Effects on Spontaneous Locomotion in Reserpine-Treated Rats.[22] Drugs were administered sc to rats treated with 5 mg/kg of reserpine 24 h prior to testing. The effect on locomotor activity was measured immediately after as described above.

Registry No. **5**, 63815-39-4; **6**, 20811-60-3; **7**, 5802-17-5; **8**, 66125-08-4; **9**, 67064-55-5; **10**, 123594-58-1; **11**, 123594-59-2; **12a**, 123594-60-5; **12a**·HCl, 123621-20-5; **12b**, 123671-94-3; **12b**·(*S*)-PhCH(OH)CO_2H, 123671-95-4; **12b**·HCl, 123671-96-5; **12c**, 123805-43-6; **12c**·(*R*)-PhCH(OH)CO_2H, 123877-16-7; **12c**·HCl, 123877-17-8; **13a**, 123594-61-6; **14a**, 123594-62-7; **14b**, 123671-97-6; **14c**, 123671-98-7; **15a**, 123594-63-8; **15a**·HCl, 123594-65-0; **15b**, 123748-66-3; **15b**·HCl, 123671-99-8; **15c**, 123748-67-4; **15c**·HCl, 123672-00-4; **16a**, 123594-64-9; **16b**, 123671-92-1; **16c**, 123671-93-2; H_2C=CHCN, 107-13-1; *p*-$MeOC_6H_4OH$, 150-76-5.

Supplementary Material Available: Tables listing fractional atomic coordinates and temperature factor parameters, bond lengths and angles, hydrogen bonding parameters, and general displacement parameter expressions (13 pages); a table of observed and calculated structure amplitudes (12 pages). Ordering information is given on any current masthead page.

Cholecystokinin-A Receptor Ligands Based on the κ-Opioid Agonist Tifluadom

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Tifluadom, a κ-opioid agonist and cholecystokinin-A (CCK-A) receptor antagonist, was utilized as a model to prepare a series of 2-(aminomethyl)- and 3-(aminomethyl)-1,4-benzodiazepines. These compounds were tested in vitro as inhibitors of the binding of [^{125}I]CCK to rat pancreas and guinea pig brain receptors. All compounds with IC_{50}'s less than 100 μM proved to have greater affinity for the CCK-A receptor, with the most potent analogue, **6e**, having an IC_{50} of 0.16 μM. The benzodiazepines described in this study are simultaneously CCK-A and opioid receptor ligands. The ramification of this dichotomy on current concepts of peptide hormone action are discussed. These results further demonstrate the versatility of the benzodiazepine core structure for designing nonpeptide ligands for peptide receptors and the ability to fine-tune the receptor interactions of these benzodiazepines by appropriate structure modifications.

The elucidation of the physiologic role of the gastrointestinal hormone cholecystokinin (CCK) has been pursued in recent years with increased vigor. This mounting interest derives, in part, from the present availability of several nonpeptidal antagonists, some of which bind to the CCK receptor as avidly as the natural ligand CCK-8.[1-5] Among these, the prototypical agent is the highly selective and orally effective CCK-A antagonist (3*S*)-(−)-*N*-(2,3-dihydro-1-methyl-2-oxo-5-phenyl-1*H*-1,4-benzodiazepin-3-yl)-1*H*-indole-2-carboxamide, **1** (MK-329, formerly L-364,718)[6] (Chart I).

The reasoning which formed the basis of the design process which led to the potent CCK-A antagonist MK-329 (**1**) was predicated on the benzodiazepine ring in the natural product CCK antagonist asperlicin and its role as a structural mimic for fragments of the CCK peptide chain.[1] In the incipient phase of the development of **1**, it was recognized that the benzodiazepine core structure was the key feature which linked asperlicin with 1,4-benzodiazepine progenitors of **1**. Prudence thus dictated that analogous ring systems be examined for CCK receptor binding affinity.[3,7,8] Indeed, after completion of these studies, several reports appeared which demonstrated that a number of anxiolytic 1,4-benzodiazepines (e.g. diazepam, lorazepam, chlordiazepoxide) antagonize the effects of CCK both in the periphery[9-11] and in the central nervous system.[12,13] However, the observed effects are weak. The benzodiazepine κ-opioid agonist tifluadom (**2**, Chart I) was assayed in these laboratories and determined to be a moderately potent, CCK-A-selective receptor antagonist.[14] This finding prompted the preparation of analogues of tifluadom in an attempt to gain insight into the structural prerequisites necessary for CCK receptor binding affinity and selectivity. Herein we detail this study, which includes the synthesis and pharmacological evaluation of the series of (2-aminoethyl)- and 3-(aminomethyl)-1,4-benzodiazepines, the physiochemical properties of which are summarized in Tables I and II, derived from the tifluadom core structure.

† Department of Microbial Pharmacometrics.

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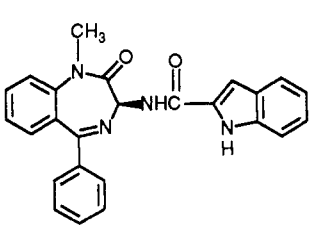
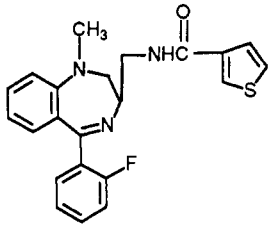
0022-2623/90/1833-0450$02.50/0

Chart I



1, MK-329



2, (*R,S*)-tifluadom
2a, (*R*)-(+)-tifluadom
2b, (S)-(−)-tifluadom

Chemistry

The 2-(aminomethyl)-1,4-benzodiazepines in this study, which are based on the κ-opioid agonist tifluadom, were synthesized according to published methods and as outlined in Scheme I. In this way, 3-chloro-1,5-benzodiazocene **3**, available in five steps from potassium phthalimide,[15,16] was converted to the *N*-acyl- and *N*-alkyl-1,4-benzodiazepines **4**, **6b–h**, and **6j–s**. 2,3,4,5-Tetrahydro-1,4-benzodiazepine derivative **6a** was obtained by reducing the N4–C5 imine bond in **2b** with sodium cyanoborohydride in the standard manner. L-Tryptophan analogue **6i** was in turn derived from **6h** by removal of the N-Boc protecting group in the usual fashion with hydrogen chloride gas.

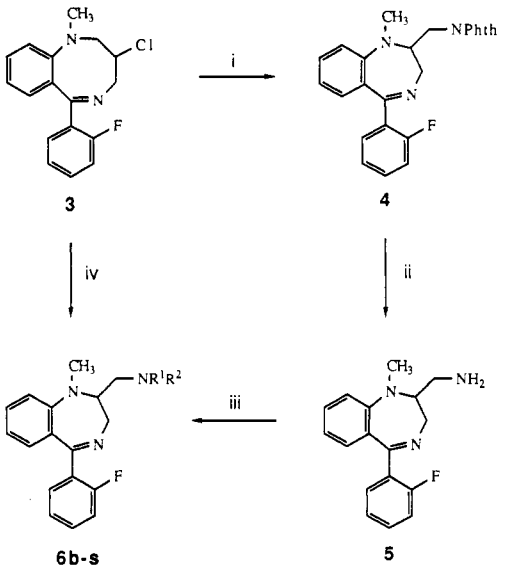
3-(Aminomethyl)-1,4-benzodiazepines **9a–d** were prepared as shown in Scheme II. Thus, 2,3-diphthalimidopropionamide **7**, obtained by acylating 2-aminobenzophenone with 2,3-diphthalimidopropionyl chloride, was reacted with 95% hydrazine in methanol to effect sequential deblockade and cyclization to give **8**. As anticipated, only the 7-membered ring product, resulting from the condensation of the intermediate 2-amino group with the ketone carbonyl, was observed; no trace of the entropically disfavored 8-membered cycle could be detected. Compound **8** was then elaborated to afford analogues **9a–d**, according to method A as detailed in the Experimental Section. Compound **8**, although stable at room temperature for an indefinite period, could be induced to eliminate ammonia. Indeed, simply refluxing a solution of **8** in 2-propanol containing triethylamine resulted in the formation of **9e**, presumably via Michael addition of unreacted **8** with the intermediate **8a**.

Reduction of the 2-amide carbonyl function in **9a** was carried out in two steps to give compound **11**, as depicted in Scheme III. Accordingly, **9a** was reacted with the Lawesson reagent[17] in toluene to yield thionamide **10**. Raney nickel reduction of **10** in ethanol then cleanly afforded **11**. The latter compound was then further elaborated at the 3-position by initial removal of the *N*-carbobenzyloxy protecting group with hydrogen bromide gas to give **12**, followed by acylation of the resulting amine salt, according to method A or B, to afford the 1,4-benzodiazepines **13a–d**.

Biology

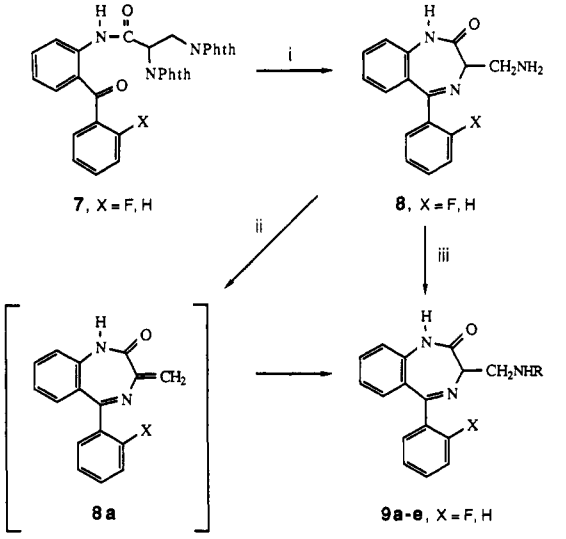
The methods employed for the determination of [^{125}I]-CCK-33 or [^{125}I]CCK-8 binding to rat pancreas and guinea pig cerebral cortex,[6] as well as [^{3}H]dihydromorphine[18] binding in rat brain and [^{3}H]naloxone[19] binding in guinea pig brain tissues, respectively, were previously described. Vaules shown are the means of triplicate determinations.

Scheme I[a]



[a] (i) Potassium phthalimide, NaI, DMF; (ii) NH_2NH_2 (95%), EtOH, 78 °C; (iii) method A or B; (iv) method C.

Scheme II[a]



[a] (i) NH_2NH_2 (95%), MeOH, 23 °C, 12 h; (ii) 2-PrOH, NEt_3, 83 °C; (iii) method A.

Discussion

The 2-(aminomethyl)-1,4-benzodiazepines which were prepared for this study (cf. Table I) were tested as inhibitors of the binding of [^{125}I]CCK to rat pancreas and guinea pig brain receptors. These data are collated in Table III, where they are compared with those of the CCK-A antagonist MK-329 (**1**) and with the opioid κ-receptor agonist tifluadom (**2**).

Since tifluadom was originally developed as a selective opioid κ-agonist,[20] we reasoned that its CCK-A receptor binding affinity may not have been optimized. Reduction

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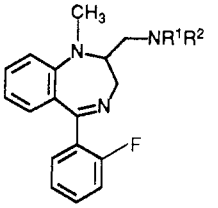
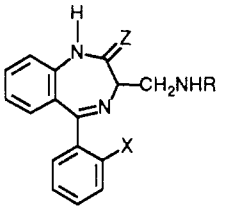
Table I. Physicochemical Data of 2-(Aminomethyl)-1,4-benzodiazepines



compd	R^1	R^2	scheme (method)[a]	% yield	mp, °C	formula[b]	anal.[c]
4		phthaloyl	1 (C)	12[d]	135–138	$C_{25}H_{20}FN_3O_2$	C, H, N
6a	H	3-thienylcarbonyl[e]	1	18	172–174	$C_{22}H_{22}FN_3OS\cdot0.2H_2O$	C, H, N
6b	H	4-thianaphthenylmethylcarbonyl	1 (B)	75	79–81	$C_{27}H_{24}FN_3OS\cdot0.2H_2O$	C, H, N
6c	H	nicotinoyl	1 (B)	53	84–87	$C_{23}H_{21}FN_4O\cdot0.4H_2O$	C, H, N
6d	H	3-indolylmethylcarbonyl	1 (B)	26	97–100	$C_{27}H_{25}FN_4O\cdot H_2O$	C, H, N
6e	H	2-indolylcarbonyl	1 (B)	80	160–161	$C_{26}H_{23}FN_4O\cdot0.7H_2O$	C, H, N
6f	H	2-fluorobenzoyl	1 (A)	93	54–57	$C_{24}H_{21}F_2N_3O\cdot0.5H_2O$	C, H, N
6g	H	4-chlorobenzoyl	1 (A)	94	83–86	$C_{24}H_{21}ClFN_3O\cdot0.66H_2O$	C, H, N
6h	H	N^{α}-Boc-L-Trp	1 (B)	54	124	$C_{33}H_{36}FN_5O_3\cdot0.3H_2O$	C, H, N
6i	H	L-Trp	1	99	219	$C_{28}H_{27}FN_5O\cdot1.5H_2O\cdot2HCl$	C, H, N[f]
6j	H	(*S*)-(+)-mandeloyl	1 (B)	73	88–90	$C_{25}H_{24}FN_3O_2\cdot0.2H_2O$	C, H, N
6k	H	(*S*)-(−)-α-methoxy-α-(trifluoromethyl)-phenylacetyl	1 (B)	91	64–67	$C_{27}H_{25}F_4N_3O_2\cdot0.75H_2O$	C, H, N
6l	H	benzylsuccinoyl	1 (B)	86	gum	$C_{28}H_{28}FN_3O_3\cdot0.3H_2O$	C, H, N
6m	H	(acetamidomethyl)thioglycolyl	1 (B)	88	71–73	$C_{22}H_{25}FN_4O_2S\cdot H_2O$	C, H, N
6n	H	N^{α}-Boc-L-Cys(Acm)	1 (B)	83	100–103	$C_{28}H_{36}FN_5O_4S\cdot0.5H_2O$	C, H, N
6o	H	isobutyloxycarbonyl	1 (A)	66	gum	$C_{22}H_{26}FN_3O_2\cdot0.6H_2O$	C, H, N
6p	H	methyl-3-phenylpropanoate	1 (C)	54	gum	$C_{27}H_{28}FN_3O_2\cdot0.6H_2O$	C, H, N
6q	H	3-(trifluoromethyl)phenyl	1 (C)	43	56–58	$C_{24}H_{21}F_4N_3\cdot0.1H_2O$	C, H, N
6r		(diethylamino)methyl	1 (C)	92	gum	$C_{22}H_{29}FN_4\cdot0.3H_2O$	C, H, N[g]
6s		diethylamino-3-(trifluoromethyl)phenyl	1 (C)	52	58–61	$C_{28}H_{30}F_4N_4\cdot0.2H_2O$	C, H, N

[a] Refers to scheme in which general synthesis of compound is outlined; method is detailed in the Experimental Section. [b] All compounds were fully characterized spectroscopically (^1H NMR, MS); ^1H NMR confirmed the presence of a solvate where indicated. [c] Combustion analyses were within ±0.4% of the theoretical value; compound purity was further verified by HPLC, which in all cases was >97%. [d] Overall yield from potassium phthalimide, six steps. [e] N4–C5 imine bond of **2** reduced; major diastereomer; absolute configuration not determined. [f] N: calcd, 12.30; found, 11.67. [g] N: calcd, 15.06; found, 14.52.

Table II. Physicochemical Data of 3-(Aminomethyl)-1,4-benzodiazepines



compd	X	Z	R	scheme (method)[a]	% yield	mp, °C	formula[b]	anal.[c]
8	F	O	H	2	90	168–169	$C_{16}H_{14}FN_3O\cdot0.2H_2O$	C, H, N
9a	F	O	benzyloxycarbonyl	2	97	90–92	$C_{24}H_{20}FN_3O_3\cdot0.25H_2O$	C, H. N
9b	F	O	3-thienylcarbonyl	2 (A)	67	237–240	$C_{21}H_{16}FN_3O_2S\cdot HCl$	C, H, N
9c	F	O	2-indolylcarbonyl	2 (A)	35	315–317	$C_{25}H_{19}FN_4O_2\cdot1.25H_2O$	C, H, N
9d	F	O	5-chloropyrazine	2 (A)	71	148–152	$C_{20}H_{15}ClFN_5O\cdot0.25H_2O$	C, H, N[d]
9e	F	O	1,3-dihydro-5-(2-fluorophenyl)-2*H*-1,4-benzodiazepin-2-one-3-ylmethyl	2	36[e]	155–158	$C_{32}H_{25}F_2N_5O_2\cdot0.35CHCl_3$	C, H, N
11	F	H	benzyloxycarbonyl	3	50	114–115	$C_{24}H_{22}FN_3O_2\cdot0.03CHCl_3$	C, H, N
13a	F	H	mandeloyl[f]	3 (B)	42	183 dec	$C_{24}H_{22}FN_3O_2\cdot0.5H_2O$	C, H, N[h]
13b	F	H	mandeloyl[g]	3 (B)	42	91–93	$C_{24}H_{22}FN_3O_2$	C, H, N
13c	F	H	3-thienylcarbonyl	3 (A)	49	105 dec	$C_{21}H_{18}FN_3OS\cdot0.1CHCl_3$	C, H, N
13d	F	H	2-indolycarbonyl	3 (A)	90	210 dec	$C_{25}H_{21}FN_4O\cdot0.15CHCl_3$	C, H, N

[a] Refers to scheme in which synthesis of compound is outlined; method is detailed in the Experimental Section. [b] All compounds were fully characterized spectroscopically (^1H NMR, MS); ^1H NMR confirmed the presence of solvate where indicated. [c] Combustion analyses were within ±0.4% of the theoretical value; compound purity was further verified by HPLC, which in all cases was >97%. [d] N: Calcd, 17.49; Found, 16.59. [e] 60% conversion. [f] More polar diastereomer; absolute configuration not determined. [g] Less polar diastereomer; absolute configuration not determined. [h] Satisfactory combustion analysis could not be obtained.

of the N4–C5 imine bond in **2** afforded **6a** and a concomitant 2 order of magnitude loss in CCK receptor binding affinity. This result supported the inviolate nature of the 1,4-benzodiazepine ring system in **2**. Accordingly, we focused our subsequent modifications on the 2-[(3-thienylcarbonyl)amino]methyl group in **2**. A number of heterocyclic and aromatic nuclei were substituted for the thienyl moiety in **2** with the result that no increase in CCK re-

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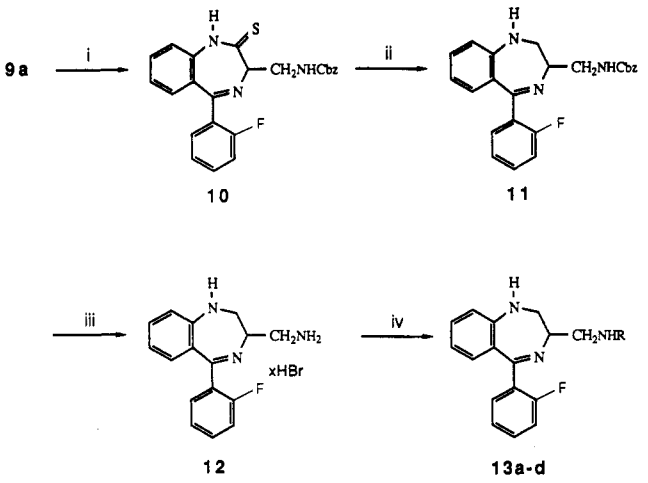
Scheme III[a]



[a] (i) Lawesson's reagent, toluene, 110 °C, 1.5 h; (ii) Raney Ni, EtOH, 23 °C, 50 h; (iii) HBr(g), CH_2Cl_2, 0–23 °C, 12 h; (iv) method A or method B.

ceptor binding affinity was realized. Nevertheless, among the analogues derived from tifluadom, the most potent compound is **6e**, in which 2-indolyl replaced the 3-thienyl group in tifluadom. This observation follows from the results obtained in other amino-1,4-benzodiazepine series in which the 2-indolyl group has been found to augment the CCK-A receptor binding affinity of the respective receptor ligand.[1,3,4] Replacement of the thienyl ring in **2** with nonaromatic groups (e.g. **6m–o**) offered no improvement in receptor binding affinity. Similarly, removal of the 2-(aminomethyl)amide carbonyl group as in **6p–s** yielded receptor ligands which did not exceed the 1 μM level in binding affinity.

An examination of the data in Table III reveals that, with the exception of the low binding affinity compounds **6m**, **6r**, and **6s**, the remaining compounds which were tested exhibited selectively for the CCK-A receptor. It should be noted that all compounds in this series are racemic and therefore no conclusions can be drawn, as with **2a** and **2b**, regarding the relative contributions of the corresponding enantiomers toward inhibiting [^{125}I]CCK pancreatic binding.

A representative sampling of the compounds listed in Table III were also tested for their ability to displace [^{3}H]dihydromorphine and/or [^{3}H]naloxone from opioid receptors in rat or guinea pig brain tissues. In line with the reported opioid affinity of **2**,[20,21] the compounds in Table III were also found to bind to opioid receptors. Interestingly, the most potent CCK receptor ligands, **6e** and **6j**, were also found to possess the highest opioid receptor affinity.

Within the context of our study, the potent opioid affinity displayed by the compounds in Table III was deemed a liability. Since the level of opioid affinity displayed by MK-329 is low (Table III), the 2-aminomethyl side chain in **2** was transposed to the 3-position on the 1,4-benzodiazepine ring to afford structural hybrids of **1** and **2** which might be more CCK selective. However, compounds **9b** and **9c** (Table IV), the best representatives of this strategy, were found to possess only marginal affinity for the CCK-A receptor. Both compounds showed greater affinity for opioid receptors in guinea pig brain tissues than for either the CCK-A or the CCK-B receptors.

(21) Kley, H.; Scheidemantel, K.; Bering, B.; Muller, W. E. *Eur. J. Pharmacol.* **1983**, *87*, 503.

Table III. Effect of 2-(Aminomethyl)-1,4-benzodiazepines on [^{125}I]CCK Receptor Binding in Pancreatic and Brain Membranes

	[^{125}I]CCK[a,b]		
compd	pancreas	brain	[^{3}H]DHM[b,c]
1	0.0008	0.245	10[g]
2[d]	0.047	>100	
2a[e]	0.029	>100	0.0011[g]
2b[f]	0.26		0.050
4	4.3	>100	
6a	4.8	>100	0.030
6b	0.45	23	0.003[g]
6c	10	100	0.450
6d	1.0		0.022
6e	0.16	15.5	0.0003 (0.0011)[g]
6f	2.9	>100	
6g	0.8	37.4	0.0005
6h	4.1	38.6	
6i	11.6	>100	0.040
6j	0.6	>100	0.007
6k	13	>100	
6l	45	55	
6m	100	100	0.120
6n	24	>100	
6o	5.8	>100	
6p	1.6	>100	0.018
6q	2.2	100	
6r	>100	>100	
6s	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]CCK-8 to CCK receptors in rat pancreatic or guinea pig brain tissues. [b] IC_{50} values were reproducible within ±20%. [c] IC_{50} (μM) for half-maximal displacement of [^{3}H]dihydromorphine from opioid receptors in rat brain tissues. [d] (±)-Tifluadom. [e] (−)-Tifluadom. [f] (+)-Tifluadom. [g] IC_{50} (μM) for half-maximal displacement of [^{3}H]naloxone from opioid receptors in guinea pig brain tissues.

Table IV. Effect of 3-(Aminomethyl)-1,4-benzodiazepines on [^{125}I]CCK Receptor Binding in Pancreatic and Brain Membranes

	[^{125}I]CCK[a,b]		
compd	pancreas	brain	[^{3}H]naloxone[b,c]
8	>100	>100	
9a	22	100	12
9b	22	100	7.5
9c	7	30	0.54
9d	15	100	
9e	14	>100	
11	28	100	0.091
13a	>100	>100	
13b	8	>100	
13c	10	74	0.093
13d	4	34	0.0086

[a] Receptor binding affinity is expressed as IC_{50}, the concentration (μM) of compound required for half-maximal inhibition of binding of [^{125}I]CCK-8 to CCK receptors in rat pancreatic or guinea pig brain tissues. [b] IC_{50} values were reproducible within ±20%. [c] IC_{50} (μM) for half-maximal displacement of [^{3}H]naloxone from opioid receptors in guinea pig brain tissues.

Reduction of the 2-amide carbonyl in **9b** and **9c** gave **13c** and **13d**, respectively. While this ploy did not result in enhanced CCK receptor binding affinity, it did increase the opioid potency of these compounds. Not surprisingly, **13d**, closest in structural similarity to **6e**, also showed the greatest affinity for the opioid receptors in guinea pig brain tissues.

Despite exhibiting only micromolar affinity for the CCK-A and CCK-B receptors, compounds **13a** and **13b** did so in a stereoselective manner. Compound **13b** inhibited [^{125}I]CCK binding in rat pancreatic membranes at a significantly lower concentration than its corresponding diastereomer **13a**. However, the effect observed here was

substantially diminished relative to MK-329 and tifluadom.

The compounds described in this paper illustrate the versatility of the benzodiazepine core structure as a base for designing nonpeptide ligands for peptide receptors and the ability to modulate the receptor interaction of these benzodiazepines by modifying their structure. In addition, their interaction with two different receptors invite consideration of proposed mechanisms by which signal transduction fails subsequent to the binding of a ligand to a receptor. In one concept, a conformational change of the ligand–receptor complex is invoked as being necessary for agonist effect after the initial binding process. Walter has proposed such a sequence of events in his "cooperative model" of oxytocin action.[22] Hruby has expressed a similar view of oxytocin and has suggested that this scenario is a general feature of peptide hormone action.[23,24] The benzodiazepines of the tifluadom class described in this work offer a contrast in action as they are at once CCK antagonists and opioid agonists.[19] Thus, the influence of molecular mobility in producing ligands may be different for the various peptide hormones and may even differ for receptor subtypes for the same peptide. Indeed, a linear heptapeptide CCK analogue[25] which has considerable conformational flexibility is reported to be a CCK-A receptor agonist while showing antagonist properties at the CCK-B receptor. In contrast, less flexible cyclic CCK analogues are CCK-B agonists.[26] The role of conformation on peptide receptor ligand bioactivity will doubtlessly continue to be an important area for further basic studies.

Whatever the mechanism, the observation of CCK and opioid receptor binding affinity within a single molecular entity offers interesting potential in view of the observations linking the two receptor groups. CCK is analgesic at certain doses while at other doses it attenuates opioid analgesia.[27] Accordingly, CCK antagonism may be expected to synergize with opioid analgesia. An improved analgesic might contain an ideal balance between these two properties.

Summary and Conclusions

We have previously demonstrated that the κ-opioid agonist tifluadom displays affinity for the CCK-A receptors.[14] The results of the present study show that analogues of tifluadom can be prepared which rival its CCK-A/CCK-B selectivity and CCK-A receptor binding affinity. However, these compounds display an even greater affinity for opioid receptors, a liability which, similar to tifluadom, limits their usefulness as selective CCK-A receptor ligands. Nevertheless, in view of reports that CCK antagonists potentiate opioid analgesia,[28,29] compounds like **6e** and **6j** may find application in this connection. Finally, our results underscore the versatility of the benzodiazepine core structure for designing nonpeptide ligands for peptide receptors and the ability to fine-tune the receptor interactions of these benzodiazepines by appropriate structure modifications. This suggests that the application of benzodiazepines as peptide receptor ligands will continue to expand.

Experimental Section

Melting points were determined in open capillaries on an Electrothermal melting point apparatus and are uncorrected. Proton nuclear magnetic resonance spectra (^{1}H NMR) were recorded on a Varian XL300 (300 MHz, FT mode) spectrometer and on a Nicolet NT-360 (360 MHz, FT mode) spectrometer, both instruments with an internal lock on the deuterium resonance of the solvent. Electron impact (EI) mass spectra were determined on a VG 7035 spectrometer and fast atom bombardment (FAB) mass spectra were run on a Finnigan-MAT 731 instrument.

Flash chromatography was performed using silica gel (E. Merck 40–63 μm). Thin-layer chromatography (TLC) and preparative thick-layer chromatography (PTLC) were carried out on E. Merck 60F-254 precoated silica gel plates (0.25, 0.5, and 2 mm thickness) with UV light, iodine vapors, or 5% phosphomolybdic acid reagent in 95% ethanol to visualize the chromatograms.

All reactions, except those performed in aqueous solvents, were carried out with use of standard techniques for the exclusion of moisture. Commercial chemicals were used as obtained without further purification, except for solvents, which were purified and dried, where appropriate, before use by standard methods.

Preparation of 1-Methyl-2-[(2-fluorobenzoyl)amino]-methyl]-5-(2′-fluorophenyl)-2,3-dihydro-1*H*-1,4-benzodiazepine (6f). Method A. A magnetically stirred solution of 1-methyl-2-(aminomethyl)-5-(2′-fluorophenyl)-2,3-dihydro-1*H*-1,4-benzodiazepine (**5**, 500 mg, 1.76 mmol) in 12 mL of tetrahydrofuran at 0 °C was treated in succession with triethylamine (245 μL, 1.76 mmol) and 2-fluorobenzoyl chloride (210 μL, 1.76 mmol) in 3 mL of tetrahydrofuran. The reaction mixture was warmed to 23 °C on overnight stirring and diluted with ethyl acetate (300 mL). The organic phase was washed with saturated sodium bicarbonate solution (2 × 50 mL) and brine. The dried ($MgSO_4$) organic extracts were concentrated under reduced pressure to give 700 mg of the crude product as an oil. PTLC (ethyl acetate–hexane, 7:3 v/v) afforded the analytical product (R_f 0.33).

Preparation of 1-Methyl-2-[[[[(4-thianaphthenyl)-methyl]carbonyl]amino]methyl]-5-(2′-fluorophenyl)-2,3-dihydro-1*H*-1,4-benzodiazepine (6b). Method B. To a solution of 1-methyl-2-aminomethyl)-5-(2′-fluorophenyl)-2,3-dihydro-1*H*-1,4-benzodiazepine, (**5**, 250 mg, 0.88 mmol) in 4 mL of methylene chloride were added thianaphthene-4-acetic acid (170 mg, 0.88 mmol) and 1-ethyl-3-[3-(dimethylamino)propyl]carbodiimide hydrochloride (169 mg, 0.88 mmol). The pH of the reaction mixture was adjusted to 8 with triethylamine and stirring was continued for 10 h. The reaction mixture was diluted with ethyl acetate (150 mL) and the organic phase was washed with saturated sodium bicarbonate solution (2 × 50 mL) and brine. The dried ($MgSO_4$) organic extracts were concentrated in vacuo to afford 300 mg of the product as an oil. PTLC (ethyl acetate–hexane, 4:1 v/v) yielded the analytical sample as a yellow solid (R_f 0.25).

Preparation of 1-Methyl-2-[[[[2(*S*)-(methoxycarbonyl)-3-phenyl]propanoyl]amino]methyl]-5-(2′-fluorophenyl)-2,3-dihydro-1*H*-1,4-benzodiazepine (6p). Method C. 3-Chloro-1-methyl-6-(2′-fluorophenyl)-1,2,3,4-tetrahydro-1,5-benzodiazocine (**3**, 150 mg, 0.5 mmol), phenylalanine methyl ester hydrochloride (324 mg, 1.5 mmol), sodium iodide (210 mg, 1.5 mmol), and potassium carbonate (414 mg, 3 mmol) were combined in 4 mL of *N*,*N*-dimethylformamide. The resulting reaction mixture was heated at 60 °C for 72 h. The solvent was removed under reduced pressure and the residual oil was partitioned between ethyl acetate and saturated sodium bicarbonate solution. The organic phase was washed with saturated sodium bicarbonate solution (2 × 50 mL) and brine and then dried ($MgSO_4$) and concentrated. Chromatography (ethyl acetate–hexane, 7:3 v/v) afforded 120 mg of the analytical sample (R_f 0.22).

***N*-[[5-(2′-Fluorophenyl)-2,3,4,5-tetrahydro-1-methyl-1*H*-1,4-benzodiazepin-2-yl]methyl]-3-thiophenecarboxamide (6a).**

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(–)-Tifluadom (**2a**, 440 mg, 1.12 mmol) was dissolved in 10 mL of glacial acetic acid and cooled to 10 °C. To this solution was added 282 mg (4.48 mmol) of sodium cyanoborohydride and the reaction mixture was stirred vigorously for 5 min. The reaction was quenched by adding this mixture to water (200 mL) and extracting the resulting suspension with ethyl acetate (3 × 75 mL). The combined organic extracts were washed with 10% sodium bicarbonate solution and brine and then dried (Na_2SO_4) and concentrated to give an approximately 3:1 mixture of diastereomers. The major diastereomer was isolated by PTLC (chloroform–methanol–concentrated ammonium hydroxide, 95:5:0.5 v/v) as a solid (R_f 0.23).

1-Methyl-2-[[[2(*S*)-amino-3-(1*H*-indol-3-yl)propanoyl]amino]methyl]-5-(2′-fluorophenyl)-2,3-dihydro-1*H*-1,4-benzodiazepine Dihydrochloride (6i). Into an ice-cold solution of ethyl acetate (2 mL) containing **6h** (50 mg, 0.087 mmol) was passed a continuous stream of hydrogen chloride gas for 30 min. Solvent and excess reagent were then removed under reduced pressure to afford the title compound.

1,3-Dihydro-5-(2′-fluorophenyl)-3(*R*,*S*)-(aminomethyl)-2*H*-1,4-benzodiazepin-2-one (8). Diphthalimide **7** (1.35 g, 2.40 mmol) was suspended in 15 mL of methanol and treated with 95% hydrazine (1.2 mL) at 23 °C. After 14 h the reaction mixture was diluted with methanol (40 mL) and filtered. The filtrate was concentrated and the residue was partitioned between methylene chloride and water. The aqueous phase was extracted with methylene chloride, and the combined organic extracts were washed with brine, dried ($MgSO_4$), and concentrated to give an oil, which crystallized on standing. Trituration with ethyl ether afforded the analytical sample as an off-white solid (R_f 0.22, chloroform–methanol–concentrated ammonium hydroxide, 90:10:1 v/v).

1,3-Dihydro-5-(2′-fluorophenyl)-3(*R*,*S*)-[[(benzyloxy)carbonyl]methyl]-2*H*-1,4-benzodiazepin-2-one (9a). A solution of methylene chloride (50 mL) containing **8** (260 mg, 0.91 mmol) and 4-(dimethylamino)pyridine (224 mg, 1.83 mmol) was treated with benzyl chloroformate (0.51 mL, 3.57 mmol) at 0 °C. The reaction mixture was warmed to 23 °C on overnight standing and was then diluted with 200 mL of methylene chloride. The resulting solution was washed with saturated sodium bicarbonate solution (2 × 50 mL) and brine and then dried ($MgSO_4$) and rotoevaporated to give an oil. Purification by PTLC (chloroform–methanol–concentrated ammonium hydroxide, 95:5:0.5 v/v) afforded the analytical sample as a white solid (R_f 0.36).

3(*R*,*S*),3′(*R*,*S*)-[Iminobis(methylene)]bis[5-(2-fluorophenyl)-1,3-dihydro-2*H*-1,4-benzodiazepin-2-one] (9e). A solution of **8** (60 mg, 0.21 mmol) and triethylamine (30 μL, 0.22 mmol) in 3 mL of 2-propanol was heated to reflux for 16 h. The volatile materials were removed under reduced pressure, and the residual oil was chromatographed (chloroform–methanol–concentrated ammonium hydroxide, 90:10:1 v/v) to afford 24 mg of **8** and 25 mg of the title compound as a white solid (R_f 0.17).

1,3-Dihydro-5-(2′-fluorophenyl)-3(*R*,*S*)-[[(benzyloxy)carbonyl]methyl]-2*H*-1,4-benzodiazepin-2-thione (10). A mixture of **9a** (1.7 g, 4.07 mmol) and Lawesson's reagent (1.23 g, 3.05 mmol) was heated to reflux in 150 mL of toluene for 1.5 h. The reaction mixture was cooled and diluted with 200 mL of ethyl acetate. The resulting solution was washed with 20% sodium hydroxide solution (3 × 50 mL) and brine, dried (Na_2SO_4), and concentrated. The crude reaction product was chromatographed (ethyl acetate–hexane, 1:1 v/v) to give the analytical product in 95% yield (R_f 0.67).

1,3-Dihydro-3(*R*,*S*)-[[[(benzyloxy)carbonyl]amino]methyl]-5-(2′-fluorophenyl)-2*H*-1,4-benzodiazepine (11). Freshly prepared W-2 Raney nickel (10 g, wet weight) was added to a solution of 150 mL of absolute ethanol containing **10** (1.85 g, 4.3 mmol). The resulting suspension was stirred vigorously for 50 h and then filtered through Celite. The catalyst and filter pad were thoroughly washed, first with ethanol and then with tetrahydrofuran, and the combined washings were rotoevaporated. Chromatography (chloroform–methanol, 96:4 v/v) afforded the analytical sample.

1,3-Dihydro-3(*R*,*S*)-(aminomethyl)-5-(2′-fluorophenyl)-2*H*-1,4-benzodiazepine (12). Hydrogen bromide gas was bubbled into an ice cold solution of **11** (700 mg, 1.73 mmol) in 100 mL of methylene chloride. After 45 min the reaction vessel was capped and the reaction mixture was warmed to room temperature on overnight stirring. The reaction vessel was vented and the volatile components were removed under reduced pressure. The residue was suspended in ethyl ether and concentrated. Repetition of the cycle two more times gave the title compound in sufficient quality for use in subsequent reactions without further purification.

Acknowledgment. It is a pleasure to acknowledge the assistance of Dr. S. M. Pitzenberger and J. Murphy (^{1}H NMR), Dr. H. Ramjit (MS), C. F. Homnick (HPLC), and J. P. Moreau (elemental analysis). We are grateful to Dr. P. S. Anderson for his support and encouragement during the course of this study and to V. Finley for preparation of the manuscript.

Registry No. **1**, 103420-77-5; **2**, 123903-75-3; **2a**, 124018-64-0; **2b**, 124018-65-1; **3**, 123903-76-4; **4**, 123903-77-5; **5**, 123903-94-6; **6a** (isomer 1), 123903-78-6; **6a** (isomer 2), 124018-66-2; **6b**, 123903-79-7; **6c**, 123903-80-0; **6d**, 123903-81-1; **6e**, 123903-82-2; **6f**, 123903-83-3; **6g**, 123903-84-4; **6h**, 102428-92-2; **6i**, 123903-85-5; **6j**, 102428-88-6; **6k**, 123903-86-6; **6l**, 123903-87-7; **6m**, 123903-88-8; **6n**, 102428-97-7; **6o**, 123903-89-9; **6p**, 102428-90-0; **pq**, 123903-90-2; **6r**, 123903-91-3; **6s**, 123903-92-4; **7** (X = H), 123903-95-7; **7** (X = F), 103343-35-7; **8**, 103343-37-9; **9a**, 103343-40-4; **9b**, 103407-24-5; **9c**, 103343-41-5; **9d**, 123903-93-5; **9e**, 103343-42-6; **10**, 123903-96-8; **11**, 103343-75-5; **12**, 123903-97-9; **13a**, 119487-27-3; **13c**, 103343-77-7; **13d**, 103343-79-9; 2,3-diphthalimidopropionyl chloride, 103343-36-8; 2-aminobenzophenone, 2835-77-0.

Supplementary Material Available: Analysis data for **4**, **6a–s**, **8**, **9a–e**, **11**, and **13a–d** (1 page). Ordering information is given on any current masthead page.