)_5Me$	A	147–149	57	$C_{23}H_{36}N_2O_2$	C, H, N	
2b	NH	1	*n*-Bu	$CONH(CH_2)_4Me$	A	128–129	66	$C_{24}H_{38}N_2O_2$	C, H, N	0
7	NH	2	H	CO_2H	B	169–170	86	$C_{16}H_{16}N_2O_4$	C, H, N	82
8	CH_2	1	H	CN	C	90–92	70	$C_{16}H_{14}N_2O$	C, H, N	0
9	CH_2	1	H	CO_2H	D	93–95	30	$C_{16}H_{15}NO_3$	C, H, N	7
10	CH_2	1	*n*-Bu	CO_2H	D	86–88	44	$C_{20}H_{23}NO_3$	C, H, N	12
11	CH_2	2	H	CO_2H	E	122–124	45	$C_{17}H_{17}NO_3$	C, H, N	100

[a] Figures represent the per cent stabilization with the test compound compared to the control; 1 mmole of the compounds was used.

TABLE II

N-SUBSTITUTED ANILINOALKANOIC ACIDS AND THEIR DERIVATIVES



No.	X	Y	Z	n	R^1	R^2	R^3	Method	Mp, °C	Bp, °C (mm)	Yield, %	Formula	Analyses	Heat coagulation test[a]
12[b]	CH_2	H	H	0		H	CO_2Na	F	>300[c]		22	$C_{16}H_{16}NNaO_2$	C, H, N	98
13	CH_2	4-Cl	H	0		H	CO_2Et	G	50–58		95	$C_{17}H_{18}ClNO_2$	C, H, N	
14	CH_2	4-Cl	H	0		H	CO_2H	G	128–130		80	$C_{15}H_{14}ClNO_2$	C, H, N	0
15	CH_2	4-Cl	4-Cl	0		H	CO_2Et	G	100–103		89	$C_{17}H_{17}Cl_2NO_2$		
16	CH_2	4-Cl	4-Cl	0		H	CO_2H	G	133–134		78	$C_{15}H_{13}Cl_2NO_2$	C, H, N	67
17	CH_2	3-CF_3	4-Cl	0		H	CO_2H	G	125–127		65	$C_{16}N_{12}ClF_3NO_2$	C, H, N	90
18	CH_2	H	H	1	H	H	CO_2Et	H		175–180 (0.8)[d]	43	$C_{18}H_{19}NO_2$	C, H, N	0
19	CH_2	H	H	1	H	H	CO_2H	H		[e]	10	$C_{16}H_{15}NO_2$	C, H, N	90
20	CH_2	4-Cl	H	1	H	H	CO_2Et	G		210–215 (0.6)[f]	73	$C_{18}H_{20}ClNO_2$	C, H, N	0
21	CH_2	H	H	1	Me	H	CO_2Me	G		159–163 (0.4)[g]	53	$C_{18}H_{19}NO_2$	C, H, N	0
22	CH_2	H	H	1	Me	H	CO_2H	G	144–149[h]		51	$C_{17}H_{19}NO_2 \cdot C_7H_9N$	C, H, N	91
23	CH_2	H	H	1	H	Me	CO_2Me	G		199–200 (0.7)[i]	75	$C_{18}H_{19}NO_2$	C, H, N	
24	CH_2	H	H	1	H	Me	CO_2H	G	110–112[j]		62	$C_{17}H_{17}NO_2 \cdot C_7H_9N$	C, H, N	
25	CH_2	H	H	2	H	H	CN	I	37–39	170–174 (0.2)[k]	78	$C_{17}H_{16}N_2$	C, H, N	0
26	CH_2	H	H	2	H	H	CO_2Et	J		170–172 (0.3)[l]	70	$C_{19}H_{23}NO_2$	C, H, N	0
27	CH_2	H	H	2	H	H	CO_2H	J	108–109		65	$C_{17}H_{19}NO_2$	C, H, N	97
28	CO	H	4-Cl	0		H	CO_2H	K	154–156		58	$C_{15}H_{12}ClNO_3$	C, H, N	94
29	CO	2,4-Cl_2	2,4-Cl_2	0		H	CO_2H	L	152–153		11	$C_{15}H_9Cl_4NO_3$	C, H, N	0
30	CO	4-Cl	2,4-Cl_2	0		Et	CO_2H	L	172–173		26	$C_{17}H_{13}Cl_3NO_3$	C, H, N	0
31	CO	H	H	1	H	H	CO_2Et	M		172–177 (0.5)[m]	74	$C_{19}H_{19}NO_3$	C, H, N	0
32	CO	H	H	1	H	H	CO_2H	M	83–84[n]		51	$C_{16}H_{15}NO_3 \cdot 0.5H_2O$	C, H, N	0
33	CO	4-Cl	H	1	H	H	CO_2Et	M		200–205 (0.45)[o]	91	$C_{18}H_{16}ClNO_3$	C, H, N	0
34	CO	4-Cl	H	1	H	H	CO_2H	M	122–125[p]		60	$C_{16}H_{14}ClNO_3 \cdot C_7H_9N$	C, H, N	
35	CO	H	H	1	Me	H	CO_2H	N	133–135		51	$C_{17}H_{17}NO_3$	C, H, N	0
36	CO	H	H	2	H	H	CN	O	69–70	156–172 (0.1)[q]	50	$C_{17}H_{16}N_2O$	C, H, N	0
37	CO	H	H	2	H	H	CO_2H	J	119–120		68	$C_{17}H_{17}NO_3$	C, H, N	0
38	CO	3-CF_3	2,4-Cl_2	2	H	H	CO_2H	L	118–119		19	$C_{18}H_{12}Cl_2F_3NO_3$	C, H, N	0
39[r]	CS	H	H	0		H	CO_2H	P	176–178		10	$C_{15}H_{13}NO_2S$	C, H, N	0
40[s]	SO_2	H	4-Me	1	H	H	CO_2H	Q	114–116		53	$C_{16}H_{17}NO_4S$	C, H, N	0

[a] See footnote *a*, Table I. [b] See ref 9. [c] Free acid obtained as gum. Melting point and analysis given for Na salt. [d] $n^{22}D$ 1.5761. [e] Obtained as gum, $n^{22}D$ 1.5860. Distilled with decomposition around 160° (0.05 mm). [f] $n^{26}D$ 1.5755. [g] $n^{26}D$ 1.5704. [h] Free acid obtained as gum. Melting point and analysis given for benzylamine salt. [i] $n^{25}D$ 1.5775. [j] $n^{20}D$ 1.5666. [k] $n^{25}D$ 1.5544. [l] Melting point and analysis given for hemihydrate. [m] $n^{25}D$ 1.5662. [n] See ref 11. [o] See ref 12.

erated oil was extracted with Et_2O which was dried (Na_2SO_4) and evaporated. The residual oil eventually solidified and was recrystallized from CCl_4–petroleum ether (bp 60–80°) yielding the substituted malonanilic acid (2.25 g).

Method E. N-Benzylsuccinanilic Acid.—A mixture of N-benzylaniline (18.0 g), succinic anhydride (20.0 g), and concentrated H_2SO_4 (0.5 ml) was stirred at 140–150° for 45 min. The mixture was poured into H_2O (400 ml) and stirred at 60° for 30 min. The cooled mixture was extracted with $CHCl_3$ (three 100-ml portions) and the extracts were washed with H_2O (two 50-ml portions), dried (Na_2SO_4), and evaporated. The residue was recrystallized from C_6H_6–cyclohexane to give the required acid (12.5 g).

Method F. N-Benzyl-N-phenylglycine Sodium Salt.—By the method described by Pfanz, *et al.*,[9] N-benzylaniline (12.2 g), chloroacetic acid (6.3 g), and NaOAc (5.5 g) gave the amino acid as a colorless gum (3.5 g) which refused to crystallize. A crystalline Na salt, mp >300°, was prepared from this in the recommended manner.

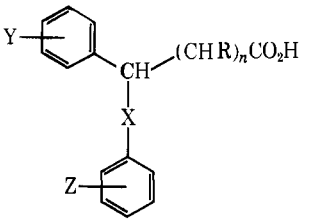
Method G. Methyl N-Benzyl-β-anilinobutyrate and the Corresponding Acid.—A mixture of methyl β-anilinobutyrate (4.0 g), benzyl chloride (4.8 g), NaOAc (4.6 g), and I_2 (two crystals) were heated at 140° for 2 hr and at 170° for a further 1 hr. The cooled mixture was triturated with Et_2O (100 ml) and filtered. The filtrate was washed with H_2O (50 ml), dried (Na_2SO_4), and evaporated. Fractionation of the residual oil *in vacuo* yielded the required ester as a light yellow oil (3.1 g).

This ester (2.1 g) was dissolved in EtOH (15 ml) and heated at 100° for 1 hr in the presence of 4 *N* NaOH (15 ml). The solution was concentrated to half-volume, diluted with water (50 ml), and acidified with 4 *N* HCl. The required acid separated as a

(9) H. Pfanz, E. Jassmann, and H. Breslauer, East German Patent 12090 (1956).

TABLE III

SUBSTITUTED PHENYLALKANOIC ACIDS



No.	X	Y	Z	n	R	Method	Mp, °C	Yield, %	Formula	Analyses	Heat coagulin test[a]
41	NH	H	3-CF_3	0		R	103–104	68	$C_{15}H_{12}F_3NO_2$	C, H, N	20
42	NH	4-*i*-Pr	3-CF_3	0		S	124–125	53	$C_{18}H_{18}F_3NO_2$	C, H, N	17
43	NH	4-*i*-Pr	2,3-Me_2	0		S	122–123	41	$C_{19}H_{23}NO_2$	C, H, N	89
44[b]	NH	H	H	1	H	T	134–135	81	$C_{15}H_{15}NO_2$	C, H, N	2
45	NH	H	H	1	*n*-Pr	T	118–120	54	$C_{18}H_{21}NO_2$	C, H, N	89
46	O	H	H	0		U	108–109	62	$C_{14}H_{12}O_3$	C, H	0
47	O	H	2,3-Me_2	0		U	116–119	35	$C_{16}H_{16}O_3$	C, H	97
48	O	4-*i*-Pr	2,3-Me_2	0		U	139–141	50	$C_{19}H_{22}O_3$	C, H	97
49	CH_2	H	4-OH	0		*c*	175–178		$C_{15}H_{14}O_3$	C, H	17

[a] See footnote *a*, Table I. [b] See ref 13. [c] Supplied by K & K Laboratories, Inc., New York, N. Y.

viscous gum which refused to crystallize. Treatment of an ethereal solution of the acid with benzylamine (3.0 ml) yielded a crystalline benzylamine salt (2.65 g) which was filtered off and recrystallized (C_6H_6).

Method H. Ethyl N-Benzyl-β-anilinopropionate and the Corresponding Acid.—Ethyl acrylate (5.0 g) was added dropwise during 30 min to a stirred solution of N-benzylaniline (9.2 g) in HOAc (25 ml) kept under N_2 at 100°. The solution was heated for a further 9 hr, cooled, and diluted with Et_2O (100 ml). The mixture was washed with H_2O (three 50-ml portions), then with 5% $NaHCO_3$ solution (four 50-ml portions), and finally with H_2O (50 ml). Fractionation of the dried (Na_2SO_4) organic solvent gave the expected ester as a pale yellow oil (6.1 g).

Hydrolysis of the ester afforded the required acid as a gum. A crystalline salt could not be prepared.

Method I. N-Benzyl-γ-anilinobutyronitrile.—A mixture of γ-anilinobutyronitrile (16.0 g), benzyl chloride (25.0 g), and K_2CO_3 (28.0 g) was stirred at 100° for 7 hr. The cooled product was partitioned between an equal volume of Et_2O and H_2O, and the Et_2O layer was dried (Na_2SO_4). Fractionation of this solution yielded the N-benzyl compound as a viscous oil which crystallized from EtOH at a low temperature.

Method J. Ethyl N-Benzyl-γ-anilinobutyrate and the Corresponding Acid.—The above nitrile (10.0 g) was dissolved in a saturated solution of HCl in EtOH (80 ml). H_2O (0.8 ml) was added and the solution was heated at 100° for 2 hr. The filtered solution was concentrated to a small volume, H_2O (75 ml) was added, and the mixture was neutralized by the addition of K_2CO_3 solution. The mixture was extracted with Et_2O (three 75-ml portions) and the combined organic extracts were washed with H_2O (75 ml) and evaporated. Distillation of the residue yielded the required ester as an almost colorless oil (8.3 g).

Hydrolysis of the ester yielded the acid as off-white needles from CCl_4.

Method K. N-(*p*-Chlorobenzoyl)-N-phenylglycine.—*p*-Chlorobenzoyl chloride (11.6 g) was added in small portions to a vigorously shaken solution of N-phenylglycine (10.0 g) in 16% NaOH solution (50 ml). After 1 hr, the solution was cooled to 5° and acidified with HCl. The precipitate was filtered off, washed with H_2O, and recrystallized from aqueous MeOH yielding the required product as colorless crystals (11.4 g).

Method L. N-(2,4-Dichlorobenzoyl)-N-(2,4-dichlorophenyl)-glycine.—2,4-Dichlorobenzoyl chloride (17.3 g) was added dropwise during 1 hr to a stirred solution of 2,4-dichloroaniline (16.2 g) and Et_3N (20.0 g) in anhydrous Et_2O (200 ml). After 3 days at room temperature, the mixture was washed successively with H_2O (two 100-ml portions), 2 *N* HCl (100 ml), and H_2O (three 100-ml portions). The organic solution was dried (Na_2SO_4) and evaporated. The residue was recrystallized from aqueous MeOH yielding N-(2,4-dichlorobenzoyl)-2,4-dichloroaniline as crystals (14.0 g, 40%), mp 158–160°.

NaH (0.8 g) was added to a stirred solution of the above (12.0 g) in dry THF (150 ml). After 3 hr at room temperature, ethyl bromoacetate (5.1 g) was added and the mixture was stirred under reflux for 8 hr. The precipitated NaBr was filtered off and the filtrate was concentrated under reduced pressure. Distillation of the residue gave ethyl N-(2,4-dichlorobenzoyl)-*p*-chloroanilinoacetate as a yellow oil (4.3 g, 29%), bp 211–213° (0.1 mm).

The ester (4.2 g) was dissolved in a solution of NaOH (4.0 g) in 80% aqueous EtOH (75 ml) and kept overnight at room temperature. Most of the solvent was removed at 30° under reduced pressure, then H_2O (50 ml) was added, and the solution was acidified with HCl. A viscous gum which separated from the solution solidified after 1 hr and was recrystallized from aqueous EtOH yielding the required acid as white crystals (3.7 g).

Method M. N-Benzoyl-β-anilinopropionic Acid.—Benzoyl chloride (3.5 g) was added dropwise during 10 min to a stirred solution of ethyl β-anilinopropionate (4.5 g) in freshly distilled Et_3N (15 ml). An exothermic reaction occurred and a white precipitate was produced. After 30 min, C_6H_6 (25 ml) was added and the mixture was filtered. The solid was washed with C_6H_6 (10 ml) and the combined organic solutions were evaporated. Distillation of the residue yielded ethyl N-benzoyl-β-anilinopropionate as a pale yellow viscous oil (5.1 g).

The ester (1.0 g) was dissolved in EtOH (8 ml) and treated with 4 *N* NaOH (8 ml). After 5 days at room temperature, the solution was evaporated to half-volume at 30° under reduced pressure. H_2O (15 ml) was added and the solution was extracted with Et_2O (two 15-ml portions). Acidification of the aqueous solution with AcOH yielded a precipitate which was filtered off, dried, and recrystallized from C_6H_6. The expected acid was thus obtained as a hemihydrate (0.65 g).

Method N. N-Benzoyl-β-anilinobutyric Acid.—PhCOCl (2.6 g) was added to 10% NaOH (18 ml) containing methyl β-anilinobutyrate[10] (3.5 g). The mixture was stirred for 2 hr, left overnight, and extracted with Et_2O (three 25-ml portions). Acidification of the aqueous solution with HCl caused a gum to separate from the solution. The gum solidified on standing and was filtered off. Recrystallization of the solid from C_6H_6–petroleum ether (bp 60–80°) yielded the pure acid (2.6 g).

Method O. N-Benzoyl-γ-anilinobutyronitrile.—The N-benzoylated nitrile was prepared from γ-anilinobutyronitrile (16.0 g) as in method M and was obtained as white crystals (13.2 g) from ethanol.

Method P. N-Thiobenzoylanilinoacetic acid was prepared by the method of Lawson and Miles[11] from N-phenylglycine ethyl ester.

Method Q. Toluene-*p*-sulfonyl-β-anilinopropionic Acid.—Toluene-*p*-sulfonanilide (18.5 g) and β-iodopropionic acid (15.0 g) gave the required acid (6.8 g) by the method of Clemo and Perkin.[12]

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(12) G. R. Clemo and W. H. Perkin, *ibid.*, **125**, 1608 (1924).

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.* ($C_{24}H_{23}NO$) 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.

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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-

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