Design of Nonpeptidal Ligands for a Peptide Receptor: Cholecystokinin Antagonists

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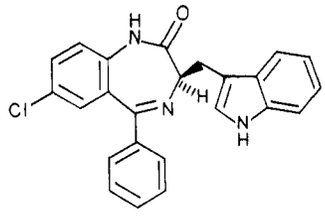
A series of 3-substituted 5-phenyl-1,4-benzodiazepines, nonpeptidal antagonists of the peptide hormone cholecystokinin (CCK), have been synthesized. Designed on the basis of facts regarding CCK, its natural-product antagonist asperlicin (**3**), and the antianxiety agent diazepam (**4**), these compounds represent a significant departure from existing CCK antagonists. They also constitute perhaps the first examples of simple, nonpeptidal ligands for a peptide receptor to arise by design rather than by screening. These compounds serve to illuminate the distinction between central and peripheral CCK receptors, as well as to provide orally effective CCK antagonists of potential pharmacological or therapeutic utility. One rationale for their receptor affinity has possible applications in the design of nonpeptidal ligands for other receptors, peptidal as well as nonpeptidal.

In a recent paper,[1] we reported the design and synthesis of a new class of potent, specific, orally effective, nonpeptidal antagonists of the peptide hormone cholecystokinin (CCK). These compounds are 3-substituted 5-phenyl-1,4-benzodiazepines. They include the initially designed 3-alkyl series, represented by **1**, and the more



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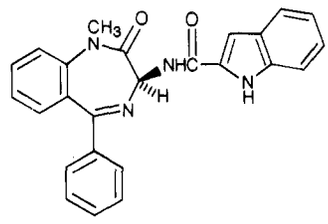

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potent 3-amido derivatives developed from them, including L-364,718 (**2**), the most potent antagonist of peripheral CCK yet reported.[1,2] In this paper, we present a detailed account of the 3-alkyl group, including design, syntheses, and structure–activity. In a subsequent paper, we will provide similar elaboration of the 3-amido series.

Cholecystokinin (CCK) is a peptidal gastrointestinal hormone and putative central neurotransmitter.[3] It has been implicated in the control of pancreatic and biliary secretion, gallbladder contraction, and gut motility[4–7] and in modulation of central dopaminergic transmission.[8,9] CCK is one of a growing list of peptides found to play key roles in normal physiology as neurotransmitters and neurohormones. For most of these peptides, clarification of their function and evaluation of their involvement in disease states are hampered by a shortage of appropriate pharmacological tools, notably a selection of potent, stable, and selective antagonists. This is the case with CCK:[10,11] much of the pharmacological investigation of CCK has relied upon the two amino acid derived antagonists proglumide and benzotript, compounds of marginal potency and uncertain specificity.[10–13]

A significant factor in the shortage of useful peptide antagonists is the peptide structure itself. To the collection of problems associated with design of analogues for bioactive molecules in general, peptides add their own particular difficulties. Primarily consequences of the properties of the peptide backbone, these include metabolic lability and poor oral absorption. Nonpeptidal analogues might circumvent these liabilities, but unfortunately, the design of such analogues remains a difficult problem for which there is as yet no rigorous solution. General principles of analysis and design through which structural information about a target peptide might be used to construct functionally equivalent nonpeptidal entities have yet to be developed.[14]

The most productive source of nonpeptidal surrogates for bioactive peptides at present is an empirical one, the screening of natural products for the desired activity. It was this approach that provided the prototypical example, the enkephalin agonist morphine,[14,15] and it was this approach that recently uncovered a new, effective antagonist for CCK, the natural product asperlicin[16–19] (**3**).

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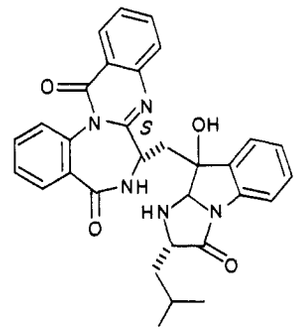

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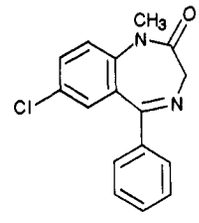
The key liability of asperlicin as a pharmacological or potential therapeutic agent is its poor oral bioavailability.[20] Failure of a synthetic analogue program[21] to overcome this lack of oral activity led us to consider a new approach to the design of nonpeptidal ligands for peptide receptors. This approach was based on the hypothesis that common structural and conformational elements shared by peptides might also be reflected in common structural requirements for their nonpeptidal surrogates.[1] In practice, such an approach would involve seeking out small, stable nonpeptides that had been previously identified as ligands for other peptide receptors. These would then be used as bases for construction of ligands for the CCK receptor. Unfortunately, such base molecules were as yet unreported.

The impetus for pursuit of this line of investigation was provided by two observations: our identification of a 1,4-benzodiazepine nucleus among the numerous substructures contained in asperlicin and, in particular, the recent reports suggesting that a peptide may be the natural ligand for the pharmacological receptor that recognizes 1,4-benzodiazepine anxiolytic agents such as diazepam **4**.[22,23] This latter suggestion cast the 5-phenyl-1,4-



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benzodiazepine structure **4** in the role of a high-affinity, orally effective, nonpeptidal ligand for a peptide receptor,

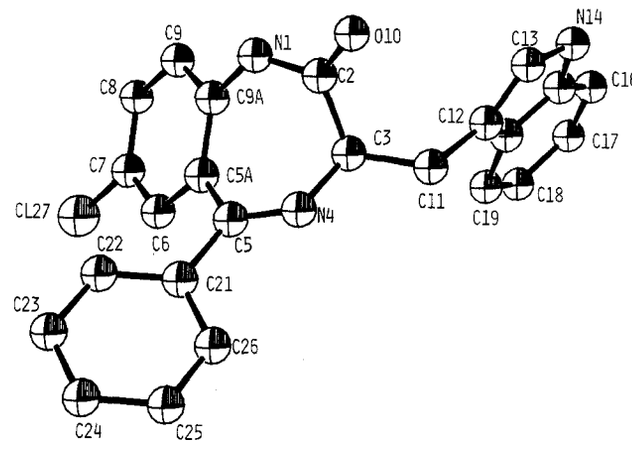
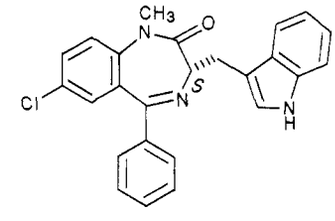


Figure 1. A computer-generated drawing of **1** derived from the X-ray coordinates with hydrogens omitted for clarity. The bond distances and angles in the benzodiazepine portion of the molecule follow closely those found in 3-hydroxy- and 3-unsubstituted-benzodiazepines.[50]

an attractive base for an attempt to develop antagonists of CCK based on our hypothesis as presented above.

In selecting a pathway for elaboration of this base, we focused on 3-substitution, a modification known to diminish the antianxiety activity of diazepam,[24,25] and a modification that the asperlicin structure implied might be compatible with CCK antagonist activity. The form such a 3-substituent might take was suggested by two factors, the presence in asperlicin of an indolinylmethyl group derived biosynthetically from L-tryptophan (L-Trp)[26] and the occurrence of L-Trp as a key amino acid in the sequence of CCK;[27] combination of the L-Trp side chain with the diazepam ring (**4**) gave **5**.



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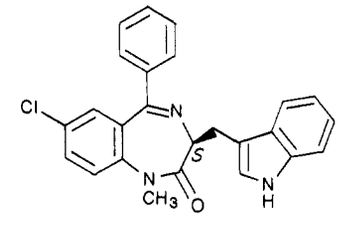

5b

To fit both asperlicin (**3**) and the diazepam-derived **5** to the same receptor site model requires accommodation of the quinazoline ring in asperlicin and the 5-phenyl substituent in **5** to the same site. Such accommodation can be achieved, as illustrated by the reoriented structure

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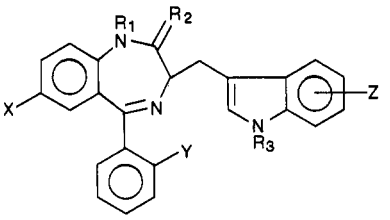
Table I. Receptor Binding Affinities for 3-(3-Indolylmethyl)benzodiazepines[a]



								IC_{50}, μM		
								[^{125}I]CCK		[^{125}I]gastrin, gastric
no.	X	Y	Z	R_1	R_2	R_3	3-stereo.	pancreas	brain	glands
1	Cl	H	H	H	O	H	*R*	3.4	>100	30
6	Cl	H	H	H	O	H	*S*	32	>100	70
7	H	H	H	H	O	H	*R*	1.2	50	50
8	H	F	H	H	O	H	*R*	0.5	80	40
9	Cl	Cl	H	H	O	H	*R*	5.0	>100	>100
10	H	*b*	H	H	O	H	*R*	48	>100	80
11	H	F	H	H	O	H	*S*	10.6	36	>100
12	H	COOH	H	H	O	H	*R*	18	16	24
13	H	F	H	H	H_2	H	*R*	4.0	>100	28
14	Cl	H	H	H	S	H	*R*	4.5	>100	>100
15	H	F	H	$C(CH_3)$=NN		H	*R*	1.2	39	12
16	H	H	5-Br	H	O	H	*RS*	2.9	~100	–
17	H	F	5-F	H	O	H	*RS*	1.4	>100	>100
18	H	F	6-F	H	O	H	*RS*	1.3	~100	>100
19	Cl	H	H	CH_3	O	H	*R*	1.4	>100	>100
20	H	H	H	CH_3	O	H	*R*	0.3	10	21
21	H	F	H	CH_3	O	H	*R*	0.27	10	13
22	H	F	H	Et	O	H	*R*	0.3	30	>100
23	H	F	H	CF_3CH_2	O	H	*R*	3.6	>100	>100
24	H	F	H	*n*-C_5H_{11}	O	H	*R*	~100	>100	>100
25	H	F	H	$(CH_3)_2CH(CH_2)_2$	O	H	*R*	~100	~30	23
26	H	F	H	c-$C_3H_5CH_2$	O	H	*R*	2.2	30	58
27	H	F	H	$(CH_3)_2N(CH_2)_2$	O	H	*R*	~100	>100	25
28	H	F	H	CH_2COOEt	O	H	*R*	8.3	>100	25
29	H	F	H	CH_2COOH	O	H	*R*	0.3	23	5
30	H	F	H	CH_2CONH_2	O	H	*R*	2.1	66	>10
31	H	F	H	$(CH_2)_2CN$	O	H	*R*	0.7	30	13
32	H	F	H	$(CH_2)_2COOH$	O	H	*R*	1.4	11	5.8
33	Cl	H	H	$PhCH_2$	O	H	*R*	>100	>100	>100
34	Cl	H	H	$PhCH_2$	O	H	*S*	>100	>100	>100
35	H	H	H	CH_3	O	CH_3	*R*	0.1	63	67
36	H	F	H	CH_3	O	CH_3	*R*	0.36	17	49
37	Cl	H	H	CH_3	O	$PhCH_2$	*R*	~150	>100	>100
38	H	F	H	CH_3	O	*p*-ClC_6H_4CO	*R*	11	>100	>100

[a] Receptor binding affinity is expressed as IC_{50}, the concentration (μM) of compound required for half-maximal inhibition of binding of [^{125}I]gastrin to guinea pig gastric glands.[1,19] [b] In this compound, the phenyl ring bearing the substituent Y is replaced by methyl.

5b. This rotation **5** → **5b** highlights the fundamental difference between the two benzodiazepines **3** and **5**, however: (3-indolylmethyl)benzodiazepines based on **3** would reflect the *S* stereochemistry of natural L-Trp whereas, to fit the same receptor model, compounds based on **5b** would require inversion to the *R* configuration of the "unnatural" enantiomer, D-Trp. On the basis of these considerations, the 3(*R*)-(3-indolylmethyl) compound **1** was synthesized as a test of this approach. The effectiveness of this compound as a CCK antagonist[1,2] gave support to the underlying hypothesis and prompted the synthesis and evaluation of the other 3-alkylbenzodiazepines shown in Tables I–IV.

The design rationale behind compound **1** took on added importance in light of these data for, as Table I illustrates, the subsequently prepared "natural" or L-Trp-based enantiomer **6**, which reflects the stereochemistry of asperlicin, is a significantly less effective CCK antagonist. As the test case, this compound would likely have been judged insufficiently active to warrent continued investigation. The design of compound **1** was thus the key step in development of the 3-substituted benzodiazepines described in this and our other reports.[1,2]

Chemistry. Scheme I summarizes the synthetic procedures used to obtain the compounds of Tables I–IV. Compound **1** was initially synthesized by heating 2-amino-5-chlorobenzophenone and D-tryptophan (D-Trp) methyl ester hydrochloride in refluxing pyridine (method A), a variant of a published procedure for synthesis of 3-unsubstituted benzodiazepines.[28] The product proved to be a selective antagonist of CCK and the key lead for our synthetic program, and its structure was therefore confirmed by X-ray crystallography (Figure 1).

This route to benzodiazepines gave poor yields of seriously contaminated products, however, and the severity of the reaction conditions led to doubts concerning the chiral purity of the purified products. These doubts were reinforced by marked deviation of the absolute values of the observed optical rotations of purified **1** ($[\alpha]^{25}_{546}$ −38.4°; $[\alpha]^{25}_{578}$ −32.7°) from those reported for the previously synthesized enantiomer ($[\alpha]^{25}_{546}$ +48.3°; $[\alpha]^{25}_{578}$ +40.4°).[29]

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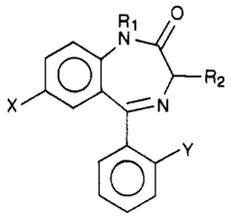
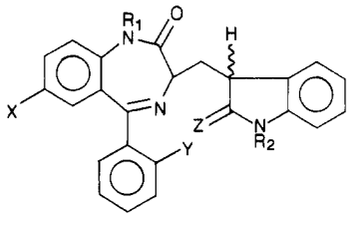
Table II. Receptor Binding Affinities for 3-Substituted Benzodiazepines[a]



no.	X	Y	R_1	R_2	3-stereo.	IC_{50}, μM [^{125}I]CCK pancreas	IC_{50}, μM [^{125}I]CCK brain	IC_{50}, μM [^{125}I]gastrin, gastric glands
39	Cl	H	H	$PhCH_2$	*R*	80	100	>100
40	Cl	H	H	$(CH_3)_2CHCH_2$	*R*	42	60	38
41	Cl	H	H	$PhCH_2OCH_2$	*R*	60	80	13
42	Cl	H	H	p-$(PhCH_2O)C_6H_4CH_2$	*R*	>100	>100	>100
43	Cl	H	H	1-naphthyl-CH_2	*RS*	23	100	>100
44	Cl	H	H	2-naphthyl-CH_2	*RS*	49	61	>100
45	H	F	H	3-thienyl-CH_2	*RS*	33	>100	14
46	H	F	H	3-thienyl	*RS*	>100	>100	>100
47	H	H	CH_3	(*E*)-3-thienyl-CH=	–	100	34	>100
48	H	H	CH_3	(*Z*)-3-thienyl-CH=	–	25	33	>100

[a] Binding affinities defined as in Table I, footnote *a*.

Table III. Receptor Binding Affinites for 3-(3′-Indolinylmethyl)benzodiazepines[a]



no.	X	Y	R_1	R_2	Z	3-stereo.[b]	IC_{50}, μM [^{125}I]CCK pancreas	IC_{50}, μM [^{125}I]CCK brain	IC_{50}, μM [^{125}I]gastrin, gastric glands
49	H	F	H	H	H_2	*R*(α)	11	48	–
50	H	F	H	H	H_2	*R*(β)	17	>100	–
51	H	H	H	H	H_2	*R*(α)	3.2	54	100
52	H	H	H	H	H_2	*R*(β)	21	28	18
53	H	H	CH_3	CH_3	H_2	*R*(α)	13	93	140
54	H	H	CH_3	CH_3	H_2	*R*(α/β)	10	48	14
55	H	F	H	Boc-L-leucyl	H_2	*R*(β)	~100	>100	>100
56	H	F	H	Boc-L-leucyl	H_2	*R*(α)	10	>100	>150
57	H	F	H	Boc-D-leucyl	H_2	*R*(β)	21	>100	>100
58	H	F	H	Boc-D-leucyl	H_2	*R*(α)	11	>100	>100
59	H	H	H	HCl-L-leucyl	H_2	*R*(α/β)	6.6	50	>100
60	H	H	H	Boc-L-leucyl	H_2	*R*(α/β)	4.7	>100	130
61	Cl	H	H	H	O	*R*	24	100	>100

[a] Binding affinities defined as in Table I, footnote *a*. [b] The absolute stereochemistries at the 3′-position (in the indoline ring) in these compounds have not been determined. The relative stereochemistries at this site are designated in the individual diastereomers as α and β.

An alternative three-step synthesis, also derived from published methods,[30] was therefore adopted for synthesis of the other 3-substituted benzodiazepines of Tables I–IV.

The alternative route (method B) involved coupling of the 2-aminobenzophenone with Boc-D-Trp using dicyclohexylcarbodiimide (DCC) or 1-ethyl-3-(3-(dimethylamino)propyl)carbodiimide (EDC) in CH_2Cl_2 followed by deblocking with HCl/EtOAc and cyclization by exposure to a buffered medium (pH 8.5) for several hours to several days. Surprisingly, purified **1** prepared by this route showed a similarly deviant optical rotation ($[\alpha]^{25}_{546}$ −39.6°; $[\alpha]^{25}_{578}$ −33.9°) indicative of ca. 15–20% racemization. HPLC assay on a chiral support, however, showed compound **1** synthesized by both methods to be >99% chirally pure (>98% ee). The reason for the optical rotation discrepancies (ca. 7°) is unclear, but treatment of the rotation sample with 1 N HCl or concentrated NH_3 gave specific rotations spanning an 80° range (HCl, $[\alpha]^{25}_{546}$ −29.1°; NH_3, $[\alpha]^{25}_{546}$ +51.7°). Clearly, the rotation of this compound is acutely sensitive to pH. These results demonstrate the risks inherent in the use of optical rotation alone as a criterion of chiral purity.

A third modification of the basic synthetic approach, treatment of 2-aminobenzophenone with D-Trp acid chloride hydrochloride followed by cyclization in basic medium (method C), provided material of equal chiral purity in somewhat improved yield. The 3-substituted benzodiazepine rings in the compounds of Table I were all prepared by one of these three methods.

The thioamide **14** was prepared from the parent amide **1** by treatment with Lawesson's Reagent according to published procedures.[31] Desulfurization over Raney nickel

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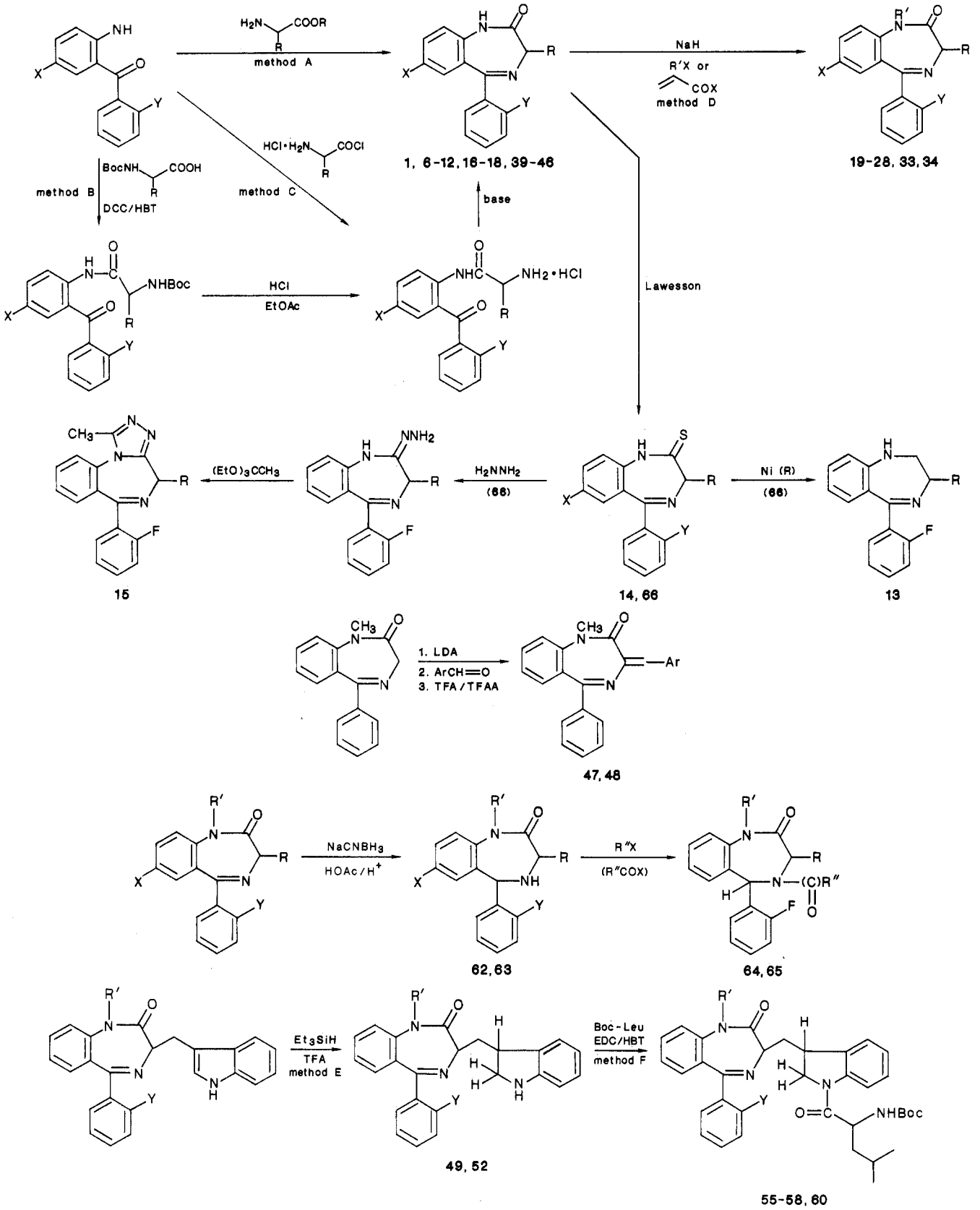
Scheme I. Methods of Synthesis of Compounds of Tables I–V[a]



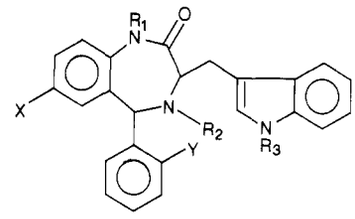
[a] R = 3-indolyl-CH_2, 5-Br-3-indolyl-CH_2, 5-F-3-indolyl-CH_2, 6-F- 3-indolyl-CH_2, $PhCH_2$, *i*-Bu, $PhCH_2OCH_2$, *p*-$(PhCH_2O)C_6H_4CH_2$, 1-naphthyl-CH_2, 2-naphthyl-CH_2, 3-thienyl-CH_2, 3-thienyl.

of the thioamide **66** similarly prepared from **8** gave the methylene compound **13**, and treatment of this same thioamide with hydrazine followed by ethyl orthoacetate according to the method of Meguro and co-workers[32]

(31) Scheibye, S.; Pedersen, B. S.; Lawesson, S.-O. *Bull. Soc. Chim. Belg.* **1978**, *87*, 229.

(32) Meguro, K.; Tawada, H.; Miyano, H.; Sato, Y.; Kunada, Y. *Chem. Pharm. Bull.* **1973**, *21*, 2382.

Table IV. Receptor Binding Affinities for 4,5-Dihydro-3-substituted-benzodiazepines[a]



no.	X	Y	R_1	R_2	R_3	3-stereo.	IC_{50}, μM [^{125}I]CCK pancreas	IC_{50}, μM [^{125}I]CCK brain	IC_{50}, μM [^{125}I]gastrin, gastric glands
62	Cl	H	H	H	H	*R*	12	>100	>100
63	Cl	H	H	H	H	*S*	75	>100	>100
64	H	F	CH_3	*p*-ClC_6H_4CO	CH_3	*R*	>100	>100	>100
65	H	F	CH_3	CH_3CO	CH_3	*R*	~100	>100	>100

[a] Binding affinities defined as in Table I, footnote *a*.

provided the triazole **15**. The N_1-alkyl derivatives **19–28**, **33**, and **34** were obtained by treatment of the parent NH compounds with sodium hydride in DMF followed by a suitable alkyl chloride (**27**), bromide, (**24–26**, **28**, **33**, **34**), or iodide (**19–23**) or by acrylonitrile (**31**) or ethyl acrylate (method D). The product of the last reaction was saponified in aqueous sodium hydroxide to give the acid **32**. Compound **28** was similarly saponified to give the acid **29** or treated with ammonia to provide the amide **30**.

Treatment of N_1-alkylated compounds with a second equivalent of sodium hydride followed by an alkyl bromide (**37**) or iodide (**35**, **36**) or an acyl chloride (**38**) gave the compounds alkylated or acylated on indole nitrogen. The positions of these alkylations were assigned on the basis of NOE experiments carried out on the products obtained from alkylation of **1** with methyl iodide (see Experimental Section).

The problem of potential racemization during alkylation was addressed by chiral purity assay of the key compounds, **21** and **29**. In these cases, the target compound was hydrolyzed in 6 N HCl at 110 °C to recover the tryptophan residue, which was then assayed for chiral purity by the method of Lam and Chow.[33] In both cases, less than 3% racemization of the recovered tryptophan was observed.

The compounds **39–46** of Table II were prepared by method B, using the appropriate Boc amino acid in place of Boc-Trp. Olefins **47** and **48** were obtained by condensation of the anion of the parent benzodiazepine, prepared as previously described,[34] with thiophene-3-carboxaldehyde, followed by dehydration of the intermediate carbinol.

The indolines **49–52** of Table III were prepared by reduction of the indoles with triethylsilane/TFA followed by chromatographic separation of diastereomers (method E). Coupling of the products with Boc-D- or Boc-L-leucine gave the acyl compounds **55–58** and **60**, and hydrolysis in acid gave deprotected amines such as **59**. Bismethylation of the indolines to give the dimethyl compounds **53** and **54** was carried out with 2 equiv of sodium hydride and methyl iodide. The oxindole **61** was obtained by oxidation of **1** with Me_2SO/HCl.[35]

An alternative mode of reduction in the benzodiazepine ring was effected on compounds such as **1** by treatment with sodium cyanoborohydride in acetic acid in the cold. The resulting compounds (e.g., **62**, **63**; Table IV) were acylated with an appropriate acid chloride to give amides such as **64** and **65**. Physical data for the compounds of Tables I–IV are presented in Table V.

Biology. The methods employed for determination of [^{125}I]CCK-33 binding to rat pancreas and guinea pig cortex, [^{125}I]gastrin binding to guinea pig gastric glands, and [^{3}H]diazepam or [^{3}H]flunitrazepam binding in rat and guinea pig brain, respectively, were as described previously.[1,19] Values shown are the means of triplicate determinations.

The in vivo activities of the compounds were determined on the basis of their ability to antagonize CCK-8 inhibition of charcoal meal gastric emptying in mice as described previously.[2] The mice were administered the test compounds at one or more dose levels ($N \geq 10$/dose) by the oral or intraperitoneal route 1 h prior to CCK-8. CCK-8 (80 μg/kg, sc) was given 5 min prior to a charcoal meal and gastric emptying determined 5 min later. ED_{50} values were based upon data obtained by using at least three dose levels and were determined by regression analysis. The data are presented in Table VI.

Discussion

The [^{125}I]CCK pancreatic binding data presented in Tables I–IV support the hypothesis underlying this work to the extent that a known, nonpeptidal ligand for one receptor has been used to construct an effective ligand for another. In this case, the benzodiazepine receptor ligand **4** has been used to generate effective ligands for the CCK receptor.

Recent reports appearing after completion of this work described pharmacological activity attributable to a CCK antagonist in 3-unsubstituted benzodiazepines such as **4**.[36-39] The mechanism of this effect was not clarified. Possible interpretations are that CCK and compounds such as **4** compete for the same receptor, perhaps even that the CCK and benzodiazepine receptors are one and the same. The compounds described in the present work afforded an opportunity to examine these possibilities at the molecular level.

(33) Lam, S. K.; Chow, F. K. *J. Liq. Chromatogr.* **1980**, *3*, 1579.
(34) Reitter, B. E.; Sachdeva, Y. P.; Wolfe, J. F. *J. Org. Chem.* **1981**, *46*, 3945.
(35) Savige, W. E.; Fontana, A. *J. Chem. Soc., Chem. Commun.* **1976**, 599.
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(38) Kubota, K.; Sugaya, K.; Sunagane, N.; Matsuda, I.; Uruno, T. *Eur. J. Pharmacol.* **1985**, *110*, 225.
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Table V. Physical Data for Compounds of Tables I–IV

no.	formula	mp, °C	% purity (HPLC)	method	yield,[b] %	MS molecular ion	anal.
1	$C_{24}H_{18}ClN_3O\cdot0.65C_4H_{10}O$	130–155[a]	>99.8	A	17	399	C, H, N, Cl
6	$C_{24}H_{18}ClN_3O\cdot0.35C_4H_{10}O$	150–154[a]	>99.6	A	13	399	C, H, N, Cl
7	$C_{24}H_{19}N_3O\cdot0.5C_3H_6O$	260–263 dec	99.6	B	25	365	C, H, N
8	$C_{24}H_{18}FN_3O$	254–256	>99.8	C	28	383	C, H, N
9	$C_{24}H_{17}Cl_2N_3O$	140–170	98.5	B	15	433	C, H, N
10	$C_{19}H_{17}N_3O\cdot0.1H_2O$	185–190	95.6	B	36	303	C, H, N
11	$C_{24}H_{18}FN_3O$	255–257	99.8	B	15	383	C, H, N
12	$C_{25}H_{19}N_3O_3\cdot0.75CHCl_3$	133–136 dec	96	C	37	409	C, H, N
13	$C_{24}H_{20}FN_3\cdot0.07CHCl_3$	101–103	96.3	–	44	369	C, H, N
14	$C_{24}H_{18}ClN_3S$	279–280	98.6	–	56	415	C, H, N, S
15	$C_{26}H_{20}FN_5\cdot0.4C_4H_{10}O\cdot0.5H_2O$	75[a]	99.1	–	41	421 (422[c])	C, H, N
16	$C_{24}H_{18}BrN_3O\cdot0.25CHCl_3\cdot0.3C_3H_6O$	175–179	99	B	18	443/445	C, H, N
17	$C_{24}H_{17}F_2N_3O\cdot0.17CHCl_3$	225–229	97.6	C	9	402[c]	C, H, N[d]
18	$C_{24}H_{17}F_2N_3O\cdot0.07CHCl_3$	257–259	89	C	8	402[c]	C, H, N
19	$C_{25}H_{20}ClN_3O$	~140[a]	98.9	D	45	413	C, H, N, Cl
20	$C_{25}H_{21}N_3O$	*a*	100	D	68	379	C, H, N
21	$C_{25}H_{20}FN_3O\cdot0.6CH_2Cl_2$	105–113[a]	99.6	D	95	397 (398[c])	C, H, N
22	$C_{26}H_{22}FN_3O\cdot0.15CH_2Cl_2$	95–113[a]	96	D	51	411	C, H, N
23	$C_{26}H_{19}F_4N_3O$	189–192	99.5	D	31	465	C, H, N
24	$C_{29}H_{28}FN_3O$	150–151	99.9	D	44	453	C, H, N[e]
25	$C_{29}H_{28}FN_3O\cdot0.2C_4H_{10}O$	198–199.5	99.9	D	35	453	C, H, N
26	$C_{28}H_{24}FN_3O\cdot0.07CH_2Cl_2$	207.5–208.5	99.6	D	26	437	C, H, N
27	$C_{28}H_{27}FN_4O$	200–201	99.6	D	49	454	C, H, N[f]
28	$C_{28}H_{24}FN_3O_3\cdot0.24CH_2Cl_2$	88–100	93	D	88	469	C, H, N
29	$C_{26}H_{20}FN_3O_3\cdot H_2O\cdot0.1C_4H_{10}O\cdot0.04C_6H_{14}$	70–90[a]	97.2	–	76	441	C, H, N
30	$C_{26}H_{21}FN_4O_2\cdot0.1CHCl_3$	143–150	96	–	49	440	C, H, N
31	$0.85C_{27}H_{21}FN_4O$ + $0.9C_3H_7NO$	97–105[a]	82.4 / 96.4 / 14.0	D	75	436 / 489	C, H, N
32	$C_{27}H_{22}FN_3O_3\cdot0.35H_2O\cdot0.55C_4H_{10}O$	75–160[a]	99.7	–	61	455	C, H, N
33	$C_{31}H_{24}ClN_3O\cdot0.5C_6H_{12}$	80[a]	100	D	75	489	C, H, N, Cl
34	$C_{31}H_{24}ClN_3O\cdot0.5C_6H_{12}$	80–100[a]	99.3	D	79	489	C, H, N, Cl
35	$C_{26}H_{23}N_3O$	*a*	99.5	D	61	393	C, H, N
36	$C_{26}H_{22}FN_3O\cdot0.2CH_2Cl_2$	98–100	99	D	66	411	C, H, N
37	$C_{32}H_{26}ClN_3O\cdot0.33H_2O$	72–80[a]	98.7	D	40	503	C, H, N, Cl
38	$C_{32}H_{23}FClN_3O_2\cdot0.3C_6H_{14}$	75[a]	99.3	D	43	536[c]	C, H, N, Cl
39	$C_{22}H_{17}ClN_2O$	154–157	100	B	37	360	C, H, N, Cl
40	$C_{19}H_{19}ClN_2O$	156–160	100	B	79	326	C, H, N, Cl
41	$C_{23}H_{19}ClN_2O_2\cdot0.25H_2O\cdot0.1C_4H_{10}O$	113–115	100	B	78	390	C, H, N, Cl
42	$C_{29}H_{23}ClN_2O_2$	97–101	98.5	B	65	466	C, H, N
43	$C_{26}H_{19}ClN_2O$	180–182	99.9	B	67	410	C, H, N, Cl
44	$C_{26}H_{19}ClN_2O$	138–142	99.7	B	48	410	C, H, N, Cl
45	$C_{20}H_{15}FN_2OS$	189–191	97.9	B	75	350	C, H, N
46	$C_{19}H_{13}FN_2OS$	219–223	98.5	B	73	336	C, H, N
47	$C_{21}H_{16}N_2OS$	196–197	99.8	–	100	344	C, H, N
48	$C_{21}H_{16}N_2OS$	194–196	99.8	–	100	344	C, H, N
49	$C_{24}H_{20}FN_3O\cdot0.4H_2O$	119–123	98.3	E	43	386[c]	C, H, N
50	$C_{24}H_{20}FN_3O\cdot0.3H_2O$	119–123	96.5	E	46	386[c]	C, H, N
51	$C_{24}H_{21}N_3O$	*a*	99.6	E	26	367	C, H, N
52	$C_{24}H_{21}N_3O\cdot H_2O$	*a*	98	E	30	367	C, H, N
53	$C_{26}H_{25}N_3O\cdot0.05CH_2Cl_2$	*a*	99.3	E	20	395	C, H, N
54	$C_{26}H_{25}N_3O$	*a*	55/45	E	99.5	395	C, H, N
55	$C_{35}H_{39}FN_4O_4\cdot0.33C_6H_{14}$	118–130[a]	97	F	26	598	C, H, N
56	$C_{35}H_{39}FN_4O_4$	130–148[a]	91	F	52	599[c]	C, H, N[g]
57	$C_{35}H_{39}FN_4O_4$	135–148[a]	87.5	F	19	598	C, H, N
58	$C_{35}H_{39}FN_4O_4$	130–145[a]	95.1	F	52	599[c]	C, H, N
59	$C_{30}H_{32}N_4O_2\cdot1.5HCl$	*a*	60/38	–	100	480	C, H, N, Cl[h]
60	$C_{35}H_{40}N_4O_4$	*a*	60/40	F	47	581[c]	C, H, N
61	$C_{24}H_{18}ClN_3O_2\cdot0.25CHCl_3$	179–182	54/43	–	24	416[c]	C, H, N
62	$C_{24}H_{20}ClN_3O\cdot HCl\cdot0.75H_2O$	198–204	90	B	16	401	C, H, N, Cl
63	$C_{24}H_{20}ClN_3O\cdot HCl\cdot0.5H_2O\cdot0.25C_2H_5OH$	198–204	98.6	B	38	401	C, H, N, Cl
64	$C_{33}H_{27}FClN_3O_2\cdot0.05C_4H_{10}O$	237–243	99	–	26	551	C, H, N, Cl
65	$C_{28}H_{26}FN_3O_2$	214–216.5	99.5	–	13	455	C, H, N

[a] Foam, mp indistinct. [b] Overall for entire synthetic sequence. [c] FAB MS, M + H. [d] C: calcd, 68.83; found, 68.38. [e] N: calcd, 9.26; found, 8.83. [f] N: calcd, 12.33; found, 11.28. [g] H: calcd, 6.57; found, 6.98. [h] N: calcd, 10.47; found, 9.97.

A preliminary survey showed some similarities in the structure–activity profile of the present compounds as ligands for the peripheral CCK receptor vs. that of compounds such as **4** as antianxiety agents. Thus, the 2′-fluoro substituent (**8**), N_1-methyl substitution (**19–21**), and triazole fusion (**15**) all enhance binding of the 3-substituted compounds of Table I to peripheral CCK receptors. These modifications have been reported to enhance antianxiety activity in structure **4** in similar fashion.[24,40] The N_1-(carboxymethyl) modification, on the other hand, is known to be detrimental to antianxiety activity in 3-unsubstituted compounds such as **4**,[25] but gives compounds (e.g., **29**) with good CCK receptor affinity in the 3-substituted series. Other N_1-alkyl substituents (**22–34**) are, for the most part, detrimental to CCK binding as they are to antianxiety

(40) Hester, J. B.; Rudzik, A. D.; Kamdar, B. V. *J. Med. Chem.* **1971**, *14*, 1078.

Table VI. Benzodiazepine and Pancreatic CCK Receptor Binding Affinities and in Vivo CCK Antagonist Potencies for Selected Benzodiazepines

compd	IC_{50}, μM [^{125}I]CCK pancreas	IC_{50}, μM [^{125}I]CCK brain	IC_{50}, μM [^{3}H]benzodiazepine brain	IC_{50} CCK (pancreas)/IC_{50} BZD (brain)	in vivo ED_{50},[b] mg/kg, CCK antagonism ip	in vivo ED_{50},[b] mg/kg, CCK antagonism po
4 (diazepam)	>100	>100	0.007	>10000	–	–
clonazepam	>100	>100	0.0017	>58000	–	–
flunitrazepam	>100	>100	0.0017	>58000	–	–
3 (asperlicin)	1.4[19]	>100[19]	50[19]	0.028	–	>300[20]
1	3.4	>100	>100	<0.03	88	>300
6	32	>100	5.6	5.7	–	>150
21	0.27	10	>100*	<0.0027	15	25
15	1.2	39	0.8*	1.5	–	76
29	0.3	23	>100	<0.003	–	42
8	0.5	80	>100	<0.005	41	104
7	1.2	50	>100	<0.012	–	>150
9	5.0	>100	>180	<0.027	119	>600
10	48	>100	>100	<0.48	–	–
16	2.9	~100	–	–	–	>150
36	0.36	17	–	–	18	39

[a] Binding affinities as defined in Table I, footnote *a*. [^{3}H]Benzodiazepine binding is IC_{50} (μM) for half-maximal inhibition of binding of [^{3}H]diazepam or (*) [^{3}H]flunitrazepam in rat or guinea pig brain, respectively. [b] In vivo activities were determined as described under Biology.

activity.[24] The ethyl (**22**) and 2-cyanoethyl (**31**) substituents, however, do appear compatible with respectable CCK receptor affinity.

Of course, these comparisons are ambiguous, since peripheral CCK receptor affinity is assayed here by direct binding, whereas reported antianxiety activity is an in vivo assay, which superimposes such factors as solubility, absorption, distribution, and metabolism on the basic receptor affinities. For a more direct comparison of the two activities, selected compounds from the 3-substituted series reported in this work were assayed for benzodiazepine receptor binding as shown in Table VI. These data show clearly that the structural requirements for high CCK and benzodiazepine receptor binding affinities in benzodiazepines are separate and distinct. The ratio of benzodiazepine to peripheral CCK receptor affinity in this group, for example, ranged from $>10^4$ in compound **4** to ca. 10^{-3} in compounds **21** and **29**. Addition of the key 3(*R*)-indolylmethyl substituent to compound **4** (cf. **1**) enhances CCK receptor affinity ca. 100-fold, while diminishing benzodiazepine binding by more than 4 orders of magnitude. Furthermore, the stereochemical preference for the two receptors is apparently opposite as indicated by the *R*/*S* pair **1**/**6** (cf. **8**/**11**, Table I). The preferred stereochemistry observed for benzodiazepine receptor binding in these compounds, 3*S*, is consistent with that reported in the literature for simple 3-methylbenzodiazepines.[41,42] Similar selectivities between brain benzodiazepine and brain CCK receptor affinities are also demonstrated by the data in Table VI. These results support the separate identities and ligand structure requirements of the brain benzodiazepine and peripheral and central CCK receptor systems.

The data in Table VI show further that in vivo CCK antagonist activity parallels peripheral CCK receptor affinity, but shows no relationship to benzodiazepine receptor affinity. These numbers indicate that the observed CCK antagonist activities of these compounds in vivo are a consequence of their interaction with peripheral CCK receptors, not benzodiazepine receptors.

CCK and CCK-like peptides are widely distributed in mammals. Receptors for CCK are found not only in the periphery,[4-7] where they can coexist with those for the closely related peptide gastrin, but in the central nervous system (CNS) as well.[3,6,7,43-45] With the exception of asperlicin,[19] the few CCK receptor antagonists known in the past have demonstrated relatively nonspecific affinity for these three different receptor types, central and peripheral CCK and gastrin.[12,13] As the data in Tables I–IV indicate, the 3-substituted benzodiazepines reported here discriminate effectively between the peripheral CCK receptor on the one hand and the central CCK and gastrin receptors on the other. Structure **35**, for example, has 100 nM affinity and >600-fold selectivity for peripheral CCK vs. central CCK or gastrin receptors.

In addition to providing CCK antagonists of superior potency (Table VI) and much-simplified structure, several of the compounds reported here also surmount the key liability of asperlicin, lack of oral bioavailability. Compounds **8**, **21**, and **36** (Table VI), for example, are orally effective CCK antagonists with po/ip ratios of 3 or less. Compound **29** provides the added advantage of good solubility in aqueous media at physiological pH.

In conclusion, we have demonstrated that a known, effective ligand for one physiological receptor, compound **4**, could be used to design and develop ligands for another such receptor. At the same time, we have succeeded for the first time in generating a potent, nonpeptidal ligand for a peptide receptor by design. As a result of this effort, we have prepared new, selective, orally active antagonists of the peptide hormone cholecystokinin. These compounds are of potential use both as pharmacological tools and as possible therapeutic agents. Our original hypothesis provides one explanation for the effectiveness of these compounds, namely, that they, like diazepam, mimic in some unknown way key structural features of the relevant peptides. An experimental examination of this possibility is in progress.

An alternate explanation is that common structural elements in these compounds bind not the site reserved

(41) Sunjic, V.; Kuftinec, J.; Kajfez, F. *Arzneim.-Forsch.* **1975**, *25*, 340.
(42) Mohler, H.; Okada, T.; Meitz, P.; Ulrich, J. *Life Sci.* **1978**, *22*, 985.
(43) *Neuronal Cholecystokinin*; Vanderhaeghen, J.-J., Crawley, J. N., Eds.; Ann. N.Y. Acad. Sci: 1985; Vol. 448.
(44) Dockray, G. J. *Nature (London)* **1976**, *264*, 568.
(45) Beinfeld, M. C. *Neuropeptides* **1983**, *3*, 411.

for the natural (peptide?) ligand, but adjacent sites specific for those elements, "accessory binding sites" as described by Ariens.[46] From the standpoint of antagonist design, this concept transcends, and may render irrelevant for this application, the distinction between peptidal and nonpeptidal receptors, offering an approach to nonpeptidal ligand design equally general for both receptor classes. This concept will be elaborated in a subsequent paper. Application of this approach to other peptidal receptor systems is currently under investigation.

Experimental Section

Melting points (Thomas-Hoover melting point apparatus) are uncorrected. Spectra were obtained as follows: IR spectra on a Perkin-Elmer 237 spectrophotometer; EI mass spectra on a VG MM 7035 mass spectrometer; FAB mass spectra on a VG MM/ZAB-HF spectrometer; ^{1}H NMR spectra on a Varian EM-390 or Nicolet NT-360 spectrometer, with Me_4Si as internal standard. HPLC was carried out on a Hewlett-Packard Model 1084B liquid chromatograph using a Waters C-18 column. Chiral separations were done on a Pirkle covalent phenylglycine column with a triethylamine phosphate buffer. Elemental analyses for carbon, hydrogen, and nitrogen were determined with a Perkin-Elmer Model 240 elemental analyzer and are within ±0.4% of the theory unless noted otherwise. Optical rotations were determined with a Perkin-Elmer Model 141 polarimeter.

Analytical TLC was carried out on 250-μm, 5 × 20 cm silica gel plates (E. Merck) using ultraviolet light/phosphomolybdic acid for visualization.

Syntheses. Specific examples presented below illustrate general synthetic methods A–F cited in Table V. Other physical data are given in that table. In general, samples prepared for physical and biological studies were dried in high vacuum (5 μm) over P_2O_5 for 18 h at temperatures ranging from ambient to 110 °C, depending on the sample melting point. Despite these measures, the majority of the compounds remained solvated (Table V). Where analytical data have been presented for such solvates, the presence of all indicated solvents has been verified by NMR.

Method A. (*R*)-7-Chloro-1,3-dihydro-3-(1*H*-indol-3-ylmethyl)-5-phenyl-2*H*-1,4-benzodiazepin-2-one (1). 2-Amino-5-chlorobenzophenone (1.2 g, 5.2 mmol) and D-tryptophan methyl ester hydrochloride (1.3 g, 5.1 mmol) were combined in dry pyridine (25 mL) and heated at reflux under nitrogen for 5 h. The mixture was evaporated in vacuo and the residue washed twice with pH 6 buffer and dissolved in ethyl acetate (50 mL). The ethyl acetate solution was dried over sodium sulfate, filtered, and evaporated in vacuo to give an oil, which was chromatographed on silica gel (230–400 mesh) eluted with 20% (v/v) ether/methylene chloride. The product fractions were evaporated in vacuo to give the title compound as a white solid, which was dried in vacuo at 100 °C: ^{1}H NMR ($CDCl_3$) δ 1.2 (t, Et_2O), 3.5 (q, Et_2O), 3.68 (1 H, dd, J_1 = 16 Hz, J_2 = 9 Hz, CH_{2a}), 3.77–3.86 (2 H, m, CH_{2b} + C_3H), 7.0–7.7 (13 H, m, aro), 8.05 (1 H, br s, indole NH), 8.68 (1 H, br s, N_1H).

X-ray Crystal Structure Analysis of 1. Suitable crystals of 1 ($C_{24}H_{18}ClN_3O{\cdot}C_3H_6O$) for X-ray diffraction studies formed from acetone with space group symmetry of $P2_12_12_1$ and cell constants of a = 8.758 (1) Å, b = 10.034 (2) Å, and c = 28.237 (2) Å for Z = 4 and a calculated density of 1.226 g/cm^3. Of the 1971 reflections measured with an automatic four-circle diffractometer equipped with Cu radiation, 1800 were observed ($I > 3\sigma(I)$). The structure was solved with a multisolution tangent formula approach and difference Fourier analysis and refined by using full-matrix least-squares techniques.[47] An ordered molecule of acetone was found in the crystal lattice. The absolute configuration was confirmed by anomalous scattering analysis, which gave an R factor of 0.074 for one enantiomer and 0.081 for the other. This difference, which is significant at the 0.005 level, was confirmed by careful remeasurement of 10 enantiomorph-sensitive reflections.[48] Hydrogens were assigned isotropic temperature factors corresponding to their attached atoms. The function $\sum w(|F_o| - |F_c|)^2$ with $w = 1/(\sigma(F_o))^2$ was minimized to give an unweighted residual of 0.058. No abnormally short intermolecular contacts were noted. Tables VII–IX containing the final fractional coordinates, temperature parameters, bond distances, and bond angles are available as supplementary material (4 pages). Figure 1 is a computer-generated perspective drawing of 1 from the final X-ray coordinates showing the absolute stereochemistry.

Method B. (*R*)-1,3-Dihydro-3-(1*H*-indol-3-ylmethyl)-5-phenyl-2*H*-1,4-benzodiazepin-2-one (7). 2-Aminobenzophenone (1.97 g, 0.01 mol), Boc-D-tryptophan (3.05 g, 0.01 mol), and dicyclohexylcarbodiimide (DCC, 10 mL of a 1 M solution in methylene chloride, 10 mmol) were combined in 15 mL of dry tetrahydrofuran stirred in an ice bath. The mixture was allowed to warm to room temperature and stirred overnight. The solids were removed by filtration, and the filtrate was evaporated in vacuo. The residue was stirred in 40 mL of ethyl acetate in an ice bath and saturated with hydrogen chloride gas for 20 min. The mixture was evaporated to dryness. The residue, in 125 mL of methanol, was treated with 30 mL of water and the pH of the mixture adjusted to 8.5–9.0 with 10% sodium hydroxide solution. The mixture was stirred at room temperature for 3 days. The mixture was evaporated in vacuo, combined with water (50 mL), and extracted with chloroform (250 mL). The chloroform solution was dried over potassium carbonate, filtered, and evaporated to dryness in vacuo. Recrystallization of the residue from a mixture of acetone (50 mL) and ether (50 mL) gave a white solid, which was dried in vacuo at 100 °C: ^{1}H NMR (CD_3OD) δ 2.13 (s, acetone), 3.55 (1 H, dd, J_1 = 15 Hz, J_2 = 8 Hz, CH_{2a}), 3.63 (1 H, dd, J_1 = 15 Hz, J_2 = 6 Hz, CH_{2b}), 3.79 (1 H, d + d, J_1 = 8 Hz, J_2 = 6 Hz), 6.95–7.75 (14 H, m aro).

Method C. (*R*)-5-(2-Fluorophenyl)-1,3-dihydro-3-(1*H*-indol-3-ylmethyl)-2*H*-1,4-benzodiazepin-2-one (8). 2-Amino-2′-fluorobenzophenone (12.5 g, 58 mmol) was stirred in 100 mL of dry tetrahydrofuran in an ice bath. D-Tryptophan acid chloride[49] hydrochloride (16 g, 62 mmol), slurried in 50 mL of tetrahydrofuran, was added over 10 min and the mixture stirred for 2 h in the ice bath. The resulting solid was filtered and then added to 200 mL of methanol containing 200 mL of water. The pH was adjusted to 8.5–9.0 with 10% sodium hydroxide, and the mixture was stirred for 3 days and then filtered. The collected solid was recrystallized from acetone/ether and dried in vacuo at 100 °C: ^{1}H NMR (CD_3OD) δ 3.54 (1 H, dd, J_1 = 15 Hz, J_2 = 8 Hz, CH_{2a}), 3.67 (1 H, dd, J_1 = 15 Hz, J_2 = 6 Hz, CH_{2b}), 3.83 (1 H, dd, J_1 = 8 Hz, J_2 = 6 Hz), 6.94–7.55 (13 H, m, aro).

Method D. (*R*)-5-(2-Fluorophenyl)-1,3-dihydro-3-(1*H*-indol-3-ylmethyl)-1-methyl-2*H*-1,4-benzodiazepin-2-one (21) and (*R*)-5-(2-Fluorophenyl)-1,3-dihydro-1-methyl-3-((1-methyl-1*H*-indol-3-yl)methyl)-2*H*-1,4-benzodiazepin-2-one (36). Compound 8 (0.85 g, 2.2 mmol) and sodium hydride (0.11 g of a 50% suspension in mineral oil, 2.3 mmol) were stirred in 10 mL of dry, degassed dimethylformamide under nitrogen in an ice bath. After 40 min, iodomethane (0.14 mL, 2.25 mmol) was added in one portion. The mixture was stirred and allowed to warm to room temperature over 20 min and then poured into 100 mL of water and extracted with methylene chloride (3 × 30 mL). The CH_2Cl_2 layers were washed with water, dried over potassium carbonate, filtered, and evaporated in vacuo. The residue was chromatographed on silica gel (250–400 mesh) eluted with 4% (v/v) diethyl ether in CH_2Cl_2. Evaporation of the product fraction gave **21** as a white solid. A small amount (3%) of **36** was also obtained by evaporation of the forerun.

Compound **21** was recrystallized from CH_2Cl_2/hexane and dried in vacuo at 40 °C: ^{1}H NMR ($CDCl_3$) δ 3.47 (3 H, s, N_1CH_3), 3.68

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(1 H, dd, J_1 = 16 Hz, J_2 = 9 Hz, CH_{2a}), 3.77–3.86 (2 H, m, CH_{2b} + C_3H), 6.98–7.65 (m, 13 H, aro), 8.03 (br s, 1 H, indole NH).

Repetition of the procedure of method D using **21** in place of **8** as starting material provided dimethyl compound **36**: ^{1}H NMR ($CDCl_3$) δ 3.47 (3 H, s, N_1CH_3), 3.60–3.70 and 3.78–3.87 (3 H, m, CH_2CH), 3.76 (3 H, s, indole NCH_3), 7.0–7.65 (13 H, m, aro).

Position of Alkylation. Assignment of the positions of N_1 and indole N hydrogen and methyl in **8**, **21**, and **36** given above were made by difference NOE studies of the corresponding 7-chloro analogues (**1** and its mono (**19**) and dimethyl derivatives). The monomethyl compound **19**, prepared from **1** by method D, gave an NMR spectrum similar to that of **21**: ^{1}H NMR ($CDCl_3$) δ 3.40 (3 H, s, N_1CH_3), 3.68 (1 H, dd, J_1 = 16 Hz, J_2 = 9 Hz, CH_{2a}), 3.77–3.86 (2 H, m, CH_{2b} + C_3H), 7.06 (1 H, dt, J_t = 7.3 Hz, J_d = 1.1 Hz, indole H_5 or H_6), 7.13 (1 H, dt, J = 7.3 Hz, J_d = 1.1 Hz, indole H_6 or H_5), 7.20 (1 H, d, J = 2.4 Hz, H_6), 7.21 (1 H, d, J = 9.0 Hz, H_9), 7.31 (br d, J = 7.3 Hz, indole H_7), 7.43 (1 H, dd, J_1 = 9.0 Hz, J_2 = 2.4 Hz, H_8), 7.63 (1 H, br d, J = 7.3 Hz, indole H_4), 7.35–7.46 and 7.54–7.59 (5 H, m, 5-phenyl), 8.0 (1 H, s, indole H_2), 8.4 (1 H, br s, indole NH). Irradiation of the N methyl as signed at δ 3.40 resulted in enhancement of only the doublet at δ 7.21, attributed to H_9, consistent with methylation at the nearby N_1 position. On this basis, the singlet at δ 3.40 was assigned to N_1 methyl and the broad singlet at ca δ 8.4 to indole NH.

A second methylation of **19** by the procedure of method D provided the dimethyl compound, which gave an NMR spectrum similar to that of **36**: ^{1}H NMR ($CDCl_3$) δ 3.41 (3 H, s, N_1CH_3), 3.64 (1 H, dd, J_1 = 13 Hz, J_2 = 5 Hz, CH_{2a}), 3.75–3.85 (2 H, m, CH_{2b} + C_3H), 3.76 (3 H, s, indole NCH_3), 7.07 (1 H, s, indole H_2 + 1 H, dt, J_t = 7.3 Hz, J_d = 1 Hz, indole H_5 or H_6), 7.19 (1 H, dt, J_t = 7.3 Hz, $J_d \sim$ 1 Hz, indole H_6 or H_5), 7.23 (1 H, d, J = 2.4 Hz, H_6), 7.24 (1 H, d, J = 8.2 Hz, H_9), 7.27 (br d, $J \sim$ 7 Hz, indole H_7), 7.45 (1 H, dd, J_1 = 8.2 Hz, J_2 = 2.4 Hz, H_8), 7.63 (1 H, br d, J = 7.3 Hz, indole H_4), 7.35–7.47 and 7.55–7.60 (5 H, m, 5-phenyl). Irradiation of the *N*-methyl signal at δ 3.41 resulted in enhancement of only the doublet at δ 7.24, attributed to H_9, again consistent with the presence of this methyl at N_1 as discussed above. Irradiation of the *N*-methyl signal at δ 3.76 resulted in enhancement of the doublet at δ 7.27, attributed to indole H_7, and the singlet at δ 7.07, attributed to indole H_2. Interaction with indole H_2 and H_7 is consistent with the presence of this methyl substituent on nearby indole nitrogen.

(*R*)-6-(2-Fluorophenyl)-4-(1*H*-indol-3-ylmethyl)-1-methyl-4*H*-[1,2,4]triazolo[4,3-*a*][1,4]benzodiazepine (15). Amide **8** (6.98 g, 18.2 mmol) and Lawesson's Reagent[31] (2,4-bis-(4-methoxyphenyl)-2,4-dithioxo-1,3,2,4-dithiaphosphetane) (4.41 g, 10.9 mmol) were combined in toluene (100 mL) and heated at reflux for 1.5 h. The solvent was removed in vacuo and the residue partitioned between ethyl acetate and 10% sodium hydroxide solution. The organic phase was washed with 10% sodium hydroxide (3 × 50 mL) and brine and then dried ($MgSO_4$) and evaporated to give an orange oil (10 g). Plug filtration of the crude product through silica gel (100 g) afforded the thioamide, **66**, which was recrystallized from ether: MS, *m/e* 401 (M + 2), 399 (M^+), 397, 367, 366, 365, 364, 270 (M + H − indole CH_2), 131, 130, 129.

The thioamide **66** (0.49 g, 1.22 mmol) and 95% hydrazine (0.24 g, 7.5 mmol) were combined in methanol (8 mL) and stirred at ambient temperature for 30 min. An additional 0.24 g of hydrazine was added, and the mixture was stirred for another 60 min and then poured into ice water (100 mL). The mixture was extracted with CH_2Cl_2 (3 × 50 mL), and the CH_2Cl_2 layers were washed with water, dried over potassium carbonate, filtered, and evaporated to dryness in vacuo.

The resulting amidrazone (0.5 g, 1.26 mmol) and triethyl orthacetate (1.15 g, 7.1 mmol) were combined in absolute ethanol (15 mL). Concentrated sulfuric acid (0.16 mL) was added and the mixture stirred at ambient temperature for 30 min. The acid was neutralized with saturated sodium bicarbonate solution and the mixture evaporated in vacuo. The residue was treated with water (30 mL) and extracted with CH_2Cl_2 (3 × 30 mL). The CH_2Cl_2 layers were combined, washed with water, dried over potassium carbonate, filtered, and evaporated to dryness in vacuo. The residue was chromatographed on silica gel (230–400 mesh) eluted with 1.5% and 4% (v/v) methanol in CH_2Cl_2, and the product fractions were evaporated to dryness in vacuo. The residue was recrystallized from ether to give the triazole **15**: ^{1}H NMR ($CDCl_3$) δ 1.2 (t, Et_2O), 2.65 (s, 3 H, CCH_3), 3.48 (q, Et_2O), 4.02 (1 H, dd, J_1 = 14 Hz, J_2 = 8 Hz, CH_{2a}), 4.16 (1 H, dd, J_1 = 14 Hz, J_2 = 5 Hz, CH_{2b}), 4.26 (1 H, dd, J_1 = 8 Hz, J_2 = 5 Hz, CH), 6.94–7.75 (13 H, m, aro), 8.2 (br s, 1 H, indole NH).

(*R*)-1,3-Dihydro-5-(2-fluorophenyl)-3-(1*H*-indol-3-ylmethyl)-2*H*-1,4-benzodiazepine (13). The thioamide **66** from preparation of **15** (178 mg, 0.44 mmol) was dissolved in absolute ethanol (20 mL) and combined with 1 spatula of moist (ethanol) Raney nickel catalyst. The resulting suspension was protected from moisture and stirred rapidly for 1 h. The reaction mixture was filtered and the filtrate concentrated to give 150 mg of a yellow oil. Purification via silica gel chromatography (chloroform/methanol/ammonia, 95:5:0.5 v/v/v) afforded **13**.

(*R*)-7-Chloro-1,3-dihydro-3-(1*H*-indol-3-ylmethyl)-5-phenyl-2*H*-1,4-benzodiazepine-2-thione (14). Compound **14** was prepared by using the procedure described for synthesis of the intermediate **66** in the preparation of **15**. The product was recrystallized from acetone/ethyl acetate (1:1): ^{1}H NMR (Me_2SO-d_6) δ 3.63 (1 H, dd, J_1 = 14 Hz, J_2 = 5 Hz, CH_{2a}), 3.71 (1 H, dd, J_1 = 14 Hz, J_2 = 8 Hz, CH_{2b}), 3.90 (1 H, dd, J_1 = 8 Hz, J_2 = 5 Hz, CH), 6.93–7.70 (13 H, m, aro).

(*R*)-2,3-Dihydro-5-(2-fluorophenyl)-3-(1*H*-indol-3-ylmethyl)-2-oxo-1*H*-1,4-benzodiazepine-1-acetic Acid (29). Ester **28** (83.2 mg, 0.177 mmol) was stirred in methanol (1 mL) with 1 M sodium hydroxide (0.18 mL, 0.18 mmol) for 24 h at room temperature. The solution was acidified with 1 M hydrochloric acid and the mixture evaporated in vacuo. The residue was taken up in methylene chloride, washed with water, dried over sodium sulfate, filtered, and evaporated in vacuo to dryness. The residue was triturated with ether followed by petroleum ether and filtered to give the product, which was dried in vacuo at 80 °C: ^{1}H NMR ($CDCl_3$) δ 2.8 (br s, OH), 3.6 (1 H, dd, J_1 = 14 Hz, J_2 = 5 Hz, CH_{2a}), 3.85–4.03 (2 H, m, CH_{2b}, CH), 4.35 (1 H, br d, J = 16 Hz, $CH_{2a}COOH$), 4.69 (1 H, br d, J = 16 Hz, $CH_{2b}COOH$), 6.95–7.70 (13 H, m, aro), 8.4 (1 H, br s, indole NH).

2,3-Dihydro-5-(2-fluorophenyl)-3-(1*H*-indol-3-ylmethyl)-2-oxo-1*H*-1,4-benzodiazepine-1-acetamide (30). Ester **28** (530 mg, 1.29 mmol) was dissolved in 50 mL of methanol. The solution was stirred in a pressure bottle and saturated with ammonia at 0 °C. The bottle was sealed, and the solution was stirred at room temperature for 48 h. The solution was concentrated in vacuo and the residue purified by flash chromatography on silica using 97:3 chloroform/methanol eluent to give 245 mg of purified amide **30**: ^{1}H NMR ($CDCl_3$) δ 3.62 (2 H, d, J = 8 Hz, CH_2–indole), 4.02 (1 H, dd, $J_1 \sim J_2 \sim$ 8 Hz, CH), 4.36 (1 H, d, J = 17 Hz, $CH_{2a}CONH_2$), 4.77 (1 H, d, J = 17 Hz, $CH_{2b}CONH_2$), 6.9–7.6 (13 H, m, aro).

(*E*)- and (*Z*)-1,3-Dihydro-1-methyl-5-phenyl-3-(3-thienylmethylene)-2*H*-1,4-benzodiazepin-2-one (47 and 48). To a cooled (−60 °C) solution of diisopropylamine (0.84 mL, 6.0 mmol) in THF (10.2 mL) was added 1.5 M butyllithium in hexane (4.0 mL, 6.0 mmol). The solution was stirred for 10 min at −60 °C and then warmed to 25 °C. The light yellow solution was recooled to −60 °C and treated with solid 1,3-dihydro-1-methyl-5-phenyl-2*H*-1,4-benzodiazepin-2-one (75 mg, 3.0 mmol) added portionwise (5 × 15 mg). The reaction mixture was permitted to warm up to 0 °C and then recooled to −60 °C. A solution of thiophene-3-carboxaldehyde (336 mg, 3.0 mmol) in THF (6 mL) was added to the deep red anion solution, the cooling bath was removed, and the reaction mixture was allowed to warm to 25 °C. The reaction was quenched with brine and extracted with ether (3×). The combined extracts were washed with H_2O (1×), dried over $MgSO_4$, filtered, and stripped to dryness in vacuo. The crude red oil was chromatographed on silica gel (10% Et_2O in CH_2Cl_2) to give the intermediate alcohol. This product (171 mg, 0.472 mmol) was heated in a refluxing mixture of trifluoroacetic acid (3 mL) and trifluoroacetic anhydride (1 mL) for 12 h. The solvent was removed in vacuo, and the residue was treated with H_2O, basified with 10% NaOH (aqueous), and extracted with ether (3×). The combined extracts were washed with H_2O (1×), dried over $MgSO_4$, filtered, and stripped to dryness in vacuo to give a crude oil. Chromatography on silica gel (2% Et_2O in CH_2Cl_2) provided the title compounds. *Z* isomer: ^{1}H NMR ($CDCl_3$) δ 3.45 (3 H, s, NCH_3), 6.56 (1 H, s, =CH), 7.10–7.98 (11 H, m, aro). *E* isomer: ^{1}H NMR ($CDCl_3$) δ 3.54 (3 H, s, NCH_3), 6.23 (1 H, s, =CH), 7.07–7.80 (11 H, m, aro).

The *E* and *Z* configurations were assigned tentatively on the basis of the lower field absorption of the olefin proton in the NMR spectrum of the *Z* isomer.

7-Chloro-1,3,4,5-tetrahydro-3(*R*)-(1*H*-indol-3-ylmethyl)-5-phenyl-2*H*-1,4-benzodiazepin-2-one Hydrochloride (62). Benzodiazepine 1 (etherate, 240 mg, 0.51 mmol) was dissolved in acetic acid (10 mL) and cooled to 10 °C. To the yellow solution was added sodium cyanoborohydride (63.6 mg, 1.01 mmol) all at once. After the reaction mixture was stirred for 15 min at 10 °C, it was diluted with H_2O (10 mL), made basic with saturated Na_2CO_3 (aqueous), and extracted with EtOAc (2 × 25 mL). The combined organic extracts were washed with brine, dried over $MgSO_4$, filtered, and evaporated to dryness in vacuo. The residue was chromatographed (silica gel, 900:10:1:1 (v/v/v/v) CH_2Cl_2/MeOH/H_2O/HOAc), and the product fractions were evaporated to dryness in vacuo. The residue was dissolved in absolute ethanol, filtered, and treated with 5.4 M HCl in ethanol until the solution was acidic. The product crystallized as fine white needles, which were dried in vacuo at 82 °C: ^{1}H NMR (CD_3OD) δ 3.2 (1 H, dd, J_1 = 14 Hz, J_2 = 5 Hz, CH_{2a}), 3.63 (1 H, dd, J_1 = 14 Hz, J_2 = 9 Hz, CH_{2b}), 3.95 (1 H, dd, J_1 = 9 Hz, J_2 = 5 Hz, $CHCH_2$), 5.72 (1 H, s, CHN), 6.8–7.7 (13 H, m, aro).

Method E. 3-((2,3-Dihydro-1*H*-indol-3-yl)methyl)-5-(2-fluorophenyl)-1,3-dihydro-2*H*-1,4-benzodiazepin-2-one (49). Compound 8 (120 mg, 0.31 mmol) was dissolved in 2 mL of trifluoroacetic acid. The resulting orange solution was treated with 0.5 mL (3.1 mmol) of triethylsilane and stirred rapidly at room temperature. After 2 h, the reaction mixture was evaporated to dryness and the residue was partitioned between water and ethyl acetate. The organic phase was washed with sodium bicarbonate solution (saturated) and brine and then dried ($MgSO_4$) and concentrated. The analytical sample was obtained via preparative thick-layer chromatography on silica gel (1:1 hexane/ethyl acetate, v/v, multiple elutions): ^{1}H NMR ($CDCl_3$) δ 2.35 (1 H, ddd, J_1 = 14 Hz, J_2 = 10 Hz, J_3 = 5 Hz), 2.98 (1 H, ddd, J_1 = 14 Hz, J_2 = 9 Hz, J_3 = 4 Hz), 3.20 (1 H, t, J = 9 Hz), 3.61 (1 H, t, J = 9 Hz), 3.70 (1 H, dd, J_1 = 9 Hz, J_2 = 5 Hz), 3.82 (1 H, br ddd, J_1 = 19 Hz, J_2 = 8 Hz, J_3 = 4 Hz), 6.66 (1 H, d, J = 9 Hz), 6.74 (1 H, t, J = 15 Hz), 7.0–7.6 (m, aro), 8.0 (1 H, s).

Method F. 3(*R*)-((1-(*N*-((1,1-Dimethylethoxy)carbonyl)-L-leucyl)-2,3-dihydro-1*H*-indol-3α-yl)methyl)-5-(2-fluorophenyl)-1,3-dihydro-2*H*-1,4-benzodiazepin-2-one (56). Compound 49 (100 mg, 0.259 mmol), *N*-Boc-L-leucine monohydrate (64.7 mg, 0.259 mmol), 1-ethyl-3-(3-(dimethylamino)propyl)carbodiimide hydrochloride (EDC, 49.8 mg, 0.259 mmol), and 1-hydroxybenzotriazole hydrate (HBT, 35.0 mg, 0.259 mmol) were combined in freshly degassed dimethylformamide (DMF, 2 mL) and stirred at room temperature. The pH of the solution was adjusted to 9.0–9.5 with triethylamine (0.108 mL, 0.777 mmol), and stirring was continued for 24 h. The mixture was evaporated in vacuo, treated with 10% Na_2CO_3 (aqueous) (20 mL), and extracted with EtOAc (2 × 30 mL). The combined extracts were washed with H_2O (20 mL) and brine (20 mL), dried over $MgSO_4$, filtered, and evaporated to dryness in vacuo. The residue was chromatographed (silica gel, 30% (v/v) EtOAc in hexane) to give **56**: ^{1}H NMR ($CDCl_3$) δ 0.85 (3 H, d, J = 6 Hz), 0.90 (3 H, d, J = 6 Hz), 1.4 (9 H, s), 1.2–1.8 (3 H, m), 2.15–2.27 (br), 3.04 (br t, J_t = 12 Hz), 3.79 (dd, J_1 = 9 Hz, J_2 = 4 Hz), 3.90–3.98 (br), 4.07–4.19 (br), 4.45 (br, dt, J_1 = 9 Hz, J_2 = 4 Hz), 5.30 (d, J = 9 Hz), 7.05–7.60 (m), 8.20 (d, J = 8 Hz), 8.25 (s).

(*R*,*R*)-7-Chloro-3-((2,3-dihydro-2-oxo-1*H*-indol-3-yl)methyl)-1,3-dihydro-5-phenyl-2*H*-1,4-benzodiazepin-2-one (61). The procedure of Savige and Fontana was employed.[35] Compound 1 (0.2 g, 0.5 mmol) was dissolved in Me_2SO (0.4 g, 5.1 mmol). To the stirred solution was added dropwise 12 N HCl (0.8 mL, 9.6 mmol) and glacial acetic acid (3 mL). The mixture was heated at 60 °C for 30 min and quenched in ice water (20 mL). The mixture was neutralized with saturated $NaHCO_3$ and extracted with 1-butanol (3 × 10 mL). The butanol layer was washed with water, dried over Na_2SO_4, filtered, and evaporated to dryness in vacuo. The residue was chromatographed on preparative silica gel plates eluted with 95:5 (v/v) chloroform/methanol. The title compound was isolated as a mixture of two diastereomers: ^{1}H NMR ($CDCl_3$) δ 2.41 (ddd, J_1 = 14 Hz, J_2 = 11 Hz, J_3 = 4 Hz), 2.79 (dt, J_d = 14 Hz, J_t = 8 Hz), 2.91 (dt, J_d = 15 Hz, J_t = 7 Hz), 3.13 (ddd, J_1 = 15 Hz, J_2 = 11 Hz, J_3 = 4 Hz), 3.94 (t, J = 7.5 Hz), 4.01 (dd, J_1 = 11 Hz, J_2 = 4 Hz), 4.26 (t, J = 7.5 Hz), 4.43 (dd, J_1 = 11 Hz, J_2 = 4 Hz), 6.8–7.6 (m), 7.26 (s), 7.83 (s), 8.57 (s), 8.61 (s).

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Supplementary Material Available: Crystallographic data including tables of the atomic positional and thermal parameters, bond distances, and bond angles for 1 (4 pages). Ordering information is given on any current masthead page.

Notes

Chemical and Enzymatic Oxidative Coupling of 5-Hydroxy-*N*,*N*-dimethyltryptamine with Amines

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As part of a program aiming to obtain a covalent labeling of serotoninergic receptors we have studied the oxidative coupling of serotonin derivatives with amino compounds. The oxidation of bufotenine (2) by MnO_2 and human ceruloplasmin followed by the Michael type addition with dansylcadaverine and dansyllysine gave a fluorescent adduct identified as fused oxazole structure 4.

Serotonin (5-hydroxytryptamine) (1) is a neurotransmitter acting in the central and peripheral nervous system. The receptors involved in the function of the serotoninergic system are still not fully known. In particular, the isolation of these sites has not yet been accomplished, since adequate methods are lacking. Many studies have

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