was carried out in a Parr hydrogenation apparatus using 5% Pd-C. The intermediate was dissolved in 10 mL of MeOH containing 10% HOAc and added to the suspension of 500 mg of the catalyst in 20 mL of MeOH. The reaction mixture was shaken with hydrogen for 16–24 h at room temperature, filtered, and evaporated to dryness. Crystallization gave the pure compounds. Physical constants are given in Table II.

***N,N'*-Bis[4-[2-hydroxy-3-[(1-methylethyl)amino]propoxy]phenyl]decanediamide (7d).** To a stirred solution consisting of 0.63 g (2 mmol) of **5**, 0.2 g (2 mmol) of triethylamine, and 20 mL of CH_2Cl_2 was added dropwise 0.24 g (1 mmol) of sebacoyl chloride. The reaction mixture was stirred for 3 h at room temperature and was washed with 5% Na_2CO_3 solution. The organic layer was dried over $MgSO_4$, filtered, and evaporated to dryness. The residue was purified by column chromatography using Al_2O_3. Crystallization from MeOH gave 0.36 g (45%) of **7d**: mp 122–125 °C; TLC R_f 0.67 (solvent C); ^{1}H NMR ($CDCl_3$) δ 0.9–1.15 (m, 12 H), 1.15–1.4 (m, 8 H), 1.4–2.0 (m, 4 H), 2.0–2.4 (m, 4 H), 2.4–2.7 (m, 4 H), 2.7–3.1 (m, 2 H), 3.6 (s, 4 H), 3.65–3.95 (m, 6 H), 6.55–7.05 (AB, 18 H), 7.9 (s, 2 H); IR (KBr) 1660 (C=O), 3300 cm^{-1} (N–H). Anal. ($C_{48}H_{66}N_4O_6$) C, H, N. The *N,N'*-dibenzyl intermediate **6d** (0.57 g, 0.72 mmol) was dissolved in 10 mL of MeOH containing 10% of HOAc and the mixture was added to the suspension of the catalyst (500 mg) in 20 mL of MeOH. The reaction mixture was shaken with hydrogen for 20 h at room temperature, filtered, and evaporated to dryness. Crystallization from EtOAC gave 0.18 g (41.2%) of the bivalent compound **7d**: mp 167–168 °C; TLC R_f 0.42 (solvent A); ^{1}H NMR (Me_2SO-d_6) 0.9 (d, 12 H), 1.2–1.5 (m, 8 H), 1.5–2.0 (m, 8 H), 2.1–2.5 (m, 4 H), 2.5–3.0 (m, 10 H), 3.8–4.0 (m, 6 H), 6.1–7.7 (m, 8 H), 9.7 (s, 2 H); IR (KBr) 1655 (C=O), 3300 cm^{-1} (N–H). Anal. ($C_{34}H_{54}N_4O_6$) C, H, N.

2,2'-(1,8-Octanediyldiimino)bis[*N*-[4-[2-hydroxy-3-[(1-methylethyl)amino]propoxy]phenyl]acetamide] (10c). To a solution, consisting of 3.9 g (10 mmol) of **8**, 1.5 g (10 mmol) of NaI, 1.06 g (10 mmol) of Na_2CO_3, and 25 mL of EtOH was added dropwise 0.74 g (5 mmol) of 1,8-octanediamine and the mixture was heated at reflux for 2 h. The reaction mixture was filtered and evaporated to give a brownish oil. The oil was dissolved in $CHCl_3$, washed with $Na_2S_2O_3$ solution, H_2O, and brine, and dried over $MgSO_4$. The organic layer was evaporated to dryness and purified by flash chromatography (silica gel) to give 0.65 g (15.3%) of a yellow oil (**9c**): TLC R_f 0.75 (solvent C); ^{1}H NMR ($CDCl_3$) δ 0.96–1.2 (m, 12 H), 1.1–1.7 (m, 12 H), 2.5–3.3 (m, 4 H), 3.4 (s, 4 H), 3.7 (d, 4 H), 3.85–4.10 (m, 6 H), 6.8–7.75 (AB, 18 H), 9.3 (s, 2 H); IR (KBr) 1700 (C=O), 3330 cm^{-1} (N–H). Anal. ($C_{50}H_{72}N_6O_6$) C, H, N. The *N,N'*-dibenzyl intermediate **9c** (1.16 g, 1.26 mmol) was dissolved in 10 mL of MeOH containing 10% of HOAc and the mixture was added to the suspension of the catalyst (500 mg) in 20 mL of MeOH. The reaction mixture was shaken with hydrogen for 20 h at room temperature, filtered, and evaporated to dryness. Crystallization from EtOAc gave 0.33 g (35.6%) of **10c**: mp 89–91 °C; TLC R_f 0.21 (solvent C); ^{1}H NMR ($CDCl_3$) δ 1.10 (d, 12 H), 1.20–1.55 (m, 12 H), 3.3 (s, 4 H), 3.85–4.0 (m, 6 H), 6.7–7.5 (AB, 8 H), 9.1 (s, 2 H); IR (KBr) 1660 (C=O), 3300 cm^{-1} (N–H). Anal. ($C_{36}H_{60}N_6O_6$) C, H, N.

Radioligand Binding Assay. All binding assays were performed in disposable culture tubes (12 × 75 mm) in triplicate according to the method described by Williams et al.[19] using membrane preparations derived from rat hearts and lungs according to the method described by Williams and Lefkowitz.[20] The protein concentration of the final membrane preparation was determined by the Lowry procedure[29] using a standardized bovine serum solution. The membrane protein ranging from 100 to 300 μg was incubated with (2–6) × 10^{-9} M of [^{3}H]DHA in a final volume of 150 μL of the assay buffer (50 mM Tris·HCl, 10 mM $MgCl_2$, pH 7.4 at 37 °C) for 12 min at 37 °C. Incubations were terminated by adding 2 mL of the ice-cold assay buffer followed by rapid vacuum filtration of the suspension through a Whatman GF/C glass fiber filter. The filter was then placed in a scintillation vial and 10 mL of scintillation cocktail (Formula-963, NEN, Boston) was added. The vial was vortexed for 5 s and radioactivity counted in a Packard Tricarb 3325 liquid scintillation spectrometer. In each experiment, "nonspecific" binding was determined by measuring the amount of radioactivity retained on filters when incubations were performed in the presence of 10^{-5} M (±)-propranolol. The "specific" binding was determined by subtracting the "nonspecific" binding from the total counts bound.

Acknowledgment. We express our appreciation to Dr. Gerald S. Jones, Jr., for his assistance and valuable discussions concerning the preparation of target compounds.

Registry No. (±)-**2**, 106247-87-4; (±)-**4**, 106163-09-1; (±)-**5**, 43203-60-7; (±)-**6a**, 106163-11-5; *meso*-**6a**, 106163-26-2; (±)-**6b**, 106163-12-6; *meso*-**6b**, 106163-27-3; (±)-**6c**, 106163-13-7; *meso*-**6c**, 106163-28-4; (±)-**6d**, 106163-14-8; *meso*-**6d**, 106163-29-5; (±)-**7a**, 106163-18-2; *meso*-**7a**, 106163-30-8; (±)-**7b**, 106163-19-3; *meso*-**7b**, 106163-31-9; (±)-**7c**, 106163-20-6; *meso*-**7c**, 106191-51-9; (±)-**7d**, 106163-21-7; *meso*-**7d**, 106163-32-0; (±)**8**, 106163-10-4; (±)-**9a**, 106163-15-9; *meso*-**9a**, 106163-33-1; (±)-**9b**, 106163-16-0; *meso*-**9b**, 106163-34-2; (±)-**9c**, 106163-17-1; *meso*-**9c**, 106163-35-3; (±)-**9d**, 106191-50-8; *meso*-**9d**, 106191-52-0; (±)-**10a**, 106163-22-8; *meso*-**10a**, 106163-36-4; (±)-**10b**, 106163-23-9; *meso*-**10b**, 106163-37-5; (±)-**10c**, 106163-24-0; *meso*-**10c**, 106163-38-6; (±)-**10d**, 106163-25-1; *meso*-**10d**, 106163-39-7; $ClCO(CH_2)_2COCl$, 543-20-4; $ClCO(CH_2)_4COCl$, 111-50-2; $ClCO(CH_2)_6COCl$, 10027-07-3; $ClCO(CH_2)_8COCl$, 111-19-3; $H_2N(CH_2)_4NH_2$, 110-60-1; $H_2N(CH_2)_6NH_2$, 124-09-4; $H_2N(CH_2)_8NH_2$, 373-44-4; 4-acetamidophenol, 103-90-2; (±)-epiclorohydrin, 13403-37-7; *N*-isopropylbenzylamine, 102-97-6; chloroacetyl chloride, 79-04-9; piperazine, 110-85-0.

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Benzimidazole Derivatives with Atypical Antiinflammatory Activity

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A number of substituted 2-[(2,2,2-trifluoroethyl)sulfonyl]-1*H*-benzimidazoles (**4**) have demonstrated antiinflammatory activity that appears to have a mechanism distinct from typical cyclooxygenase inhibiting nonsteroidal antiinflammatory drugs. Several of these compounds inhibit adjuvant-induced arthritis in rats at 25 mg/kg while showing no activity in the carrageenan paw edema model at up to 100 mg/kg. Two compounds, **4a** and **4b**, showed no significant inhibition of cyclooxygenase in vitro at concentrations as high as 5 × 10^{-5} M. All compounds **4** active in adjuvant-induced arthritis were also found to inhibit release of lysosomal enzymes from neutrophils, raising the possibility that their antiinflammatory effect is at least partially mediated by an effect on neutrophil function.

A number of reports have appeared in the literature of antiinflammatory heterocycles bearing (polyfluoroalkyl)-sulfonyl or (polyfluoroalkyl)thio side chains.[1-3] A common feature of these structures (**1–3**) is a sulfonylurea or

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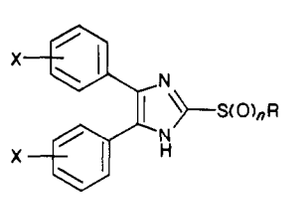
Table I. Activity of Benzimidazole Derivatives 4

no.	X	R	mp, °C	recrystn solvent[a]	anal.	synth method for 7	rat adjuvant arthritis: % inhibn[b]	neutrophil enzyme release[c]
4a	5-CH_3	CH_2CF_3	160–161	CH	C, H, N	B	52[d]	16
							72[d] (50 mg/kg)	
4b	H	CH_2CF_3	174–176	CH	C, H, N	A	52[d]	60
4c	5-F	CH_2CF_3	160–162	EW	C, H, N	B	43[d]	39
4d	5-CH_2CH_3	CH_2CF_3	122.5–124.5	CH	C, H, N	B	34[d]	9
4e	5-Cl	CH_2CF_3	161–162.5	CH	C, H, N	A	28[d]	66
4f	5-CH_3, 6-F	CH_2CF_3	164.5–166.5	EW	C, H, N	B	44[d]	20
4g	5,6-Cl_2	CH_2CF_3	190–192	T	C, H, N	A	24[d]	41
4h	5,6-$(CH_3)_2$	CH_2CF_3	208–209	EW	C, H, N	B	18	>100
4i	5-CO_2CH_3	CH_2CF_3	211–212	M	C, H, N	B	11	>100
4j	5-CF_3	CH_2CF_3	163.5–166	T	C, H, N[e]	B	0	82
4k	5-*n*-Pr	CH_2CF_3	131–132	CH	C, H, N	B	0	>100
4l	5-CH_3	CH_2CH_3	130–132	T	C, H, N	B	17	>100
4m	5-Cl	CF_2CF_3	157–159	EW	C, H, N	C	0	>100
4n	5-Cl	$CF_2CF_2CF_3$	189–192	CH	C, H, N	C	0	92
4o	5-CH_3	CF_2CF_3	183–185	CH	C, H, N	C	8	>100
indomethacin							69[d] (1 mg/kg)	>100
ibuprofen							73[d]	>100

[a] EW = ethanol–water, T = toluene, M = methanol, CH = methylene chloride–hexane. [b] Results are for 25 mg/kg po unless otherwise indicated. [c] Results given are IC_{50} values in micromoles. A value of >100 indicates 0–49% inhibition was observed at 100 micromolar concentration. IC_{50} values were extrapolated from six-point concentration curves (1–100 μM test compound), duplicate wells for each concentration. [d] Decrease in paw volume statistically significant compared to control by using Duncan's multiple range test for variable, $p < 0.05$. [e] C: calcd, 36.16; found, 36.60.

thiourea incorporated in the molecule. In the search for new antiinflammatory compounds we prepared a series of previously unreported benzimidazole derivatives **4**. Several of these compounds show an unusual pharmacological profile, in that they are active in a commonly used antiinflammatory assay (rat adjuvant arthritis), while, unlike nonsteroidal antiinflammatory drugs (NSAIDs), they apparently do not inhibit cyclooxygenase either in vivo or in vitro. The active compounds also appear to effect neutrophil function.



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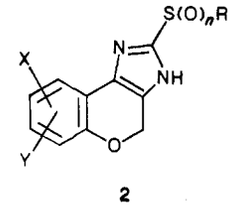

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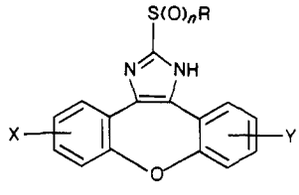

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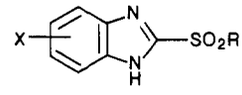
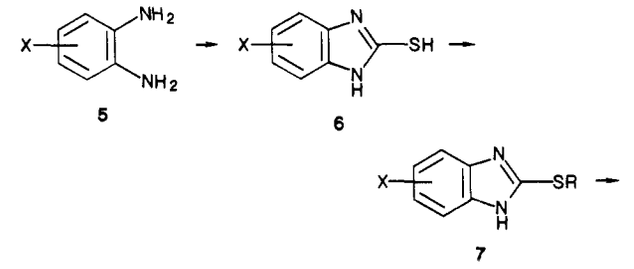


4

Chemistry

Compounds were prepared by the procedure outlined in Scheme I. Substituted 2-mercaptobenzimidazoles **6** were prepared from the corresponding diaminobenzenes and carbon disulfide.[4] Substituted diaminobenzenes **5** were either available commercially or prepared from available precursors by using the methods described by Stephens and Bower[5] and Wright et al.[6] Alkylation of **6** was carried out by irradiation in liquid ammonia in the case of perfluoroalkyl iodides. Otherwise it was done with the appropriate alkyl iodide in DMF in the presence of either DBN or NaH. 3-Chloroperoxybenzoic acid was used for oxidation to sulfones **4**.

Scheme I



Results and Discussion

Our initial interest in this series arose from the finding that several compounds showed activity in the rat adjuvant arthritis model, but were inactive in the rat carageenan paw edema (CPE) model. This suggested a profile different from classical cyclooxygenase-inhibiting NSAIDs, which typically show activity in both screens,[7] and also possibly different from **1** (X = Y = 4-F, n = 2, R = CF_2CF_2H), which is reported to be active in the CPE model and to inhibit cyclooxygenase.[8] All compounds of formula **4** tested were found inactive in the CPE model at up to 100 mg/kg (**4d**, **4c**, and **4n** were not tested). Results for the adjuvant arthritis model are in Table I. While not nearly as potent as indomethacin, the more active compounds approach the activity level of ibuprofen.

To verify that these compounds do not inhibit prostaglandin synthesis, **4a** and **4b** were tested in an in vitro cyclooxygenase assay using human platelets. In this assay,

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we found indomethacin, a strong inhibitor, had an IC_{50} value of 1×10^{-8} M, while aspirin, a weak inhibitor, had an IC_{50} value of 1.5×10^{-5} M. Compounds **4a** and **4b** showed no significant inhibition at concentrations of 1×10^{-8} to 5×10^{-5} M.

We have been routinely screening compounds for their ability to inhibit the release of lysosomal enzymes from human neutrophils. Some of these enzymes have been implicated in contributing to the inflammation process.[9,10] We found several compounds of formula **4** active in this neutrophil enzyme release (NER) screen. Furthermore, the compounds active in adjuvant arthritis were all among the better compounds in NER (Table I). This raises the possibility that inhibition of neutrophil enzyme release might contribute to the antiinflammatory effect of these compounds. Both of the representative NSAIDs were inactive in this model.

The activity of **4** is dependent on the nature of both R and X. Among the R substituents tested, activity is only observed with the trifluoroethyl group. Activity drops off with increasing size of the 5-substituent (see **4b**, **4a**, **4d**, and **4k**). Among the 5,6-disubstituted compounds (**4f**, **4g**, and **4h**) activity is retained with a fluorine in the 6-position and falls off with a methyl or chlorine substituent.

Compound **4a** (BI-L-45 XX) was examined further as a candidate for development. Additional effects on neutrophil function were found both in vivo and in vitro.[11] However the compound was found to be toxic in rats when given at doses over 100 mg/kg for 14 days or longer.

Experimental Section

Melting points were taken on a Büchi 510 melting point apparatus and are uncorrected. ^{1}H NMR spectra were all consistent with molecular structures and were recorded on a Bruker 250 WM spectrometer. Elemental analyses were performed at Micro-Tech Laboratories, Inc., Skokie, IL, and were within 0.4% of the calculated values unless otherwise indicated.

Procedure A. 5,6-Dichloro-2-[(2,2,2-trifluoroethyl)thio]-1*H*-benzimidazole. 5,6-Dichloro-2-mercaptobenzimidazole (25.1 g, 0.10 mol) in 150 mL of DMF was added, under nitrogen atmosphere and with cooling on ice, to 50% sodium hydride in mineral oil (5.76 g, 0.12 mol) that had been washed once with petroleum ether and suspended in 50 mL of DMF. After 30 min, 2-iodo-1,1,1-trifluoroethane (25 g, 0.119 mol) in 25 mL of DMF was added, and the reaction mixture was stirred 22 h at ambient temperature. The reaction mixture was poured into 1000 mL of water and extracted with ETOAc (1 × 500 mL, 1 × 250 mL). The organic extracts were washed with saturated NaCl, dried (Na_2SO_4), and concentrated in vacuo. The crude product was passed through a silica gel column, eluting with toluene–ETOAc (90:10). Fractions containing the product were concentrated, and the product was recrystallized from toluene–ligroin, giving 6.9 g (0.023 mol, 23%) 5,6-dichloro-2-[(2,2,2-trifluoroethyl)thio]-1*H*-benzimidazole, mp 144–146 °C. Anal. ($C_9H_5Cl_2F_3N_2S$) C, H, N.

Procedure B. 5-Methyl-2-[(2,2,2-trifluoroethyl)thio]-1*H*-benzimidazole. A mixture of 2-mercapto-5-methylbenzimidazole (10 g, 0.061 mol), 1,5-diazabicyclo[4.3.0]non-5-ene (DBN) (9.1 g, 0.073 mol), and 1-iodo-2,2,2-trifluoroethane (15.4 g, 0.073 mol) in 100 mL of DMF was heated at 45–55 °C under a nitrogen atmosphere. Over the next 5 h additional amounts of 1-iodo-2,2,2-trifluoroethane (12.8 g, 0.061 mol) and DBN (2 g, 0.016 mol) were added. After heating for 9 h, the reaction mixture was poured into 600 mL of ice and water. The precipitated product was collected by filtration, dried, and recrystallized from toluene–ligroin, giving 8.75 g of 5-methyl-2-[(2,2,2-trifluoroethyl)thio]-1*H*-benzimidazole (0.036 mol, 58%), mp 159–161 °C. Anal. ($C_{10}H_9F_3N_2S$) C, H. N.

Procedure C. 5-Chloro-2-[(pentafluoroethyl)thio]-1*H*-benzimidazole. 5-Chloro-2-mercaptobenzimidazole (7.5 g, 0.041 mol) and pentafluoroethyl iodide (33 g, 0.134 mol) were combined in 70 mL of THF and 250 mL of liquid ammonia. The reaction was under a N_2 atmosphere, with a dry ice condenser attached. The reaction mixture was irradiated with an immersable mercury lamp (Model SCT-1, Ultra-Violet Products, Inc.) for 10 h. The ammonia was allowed to evaporate and the remaining solvent removed by rotary evaporation. The residue was partitioned in ETOAc and water, and the organic phase was dried and concentrated. The residue was recrystallized twice from ETOH–water, giving 7.1 g (0.023 mol, 56%) of 5-chloro-2-[(pentafluoroethyl)thio]-1*H*-benzimidazole, mp 157–159 °C. Anal. ($C_9H_4ClF_5N_2S$) C, H, N.

General Procedure for Oxidation to Sulfone. 5-Methyl-2-[(2,2,2-trifluoroethyl)sulfonyl]-1*H*-benzimidazole (4a). To a solution of 5-methyl-2-[(2,2,2-trifluoroethyl)mercapto]-1*H*-benzimidazole (7.75 g, 0.032 mol) in 100 mL of ETOAc was added 85% *m*-chloroperoxybenzoic acid (18.3 g, 0.09 mol) in portions. After stirring for 4.5 h, the reaction mixture was diluted with 200 mL of ETOAc and extracted with saturated $NaHCO_3$ solution (4 × 75 mL), dried, and concentrated. The residue was recrystallized from CH_2Cl_2–hexane, giving 4.66 g of 5-methyl-2-[(2,2,2-trifluoroethyl)sulfonyl]-1*H*-benzimidazole (0.017 mol, 53%), mp 160–161 °C. Anal. ($C_{10}H_9F_3N_2O_2S$) C, H, N.

Biological Methods. Aduvant-Induced Arthritis. Arthritis[12] was induced in male Lewis rats (150–170 g) by injection of a heat-killed *Mycobacterium butyricum*. Each animal was given 0.1 mL of a 5 mg/mL adjuvant suspension in light mineral oil injected subcutaneously into the plantar surface of the right hind foot. Ten rats were used in each test group. Animals were dosed orally with a suspension of test compound in 1% acacia in distilled water immediately after adjuvant injection and once daily for a total of 14 days. Foot volumes were measured 24 h after the final dose by mercury displacement to the level of the lateral malleolus. Mean displacement volumes and their standard errors were calculated for the noninjected hind paws. An untreated arthritic control group was used as comparison for test compound effect. Results are expressed as percent inhibition of paw swelling in the drug treated group compared to the untreated arthritic controls.

Carrageenan-Induced Paw Edema.[13] Edema was produced in the right hind paw of male CD rats (150 ± 10 g) by the subplantar injection of 0.1 mL of a 1% carrageenan suspension in saline. Ten rats were used in each test group. Test compound or its vehicle (1% acacia in distilled water) was administered orally 1 h prior to carrageenan injection. Paw volume was determined by calculating the amount of mercury displaced after immersing the paw to the level of the lateral malleolus. Foot volumes were measured just prior to test compound administration and again 3 h after carrageenan injection, and the difference was designated as edema volume.

Neutrophil Enzyme Release Assay. Human neutrophils were prepared from freshly drawn, heparinized venous blood[14] and suspended in Hanks Balanced Salts Solution–Hepes buffer (pH 7.3; GIBCO Laboratories, Grand Island, NY) to a cell density of 8×10^6 cells/mL. Drugs were solubilized in Me_2SO and diluted (in buffer) into 2.0 mL of prewarmed neutrophil suspension such that the final Me_2SO level was <0.05%. These suspensions (+ or − drug) were incubated at 37 °C for 15 min, aliquotted (0.1 mL) in duplicate into microtitre wells containing 0.1 mL of stimulus (20 μM fMLP and 10 μg/mL cytochalasin B) or control (fMLP or buffer alone), and incubated for 10 min at 37 °C. The microtitre plates were centrifuged (5 min, 200*g*) and the supernatants tested for released enzyme, with lysozyme as a marker. Lysozyme activity was determined turbidometrically in microtitre wells, adapted from the method of Shugar.[15] The decrease in turbidity was monitored at 450 nm over time (Microtitre Plate Reader, Dynatech Instruments, Alexandria, VA) and the slope determined. The "% lysozyme released" was determined for each

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condition relative to a control suspension lysed with 0.05% Triton-X-100 (30 min on ice, centrifuged at 400g, 4 °C). The "% inhibition" of lysozyme release was determined as follows:

$$\% \text{ inhibition} = 100 - [(\% \text{ lysozyme released + drug})/(\% \text{ lysozyme released - drug})]$$

Cyclooxygenase Inhibition Assay. Human platelets (1×10^7 platelets/0.5 mL) in Ca^{2+}-free pH 7 buffer were incubated with test compound 15 min at 37 °C with shaking. [^{14}C]Arachidonic acid (0.25 μCi, 0.155 μg/mL) was added and the mixture incubated another 30 min. The reaction was terminated by addition of 0.025 mL of 0.8 N HCl. The mixture was then extracted with ethyl acetate–methylene chloride (2:3) supplemented with 30 μg/mL cold arachidonic acid to reduce degradation of the metabolites. After concentrating the organic phase, the number of microliters containing 1×10^5 cpm was determined and that volume applied to a silica gel plate. The plate was developed in methylene chloride–methanol–acetic acid–water (90:8:1:0.8), air-dried, and counted on a Berthold linear TLC analyzer. The integrated area of the prostaglandin–thromboxane envelope was determined and compared with the control (no drug).

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Synthesis and Binding Affinities of Analogues of Cholecystokinin-(30–33) as Probes for Central Nervous System Cholecystokinin Receptors

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CCK-30–33 has been identified as the minimum fragment of CCK with nanomolar affinity for the *central* CCK receptors, as assayed by displacement of [^{3}H]-Boc-β-alanyl-CCK-30–33 (pentagastrin) in homogenized mouse cerebral cortex. Examination of binding using this assay in the two series Boc-Trp-X-Phe-NH_2 when X = Met-Asp (Boc-CCK-30–33), Gly-Asp, Met-Gly, and Gly-Gly and when X = $(CH_2)_n$ (n = 0–4) reveals that modification of the tetrapeptide reduces affinity to a maximum of micromolar affinity (Boc-Trp-Gly-Asp-Phe-NH_2; $K_i = 2 \times 10^{-6}$ M), whereas in the series when n = 0 and 2 pentamolar affinity is still retained (Boc-Trp-Phe-NH_2, $K_i = 7 \times 10^{-5}$ M; Boc-Trp NH CH_2-CH_2-CO-Phe-NH_2, $K_i = 3 \times 10^{-5}$ M). Modification of the tetrapeptide CCK-30–33 reduces affinity 1000-fold, whereas di- and tripeptide fragments are identified that reduce affinity only a further 10-fold. This structure–activity relationship establishes a basis to design "peptoid" analogues of CCK that have therapeutic potential.

Several fragments of the polypeptide cholecystokinin (CCK) have been identified in both brain and gut of several species.[1-5] It appears that the C-terminal octapeptide CCK-26–33 retains all peripheral CCK-like activity, including gall bladder contraction, gastric acid secretion, and pancreatic secretion.[6] However, CCK-30–33, the C-terminal fragment common with the gastrin family including CCK, gastrin, and caeurulin, appears to retain these activities but has more selective effects in releasing hormones from endocrine pancreas.[3-7]

Recently, CCK fragments have been found in the brain,[4] and CCK has been implicated as a putative satiety factor.[4,5,8] Specific high-affinity sites have been located in many brain areas in rats and mice, including cerebral cortex.[3,4,9] CCK-26–33, -27–33, and -30–33 have all been shown to bind to these high-affinity sites.[9] It appears that the C-terminal tetrapeptide CCK-30–33 is the minimum fragment required for nanomolar affinity in the brain, but the full octapeptide (sulfated CCK-26–33) is required for nanomolar affinity in pancreatic tissue.[3]

This paper describes some structure–activity work on analogues of CCK-30–33 in order to characterize the chemical features necessary for binding to central nervous system (CNS) receptors, using the mouse brain cerebral cortex binding assay previously described.[9] The results are summarized in Table I.

Chemistry. The dipeptide 11 and the methylene-bridged Boc-Trp-HN$(CH_2)_n$CO-Phe-NH_2 (n = 1–4) analogues **12–15** (see Table I) were synthesized by coupling the *N*-Boc-Trp with the corresponding $H_2N(CH_2)_n$CO-Phe-NH_2 by the mixed-anhydride method with ethyl chloroformate and triethylamine. β-Alanyl-Phe-NH_2 and (δ-aminovaleryl)-Phe-NH_2 were obtained by deprotection of CBZ-β-alanyl-Phe-NH_2 and CBZ-δ-valeryl-Phe-NH_2, respectively, by catalytic hydrogenation over 10% Pd/C in acetic acid. (γ-Aminobutyryl)-Phe-NH_2 was obtained

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