angiotensinogen), 50 μL of maleate buffer (pH 6.0), 5 μL of phenylmethanesulfonyl fluoride (PMSF), and 2 μL of an appropriate concentration of inhibitor in a dimethylsulfoxide (DMSO) vehicle. Incubation was for 60 min at 37 °C. Following incubation, each mixture was analyzed (in duplicate) for angiotensin I via radioimmunoassay using ^{125}I-labeled angiotensin I and carried out in tubes coated with rabbit antiangiotensin I antibody (Gamma Coat RIA Kit, Dade Clinical Assays). Monkey plasma renin activity ranged from 3–8 ng Al per mL per h. Values for inhibitor tubes were compared to vehicle (DMSO) control tubes to estimate percent inhibition. At the concentration used, DMSO inhibits the generation of angiotensin I by <10%. The inhibition results were expressed as IC_{50} values, which were obtained by plotting six inhibitor concentrations and estimating the concentration producing 50% inhibition using nonlinear regression analysis.

Inhibition of bovine cathepsin D (Sigma) activity was assessed in duplicate by the hydrolysis of bovine hemoglobin (2× crystallized, Sigma) at pH 3.2 and 37 °C (modified from Aoyogi et al.[30] and Kokubu et al.[31] Net absorbance (at 280 nm) was measured in acid-precipitated supernatant fractions of inhibited vs uninhibited control assays. The IC_{50} values were determined as described above.

Conscious, High-Renin, Normotensive Monkey Model. Male Cynomolgus monkeys weighing between 4.9 and 7.7 kg were placed on a low-sodium diet (Bio-Serv Inc., Frenchtown, NJ) 7–10 days prior to testing. Each monkey was then treated with furosemide (Lasix, INJ 5%, Hoechst-Roussel) at 2 mg per kg per day IM for four consecutive days prior to testing.

Solutions were prepared using a vehicle of 7.5% DMA, 30% Tween 80, and 62.5% H_2O. Concentrations were adjusted to allow the total dose to be administered in a volume of 2 mL/kg. The solution was administered by oral gavage using a 16-French rectal-colon tube (Davol, Cranston, RI). The monkeys were instrumented with vascular access ports (Norfolk Medical Products, Skokie, IL) for intraarterial blood pressure monitoring. Blood pressure was measured using a computer data acquisition system. Monkeys selected for these studies had been trained to rest quietly in a basic macaque restrainer (Primate Products, Woodside, CA).

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Hybrid Cholecystokinin-A Antagonists Based on Molecular Modeling of Lorglumide and L-364,718

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A series of novel nonpeptide cholecystokinin-A (CCK-A) antagonists have been synthesized. Designed on the basis of the structural homology between lorglumide and L-364,718, as investigated with molecular modeling, these compounds constitute a link between the *N*-acylglutamic acid and 3-amino-5-phenyl-1,4-benzodiazepin-2-one derived antagonists. The prepared compounds were tested in vitro as antagonists of the binding of [^{3}H]-(±)-L-364,718 and [^{3}H]-CCK-8(S) to rat pancreas and guinea pig brain membranes, respectively. All compounds proved to be selective for the (peripheral) CCK-A receptor, the most potent analogue, **6**, having a K_i value of 90 nM. The structure–activity profile of the series of hybrid compounds relates closest to that of the *N*-acylglutamic acid derived antagonists.

Introduction

Cholecystokinin (CCK) is a gastrointestinal peptide hormone that stimulates biliary and pancreatic secretion, gastrointestinal motility, and gallbladder contraction.[1] The identification of CCK in[2] and isolation from[3] the mammalian brain has increased the interest for this peptide considerably. It is now well-recognized that CCK has a neurotransmitter[4,5] or neuromodulator[6] function in the central nervous system (CNS), especially in the modulation of dopamine-mediated neurotransmission.[7-9] With various radiolabeled probes, at least two CCK receptor subtypes have been characterized.[10,11] Whereas CCK-B receptors are confined to the CNS, CCK-A receptors have been detected in both gastrointestinal[10] and CNS[12,13] tissues. Albeit the density of type A receptors in the CNS is low, their physiological relevance has been demonstrated. For

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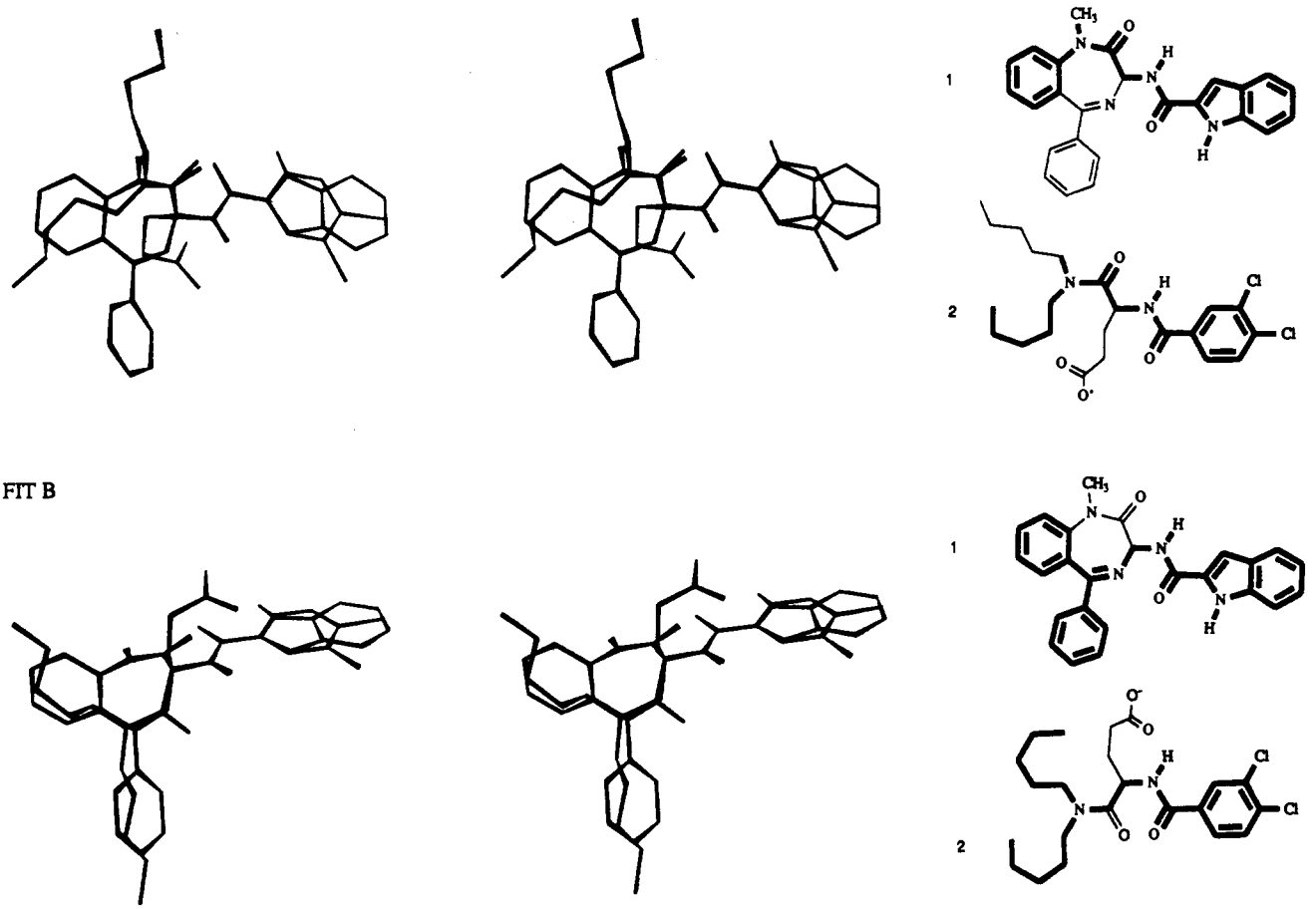
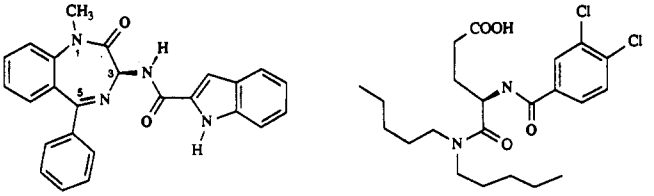


Figure 1. Superpositions of (*S*)-L364,718 (1) and (*R*)-lorglumide (2). The bold lines in the separate 2D presentations of 1 and 2 indicate putative homology segments.

example, the CCK-facilitated dopamine efflux from rat nucleus accumbens is reported to be mediated by CCK-A receptors.[14]

The recent progress in the field of CCK physiology is largely made possible by the development of potent and selective antagonists. We investigated two potent CCK-A antagonists, (*S*)-L-364,718[15] (1) and (*R*)-lorglumide[16] (2), with computer-assisted molecular modeling. These compounds are representatives of two major classes of nonpeptide CCK-A antagonists: the 3-substituted 1,4-benzodiazepin-2-ones and *N*-acylglutamic acid derivatives, respectively. The mutual displacement of 1 and 2 from the CCK-A receptor suggests a functional similarity somehow embedded in the structure of these ligands. This was investigated by superimposing putatively related lorglumide and L-364,718 moieties. The resulting homology model was used as a guideline to develop hybrid compounds. Since only functionally related moieties of



(S)-L-364,718 1 (R)-lorglumide 2

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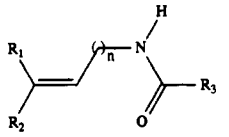
Table I. Structure and Receptor Binding Affinity for CCK-A Antagonists



compd	R_1	R_2	R_3	n	K_i,[a] μM CCK-A
1	(*S*)-L-364,718				0.0007 ± 0.0001
2	lorglumide				0.049 ± 0.003
3	Ph	Ph	2-indolyl	1	0.34 ± 0.05
4	*p*-Cl-Ph	*p*-Cl-Ph	2-indolyl	1	0.20 ± 0.05
5	*p*-MeO-Ph	*p*-MeO-Ph	2-indolyl	1	>4
6	Ph	Ph	*p*-Cl-PhNH	1	0.09 ± 0.01
7	Ph	Ph	*m*-Me-PhNH	1	0.8 ± 0.2
8	Ph	Ph	*p*-Cl-Ph	1	>2
9	Ph	Ph	*m*,*p*-Cl_2-Ph	1	0.40 ± 0.05
10	Ph	Ph	1-naphthyl	1	1.0 ± 0.2
11	Ph	Ph	2-naphthyl	1	0.45 ± 0.07
12	Ph	Ph	2-quinolyl	1	0.47 ± 0.08
13	Ph	Ph	3-quinolyl	1	>2
14	Ph	Ph	4-quinolyl	1	>2
15	Ph	Ph	2-(1,7-propylene)indolyl	1	>2
16	*n*-pentyl	*n*-pentyl	*p*-Cl-PhNH	1	0.20 ± 0.04
17[b]	Ph	Ph	2-indolyl	1	0.29 ± 0.04
18[c]	Ph	Ph	*p*-Cl-PhNH	1	1.4 ± 0.5
19	Ph-*o*-CH_2-*o*-CH_2-Ph		*p*-Cl-PhNH	1	>4
20	H	Ph	2-indolyl	1	0.18 ± 0.06
21	H	Ph	*p*-Cl-PhNH	1	>4
22	Ph	Ph	2-indolyl	2	>2
23	Ph	Ph	*p*-Cl-PhNH	2	0.40 ± 0.08

[a] Inhibition of [^{3}H]-(±)-L-364,718 binding to rat pancreas membranes. Values lower than 2 μM are means of three independent experiments ± SEM. [b] This compound contains a single carbon–carbon bond instead of the vinylic bond. [c] This compound, depicted in Scheme III, contains a single nitrogen–carbon bond instead of the vinylic bond.

1 and 2 were included, this approach yielded compounds of relatively simple structure. Radioligand binding studies demonstrated CCK-A receptor affinities in the micromolar to nanomolar range for these new materials. Here we report on the design, synthesis, and biological evaluation of this new class of hybrid CCK-A antagonists.

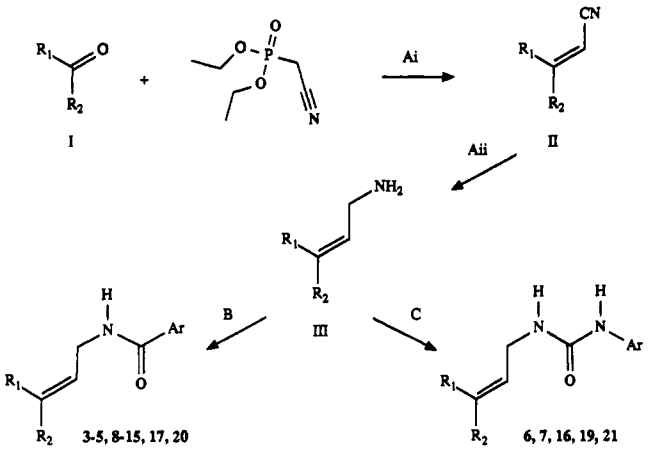
Results

Molecular Modeling. In Figure 1, two superpositions of (*R*)-lorglumide on (*S*)-L-364,718 are depicted that highlight the structural resemblance of these compounds. In both fits A and B the 3,4-dichlorobenzamide residue of lorglumide overlaps with the indole-2-carboxamide function of L-364,718. However, the overlap with the benzodiazepine moiety differs among these fits.

Fit A displays a match of identical atoms on the 1–3-positions of the diazepine ring. One of the *N*-pentyl chains of lorglumide penetrates the space occupied by the benzo ring of L-364,718. Fit B is characterized by the overlap of lorglumide with the 3–5-positions of the diazepine ring. Here, the matched atoms are not identical. However, the superimposed imine and amide groups are isosteres: both groups preferably adopt rigid planar conformations and direct the attached atoms in almost identical positions. Furthermore, the *N*-pentyl chains are imposable on both the benzo group and the 5-phenyl ring of the benzodiazepine structure. These fits argue two homology models, marked by the bold printed lines in the schematic 2D presentations.

Synthesis. Schemes I–III summarize the synthetic procedures used to obtain the novel compounds listed in Table I. The key compounds *N*-(3,3-diphenyl-2-propenyl)indole-2-carboxamide (**3**) and *N*-(4-chlorophenyl)-*N'*-(3,3-diphenyl-2-propenyl)urea (**6**) were prepared as outlined in Scheme I. The anion of diethyl (cyanomethyl)phosphonate was reacted with benzophenone (I) by a Horner–Emmons reaction in dry THF.

Scheme I. Preparation of Compounds of Table I[a]



[a] (A) (i) NaH, THF, 48 h; (ii) $LiAlH_4$, $AlCl_3$, ether; (B) ArCOOH, 2-chloro-1-methylpyridinium iodide, Et_3N, CH_2Cl_2; (C): ArNCO, CH_3CN.

Quantitative conversion to the nitrile II required 2 days stirring at room temperature. Since elevated reaction temperatures proved to result in byproduct formation and lower yields, the slow reaction at room temperature was preferred. The nitrile was reduced with equimolar amounts of $LiAlH_4$ and $AlCl_3$, as reported by Nystrom;[17] the extraction procedure however was modified. Conversion of the intermediate amine III, isolated as free base, to **3** and **6** involved either acylation with indole-2-carboxylic acid, procedure B, or coupling to 4-chlorophenyl isocyanate, procedure C. In the former reaction 2-chloro-1-methylpyridinium iodide served as a coupling

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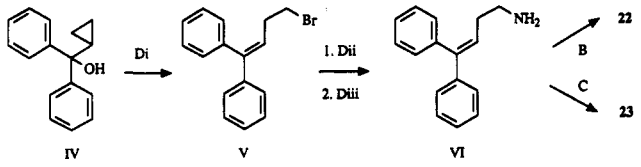
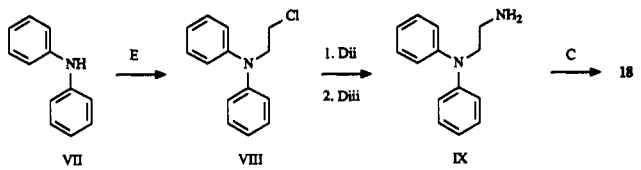
Scheme II. Preparation of Compounds **22** and **23**[a]



[a] (D) (i) HBr 47%; (ii) sodium phthalimide, 18-crown-6, toluene; (iii) hydrazine hydrate, ethanol; (B) indole-2-carboxylic acid, 2-chloro-1-methylpyridinium iodide, Et_3N, CH_2Cl_2; (C) 4-chlorophenyl isocyanate, CH_3CN.

Scheme III. Preparation of Compound **18**[a]



[a] (E) Chloroacetic acid, $NaBH_4$, toluene; (D) (ii) sodium phthalimide, 18-crown-6, toluene; (iii) hydrazine hydrate, DMF; (C) 4-chlorophenyl isocyanate, CH_3CN.

agent.[18] When dicyclohexylcarbodiimide was employed for this purpose, yields were low because of extensive and diverse byproduct formation. No attempt was made to determine the nature of these products. Compounds **4**, **5**, **7–17**, and **19–21** were prepared by adaptation of the reactants in Scheme I. 4,4′-Dichloro- and 4,4′-dimethoxybenzophenone, 6-undecanone, dibenzosuberenone, and *trans*-cinnamonitrile served as starting compounds in the synthesis of **4**, **5**, **16**, and **19–21**; for compounds **7–15**, the appropriate isocyanate, benzoic acids, or naphthyl/quinolyl/indolyl carboxylic acids were reacted with amine III in the final step. **17** was prepared from commercially available 3,3-diphenylpropylamine.

4,4-Diphenyl-3-buten-1-amine (VI), synthon for compounds **22** and **23**, was obtained as outlined in Scheme II. Solvolysis of cyclopropyldiphenylcarbinol (IV) in concentrated hydrobromic acid cleanly afforded the corresponding bromide V. Conversion of this halogen to the amine VI involved reaction with potassium phthalimide in the presence of 18-crown-6,[19] followed by liberation of the amine group on treatment with hydrazine hydrate in ethanol.[20] Coupling of VI with either indole-2-carboxylic acid or 4-chlorophenyl isocyanate gave the corresponding amide **22** and urea **23**.

N-(2-Aminoethyl)-*N*-phenylaniline (IX), synthon for **18**, was prepared in three synthetic operations according to Scheme III. Diphenylamine was alkylated with chloroacetic acid by addition of $NaBH_4$ to yield the chloride VIII, as reported by Marchini et al.[21] The previously described procedures D and C completed the synthesis of **18**.

Final products were purified by flash chromatography on silica gel with mixtures of either ether and petroleum ether (bp 40–60 °C) or acetone and hexane as eluting solvents. Except for the glassy **5**, all compounds were further purified by crystallization from various solvents or solvent mixtures.

Biological Evaluation. The compounds synthesized in this study were tested as antagonists of the specific equilibrium binding of [^{3}H]-(±)-L-364,718 to rat pancreas membranes. These data are summarized in Table I. Also, displacement of sulphated [^{3}H]-CCK-8 from guinea pig brain receptors was investigated. Since none of the compounds tested showed CCK-B receptor affinities better than 10 μM, these data are not included in Table I.

Discussion

Molecular Modeling. For the construction of the fits in Figure 1, the indole-2-carboxamide residue of (*S*)-L-364,718 and the 3,4-dichlorobenzamide function of (*R*)-lorglumide were superimposed. This procedure is validated by the high affinity of the CCK-A receptor antagonist A-64,718, a lorglumide derivative in which the 3,4-dichlorobenzamide residue is replaced by the indole-2-carboxamide residue.[22] The overlap was optimized by matching the relatively flexible lorglumide molecule to the L-364,718 template. The template conformation of L-364,718 was constructed from X-ray data as published by Evans et al.[23] By rotation of the free torsion angles adjacent to the exocyclic amide bond, this template may deviate from the receptor-bound conformation. However, this has little effect on the homology indicated by fits A and B: since this amide bond is fitted to one of lorglumides amide counterparts, analogous fits can be constructed for any rotamer of L-364,718.

The conformations of the pentyl chains in fits A and B were chosen arbitrarily: they can be matched to the aromatic rings in many other ways. Conformations in which the aliphatic chains mimic the planar shape of the aromatic rings are energetically unfavorable due to hydrogen–hydrogen repulsions in staggered methylene units. Since a hydrophobic receptor pocket seems to be involved in ligand binding, it is unlikely that mimicking to this extent is necessary. More extended and, hence, lower energy pentyl chain conformations may very well be able to interact favorably with the hydrophobic receptor pocket as well.

Both superpositions A and B show overlap of groups with comparable physicochemical properties. However, when the extent and nature of overlap and the correlation of the overlap to available biological data are taken into account, considerable differences become clear.

In fit B, the large hydrophobic overlap of the pentyl chains of lorglumide and the benzo and 5-phenyl groups of L-364,718 is remarkable. Essentially the same superposition was proposed by Kerwin et al. and Freidinger et al. to indicate the functional homology between the two antagonists.[22,24] In fit A, the hydrophobic overlap is less pronounced: a butylene fragment of the pentyl chain trans to the amide bond cannot be matched to the L-364,718 structure. Since Makovec et al. have demonstrated both

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pentyl chains are essential for high CCK-A receptor affinity,[16] fit A is contradictory to the structure–activity relationships (SAR) available on lorglumide. The close match of the N-terminal amide group of lorglumide on the diazepine amide group in fit A is a positive property of this fit. However, the latter group can be replaced by a triazolo ring without any concomitant loss of affinity,[25] illustrating it is not directly involved in receptor binding.

The above-mentioned considerations favor fit B as the better model to describe the functional interrelationship between lorglumide and L-364,718. Therefore, we selected the homology apparent in this fit as a working model for the development of hybrid CCK-A receptor antagonists.

Chemistry. Except for the central amide bonds, there is no *exact* structural homology between lorglumide and L-364,718. Since the latter compound is by far the more potent CCK-A antagonist (Table I), we decided to conserve its structure to a maximum extent, from which the hybrid compound **3** emerged. Incorporation of the imine bond (N_4–C_5) proved synthetically difficult. Therefore, an imine isostere was selected instead. From fit B, it can be assumed that the identical substituent directing effect rather than the electronic properties of the fitted imine (L-364,718) and amide (lorglumide) groups contribute to the receptor affinity. By employing the easily accessible vinyl function, compound **3** could be synthesized in only three steps.

Receptor Binding and Evaluation of Structure–Activity Relationships (SAR) of the Novel Compounds. As listed in Table I, compound **3** displays submicromolar affinity for peripheral CCK receptors. Because of this promising result, it was regarded as a lead for the synthesis of a series of derivatives. Since **3** was developed from L-364,718 and lorglumide, their respective SAR's may be expected to be at least in part paralleled in this lead. Substituents known from L-364,718 or lorglumide derivatives were introduced to verify this assumption. Also, modifications unique to the structure of **3** were performed in an attempt to enhance the affinity.

Compounds **8–13** bear carboxamide substituents previously incorporated in the lorglumide series.[16,22] Some parallelism with this family of antagonists is visible, for example in the relatively low affinity of compounds in which the long axis of the naphthyl (**10**) or quinolyl (**14**) group is orthogonal to the connecting bond. Also, *m,p*-dichloro substitution (**9**) is superior to *p*-monochloro substitution (**8**) in both series of antagonists. However, some marked deviations are obvious. 3-Quinolyl substitution, which yields optimal results in the *N*-acylglutamic acid series, proved unsuccessful in the hybrid compound **13**. In addition, the *m,p*-dichlorophenyl substituent is only a mediocre compound in the lorglumide series, whereas it displays the highest affinity of compounds **8–13**. Thus, the rank order of affinity upon various substitutions is not parallel. In compound **16**, the geminal phenyl rings are replaced by pentyl chains, which yields a close resemblance of the lipophylic moieties of **16** and lorglumide. The only marginally differing affinities of **16** and **6** validate the conclusion that these groups, as present in L-364,718 and lorglumide, respectively, interact with identical receptor sites.

The *N*-(*p*-Cl-phenyl)urea compound **6** was prepared in an attempt to enhance the CCK-B affinity. The *R*-isomer of L-365,260, a close analogue of L-364,718 that bears this substituent, is a CCK-B selective antagonist with nanomolar affinity.[28] In our series, however, no significant enhancement of CCK-B affinity was observed. Instead, CCK-A affinity improved approximately 4-fold to 90 nM. Several arguments can be advanced that may account for the observed deviations from the benzodiazepine SAR's. The rigid and strained benzodiazepine ring system may serve as a template that induces overall conformational properties highly appropriate for receptor binding. In contrast, the 3,3-diphenyl-2-propenylamine moiety of compound **6** is highly flexible. The correct conformation for binding to the CCK-B receptor is therefore not assured beforehand and in fact may not be part of the low-energy conformational space at all. In addition, conformational deviations are likely to result from the absence of a chiral center, as opposed to the chiral L-364,718. With regard to the observed CCK-A affinity of **6**, it should be noted that the *S*-isomer of L-365,260 is a potent and selective CCK-A antagonist. Intrinsically, the *N*-(*p*-Cl-phenyl)urea group is therefore fully adequate to interact favorably with the peripheral type receptor. The unexpected superior CCK-A affinity of **6** to that of compound **3** can only mean that the mode of binding of the hybrid compounds is not fully analogous to that of the C_3-substituted benzodiazepine compounds from which they were designed.

The 3,3-diphenyl-2-propenylamine moiety of **3** was modified in several ways in an attempt to increase CCK-A affinity. Whereas 4,4′-dichloro substitution (**4**) had little effect, the 4,4′-dimethoxy analogue (**5**) displayed a marked decrease of CCK-A affinity. Only symmetrically substituted analogues were prepared: applied to monosubstituted benzophenones, Horner–Emmons condensations (Scheme I) would result in the unwanted formation of cis/trans mixtures. Omission of the *cis*-phenyl ring, as in compounds **20** and **21**, results in a marked deviation from the SAR's of the other hybrid compounds: as for the 1,4-benzodiazepin-2-one-derived antagonists, the indole-2-carboxamide group yields more potent CCK-A antagonists than the *N*-(*p*-Cl-phenyl)urea group. Thus, the omission of the *cis*-phenyl ring seems to result in a binding mode that is more closely related to that of the substituted 1,4-benzodiazepin-2-ones. **20** is the lowest molecular weight compound with submicromolar affinity for CCK-A receptors known to us. Compound **17**, in which the C_2–C_3 double bond of the lead is replaced by a single carbon–carbon bond, is equipotent with the lead. The change from sp^2 to sp^3 hybridization affects the angle between the phenyl rings and their orientation relative to the rest of the molecule. Also, an additional degree of freedom is introduced. In this light, the retained affinity is remarkable. Compound **18** forms an intermediate between the conformational characteristics of **3** and **17**: as in **17** an additional degree of freedom is present, but the planar diphenylamine moiety relates closer to **3**. Still, compound **18** is less potent than either of these close analogues, for which most likely the physicochemical properties of the additional nitrogen atom are responsible. The addition of a methylene group between the amine and vinyl functions, as in compounds **22** and **23**, only slightly lowers the affinity of the hybrid compounds. Limitation of the rotational freedom of the geminal phenyl rings, however, as in compound **19**, results in a marked loss of affinity for CCK-A receptors. We performed a molecular modeling study which indicated that all energetically feasible conformers of the tricyclic molecule **19** have the *trans*-phenyl ring in an almost orthogonal orientation to the 5-phenyl ring of low-energy L-364,718 conformers. Since it can be

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Table II. Physical and Chemical Data for Compounds of Table I

compd	formula	analysis[a]	mp, °C	MS	scheme (proc)[b]
3	$C_{24}H_{20}N_2O \cdot 0.1H_2O$	C,H,N	149	352	I (A,B)
4	$C_{24}H_{18}Cl_2N_2O \cdot 0.1H_2O$	C,H,N,Cl	199	421	I (A,B)
5	$C_{26}H_{24}N_2O_3$	C,H,N	153–162	412	I (A,B)
6	$C_{22}H_{19}ClN_2O \cdot 0.25H_2O$	C,H,N,Cl	167.5–169	362	I (A,C)
7	$C_{23}H_{22}N_2O$	C,H,N	130.5	342	I (A,C)
8	$C_{22}H_{18}ClNO$	C,H,N	180–181	347	I (A,B)
9	$C_{22}H_{17}Cl_2NO$	C,H,N	98–99	381	I (A,B)
10	$C_{26}H_{21}NO \cdot 0.05(i\text{-}Pr)_2O$	C,H,N	149	363	I (A,B)
11	$C_{26}H_{21}NO \cdot 0.03(i\text{-}Pr)_2O$	C,H,N	159–160	363	I (A,B)
12	$C_{25}H_{20}N_2O$	C,H,N	112–113	364	I (A,B)
13	$C_{25}H_{20}N_2O \cdot 0.1H_2O$	C,H,N	169–170	364	I (A,B)
14	$C_{25}H_{20}N_2O$	C,H,N	108.5–110.5	364	I (A,B)
15	$C_{27}H_{24}N_2O$	C,H,N	130	392	I (A,B)
16	$C_{20}H_{31}ClN_2O \cdot 0.3H_2O$	C,H,N	68–69	350	I (A,C)
17	$C_{24}H_{22}N_2O$	C,H,N	128–130	354	I (B)
18	$C_{21}H_{20}ClN_3O$	C,H,N	172–174	365	III
19	$C_{24}H_{21}ClN_2O$	C,H,N	204–204.5	388	I (A,C)
20	$C_{18}H_{16}N_2O \cdot 0.3H_2O$	C,H,N	192–194	276	I (A,B)
21	$C_{16}H_{15}ClN_2O$	C,H,N	178–179	286	I (A,C)
22	$C_{25}H_{22}N_2O \cdot 0.05H_2O$	C,H,N	188	366	II (D,B)
23	$C_{23}H_{21}ClN_2O \cdot 0.05H_2O$	C,H,N,Cl	152–153	376	II (D,C)

[a] Combustion analyses were within ±0.4%, except for 16 (H: calcd, 8.94; found, 8.29). All compounds were characterized with ^{1}H NMR, which confirmed the presence of solvate where indicated. [b] Refers to the scheme in which the synthesis of the compound is outlined; procedures are detailed in the Experimental Section.

assumed that the orientation of this phenyl ring is optimal in L-364,718, the decreased affinity of the locked analogue of 6 demonstrates the importance of this *trans*-phenyl ring for receptor binding.

Summary and Conclusions

The compounds prepared in this study constitute a link between the *N*-acylglutamic acid derivatives and 3-substituted 1,4-benzodiazepin-2-ones. These hybrid ligands display submicromolar affinity to CCK-A receptors and are selective for this receptor subtype. The *trans*-phenyl ring of the diphenylpropenyl moiety seems particularly important for binding; omission of the *cis*-phenyl ring changes the SAR's of the hybrid compounds but can yield affinities comparable to the geminal diphenyl compounds. In general, none of the synthesized hybrid compounds approaches the sub-nanomolar affinity displayed by L-364,718 (1). Still, except for the omission of the N_1–C_2 amide bond, lead compound 3 and L-364,718 are structurally closely related. The conformational properties of these compounds, however, are entirely different. We reason that the rigid and strained 7-membered diazepine ring system stabilizes conformations in which the disposition of attached pharmacophores is optimal for receptor interaction. The hybrid compounds presented here lack both the rigidity and (deforming) ring strain, resulting in a decreased fraction of ligands in the bioactive conformation. In addition, the absence of a chiral center is likely to result in a further deviation from the conformational space and local minima of the idealized benzodiazepine.

Both the affinities and the SAR's of these compounds are more comparable to the *N*-acylglutamic acid antagonists. Lorglumide is only 2-fold more potent than the hybrid compound 6. This difference is remarkably small, since 6 does not contain any functionality comparable to the glutamic acid side chain. We are currently undertaking the introduction of this side chain in our hybrid compounds. This effort may further elucidate the relationship between these antagonists and yield more potent antagonists.

Experimental Section

Molecular Modeling. Molecules were visualized and manipulated with the molecular modeling package Biograf (BioDesign, Inc.) running on a Silicon Graphics Personal Iris workstation. Minimizations were computed with the DREIDING force field[26] using the conjugate gradient method. Superpositions of (*R*)-lorglumide and (*S*)-L-364,718 were constructed as follows. Bond lengths and bond angles of the crystal structure of (*S*)-L-364,718[23] were minimized with DREIDING. Since X-ray data are not available on (*R*)-lorglumide, this compound was constructed in Biograf and subsequently minimized. (*R*)-Lorglumide was manually superimposed on (*S*)-L-364,718 by adjusting the appropriate dihedral angles. Subsequently, the resultant lorglumide conformers were minimized to their nearest local energy minimum and superimposed again. For fit B, the exocyclic bond of L-364,718 at C_3 was slightly rotated in order to obtain a better overlap with low-energy conformers of lorglumide.

Synthesis. Commercial chemicals were used without further purification. All reactions were carried out under nitrogen atmosphere. Where appropriate, solvents were purified and dried by standard methods before use. Petroleum ether refers to low-boiling petroleum ether, 40–60 °C. All compounds in Table II were dried in vacuo (1 mmHg) over NaOH and silica gel at 80–120 °C, depending on the compound's melting point.

Flash chromatography at 1.5 atm was performed on silica gel, 0.040–0.063 mm (Merck). Melting points were determined in a Büchi capillary melting point apparatus and are uncorrected. ^{1}H NMR spectra were recorded on a JEOL FX-200 pulsed Fourier transform instrument in $CDCl_3$. Chemical shifts are measured in ppm with Me_4Si as internal standard. Mass spectra were recorded on either a Finnigan MAT ITD-700 or a TSQ-70 mass spectrometer. Elemental analyses, performed by TNO (Zeist, The Netherlands), were within ±0.4% of the theoretical values unless noted otherwise. Where analytical data (Table II) have been presented for solvates, their presence has been verified with NMR.

In this section the synthesis of *N*-(3,3-diphenyl-2-propenyl)-indole-2-carboxamide (3), *N*-(4-chlorophenyl)-*N'*-(3,3-diphenyl-2-propenyl)urea (6), 4,4-diphenyl-3-buten-1-amine (intermediate VI), and *N*-(2-chloroethyl)-*N*-phenylaniline (intermediate VIII) are described. These examples represent the general synthetic methods A–E by which all compounds were prepared.

Method Ai: 2,2-Diphenylacrylonitrile (Intermediate II). NaH (55% suspension in mineral oil, 6.00 g, 110 mmol) was rinsed three times with dry petroleum ether and suspended in 150 mL of THF. At 0 °C, a solution of diethyl (cyanomethyl)phosphonate (21.0 g, 119 mmol) in 100 mL of THF was added in 5 min. After 20 min of stirring at room temperature, the reaction mixture was

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cooled in an ice bath and a solution of benzophenone (20.0 g, 110 mmol) in 100 mL of THF was added in one portion. The reaction mixture was stirred at room temperature for 72 h and then quenched by careful dropwise addition of H_2O at 0 °C. The reaction mixture was neutralized with saturated aqueous NH_4Cl and extracted with 250 mL (1×) and 75 mL (2×) of ether/petroleum ether (1:1). The combined extracts were washed with H_2O (150 mL, 3×) and brine (100 mL), dried over $MgSO_4$, filtered, and evaporated to dryness in vacuo to give II in quantitative yield (22.54 g): ^1H NMR ($CDCl_3$) δ 5.73 (1 H, s, vinylic proton), 7.24–7.48 (10 H, m, aro).

Method Aii: 3,3-Diphenyl-2-propen-1-amine (Intermediate III). $AlCl_3$ (16.13 g, 121 mmol) was carefully dissolved in dry ether (150 mL) and added in one portion to a suspension of $LiAlH_4$ (4.60 g, 121 mmol) in dry ether (150 mL) at 0 °C. After 5 min, a solution of II (22.54 g, 110 mmol) in 100 mL of ether was added dropwise. The reaction mixture was warmed to room temperature, stirred for 2 h, and carefully quenched by dropwise addition of H_2O at 0 °C. After addition of 300 mL of H_2O, the pH was adjusted to 1 with concentrated HCl. The resulting precipitate of gray salts was filtered, washed with 200 mL of CH_2Cl_2, and resuspended in 200 mL of water and 200 mL of ether. The pH was adjusted to 14 by addition of sodium hydroxide pellets under firm stirring. During the basification, the salts completely solubilized in the ether phase. The separated water phase was extracted with ether (50 mL, 2×). The combined ether fractions were washed with water (125 mL, pH 14, 2×) and brine, dried with $MgSO_4$, filtered, and evaporated to give III: yield 21.62 g (94.1%): ^1H NMR ($CDCl_3$) δ 1.51 (2 H, br s, NH_2), 3.34 (2 H, d, J = 7 Hz, CH_2), 6.14 (1 H, t, J = 7 Hz, vinylic proton), 7.12–7.36 (10 H, m, aro).

Method B: *N*-(3,3-Diphenyl-2-propenyl)indole-2-carboxamide (3). To a solution of 60 mL of CH_2Cl_2 containing 0.50 g of III (2.4 mmol), 0.39 g of indole-2-carboxylic acid (2.4 mmol), and 0.58 g of triethylamine (5.7 mmol) was added 0.73 g of 2-chloro-1-methylpyridinium iodide (2.9 mmol). The reaction mixture was stirred at room temperature for 10 min. Subsequently 100 mL of ether and 75 mL of H_2O were added. The water phase was neutralized with a saturated NH_4Cl (aqueous) solution and separated. The organic phase was washed with H_2O (75 mL, 3×) and brine (75 ml), dried over $MgSO_4$, filtered, and evaporated to dryness. The residual oil was chromatographed (acetone–hexane elution, 25:75 v/v) and crystallized from hexane to yield 36% (0.30 g) of 3: ^1H NMR ($CDCl_3$) δ 4.20 (2 H, br t, CH_2), 6.18 (1 H, t, J = 7 Hz, vinylic proton), 6.51 (1 H, br t, amide NH), 6.80–7.60 (15 H, m, aro), 10.34 (1 H, br s, indole NH).

Method C: *N*-(4-Chlorophenyl)-*N'*-(3,3-diphenyl-2-propenyl)urea (6). A solution of III (0.70 g, 3.4 mmol) and 4-chlorophenyl isocyanate (0.51 g, 3.4 mmol) in 20 mL of CH_3CN was stirred at room temperature for 2 h; 50 mL of ether and 50 mL of H_2O were added. The organic phase was washed with H_2O (50 mL, 2×) and brine (75 mL), dried over $MgSO_4$, filtered, and evaporated to dryness. The crude product was purified by chromatography (ether–petroleum ether elution, 1:1 v/v) and crystallization from acetonitrile to yield 50% (0.61 g) 6: ^1H NMR ($CDCl_3$) δ 3.90 (2 H, br t, CH_2), 5.06 (1 H, br t, urea proton CH_2NH), 6.07 (1 H, t, J = 7 Hz, vinylic proton), 6.67 (1 H, br s, urea proton NH-Ar), 7.10–7.34 (14 H, m, aro).

Method Di: 4-Bromo-1,1-diphenyl-1-butene (Intermediate V). A suspension of cyclopropyldiphenylcarbinol (IV, 5.00 g, 22.3 mmol) in HBr (48%, 2 mL) was stirred vigorously at 4 °C for 4 h and extracted with CH_2Cl_2 (20 mL, 3×). The combined organic extracts were washed with H_2O (50 mL, 3×) and brine (50 mL), dried over $MgSO_4$, filtered, and evaporated to dryness to yield 96.9% (6.21 g) of oily residue V: ^1H NMR ($CDCl_3$) δ 2.64 (2 H, double t, J = 7 Hz (2×), CH_2CH_2Br), 3.37 (2 H, t, J = 7 Hz, CH_2CH_2Br), 6.06 (1 H, t, J = 7 Hz, vinylic proton), 7.13–7.40 (10 H, m, aro).

Method Dii: 4,4-Diphenyl-3-butenyl-1-phthalimide. A solution of 30 mL of toluene containing V (6.21 g, 21.6 mmol), potassium phthalimide (0.77 g, 26.1 mmol), and 18-crown-6 (0.57 g, 2.2 mmol) was heated to 100 °C and stirred for 5 h. To the reaction mixture were added H_2O (50 mL) and CH_2Cl_2 (100 mL). The aqueous phase was separated and extracted with CH_2Cl_2 (50 mL, 2×); the organic extracts were combined, washed with H_2O (50 mL, 3×) and brine (50 mL), dried over $MgSO_4$, filtered, and evaporated. Crystallization from 200 mL of methanol yielded 74.9% (5.71 g) of 4,4-diphenyl-3-butenyl-1-phthalimide: ^1H NMR ($CDCl_3$) δ 2.53 (2 H, double t, J = 7 Hz (2×), CH_2CH_2N), 3.79 (2 H, t, J = 7 Hz, CH_2CH_2N), 6.06 (1 H, t, J = 7 Hz, vinylic proton), 7.00–7.82 (14 H, m, aro).

Method Diii: 4,4-Diphenyl-3-buten-1-amine (VI). To a suspension of 5.00 g (14.2 mmol) of 4,4-diphenyl-3-butenyl-1-phthalimide in 30 mL of ethanol was added hydrazine hydrate (80% wet weight hydrazine monohydrate, 0.89 g, 14.2 mmol). The reaction mixture was refluxed resulting in the dissolution of the phthalimide. After 30 min of refluxing, phthalyl hydrazide started to precipitate. After 2 h, the solution was cooled to 20 °C. The thick suspension was diluted with 30 mL of ethanol and acidified to pH 3 with concentrated HCl. The precipitate was filtered and washed with ethanol (5 mL, 3×). The filtrate was evaporated to 5 mL, diluted with 25 mL of H_2O, and filtered again. The filtrate was evaporated to dryness. The residue was dissolved in 20 mL of absolute ethanol and 20 mL of dry ether and stored at 5 °C for 16 h. The white crystallite was filtered and dried in vacuo. The filtrate was evaporated, and the residue was dissolved in 10 mL of hot ethanol to perform a second crystallization. Ether (30 mL) was added to the solution. After 18 h, a second batch of white crystals was isolated by filtration. The combined crystallites provided 55.2% (2.03 g) of amine VI as an HCl salt: ^1H NMR (CH_3OH) δ 2.47 (2 H, double t, J = 7 Hz (2×), CH_2CH_2N), 3.00 (2 H, t, J = 7 Hz, CH_2CH_2N), 6.08 (1 H, t, J = 7 Hz, vinylic proton), 7.15–7.42 (14 H, m, aro).

Method E: *N*-(2-Chloroethyl)-*N*-phenylaniline (VIII). To a solution of 3.67 g (38.8 mmol) $ClCH_2COOH$ in 100 ml dry toluene was added 1.13 g (29.9 mmol) $NaBH_4$ portionwise at room temperature. When the evolution of gas ceased, 1.00 g diphenylamine (VII, 5.90 mmol) was added. The reaction mixture was refluxed for 3 h, cooled, and made alkaline with 2 N NaOH. The separated organic layer was dried with brine (50 mL) and $MgSO_4$, filtered, and evaporated. The crude yellow oil (1.42 g) was purified by flash chromatography (ether–petroleum ether elution, 1:99 v/v) which yielded 0.87 g (3.92 mmol, 66.4%) of pure VIII: ^1H NMR ($CDCl_3$) δ 3.66 (2 H, t, J = 7 Hz, CH_2Cl), 4.00 (2 H, t, J = 7 Hz, NCH_2), 6.9–7.2 (10 H, m, aro).

Receptor Binding. CCK-A receptor affinities were determined by displacement of ^3H-(±)-L364,718 from rat pancreas membranes.[27] ^3H-(±)-L-364,718 (specific activity 87 Ci/mmol) was purchased from New England Nuclear, Dreiech, FRG. Lorglumide and (*S*)-L-364,718 were kindly donated by Rotta research (Monza, Italy) and Merck, Sharp & Dohme Research Laboratories (West Point, NY), respectively.

Membrane Preparation. Membranes from male rat (Wistar) pancreas were prepared by homogenization of the tissue in 20 volumes of isolation buffer, using an Ystral X1020 homogenizer (setting 5). The isolation buffer consisted of 10 mM Hepes (pH 7.4 at 20 °C), 5 mM $MgCl_2$, and 130 mM NaCl. The homogenate was centrifuged at 50000*g* for 15 min, and the supernatant was discarded. The pellet was washed by resuspension in the original volume of buffer and centrifuged as described above. The final pellet was resuspended in 40 volumes of the isolation buffer enriched with 0.02% bacitracin and 0.01% soybean trypsin inhibitor. The preparation procedure was performed at 4 °C. Aliquots of 1 mL of membrane suspension were stored at −80 °C until use.

Binding Assays. Binding experiments were performed in an assay buffer consisting of 10 mM Hepes (pH 7.4 at 20 °C) and 5 mM $MgCl_2$. To enhance the solubility of the compounds, ethanol was added up to a final assay concentration of 0.3% maximum, which had little influence on specific binding. To duplicate, polypropylene incubation tubes were added, in a total volume of 0.5 mL, either (a) 50 μL of various concentrations of ligand in assay buffer (displacement studies), or lorglumide (10 μM final

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concentration, nonspecific binding), or assay buffer (control); (b) 50 μL of radioligand in assay buffer with 0.05% CHAPS; (c) 200 μL of assay buffer; (d) 200 μL of membranes in assay buffer. After 30 min of incubation at 37 °C, 1 mL of ice-cold buffer was added followed by rapid filtration under reduced pressure over Whatman glass fiber GF/B filters, presoaked with buffer, by means of a Millipore sampling manifold. Filters were washed with 1 mL of buffer used to wash the incubation tubes and three times with 2 mL of ice-cold buffer. Filters were dried at 75 °C for 45 min. The radioactivity trapped on the filters was counted in 3.5 mL of Packard Emulaifier Safe after at least a 2 h extraction time.

Data Analysis. Specific binding was defined as the difference between total binding and nonspecific binding in presence of 10 μM lorglumide. IC_{50} values were determined from pseudo-Hill plots of the displacement curves. Inhibitor dissociation constants (K_i) were calculated from the Cheng-Prusoff equation: $K_i = IC_{50}/(1 + [L^*]/K_d)$. L^* denotes the concentration and K_d the equilibrium dissociation constant of the radioligand. A K_d value of 0.21 nM was determined for ^{3}H-(±)-L364,718 binding to rat pancreas membranes.

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Registry No. 1, 103420-77-5; 2, 118919-27-0; 3, 138354-37-7; 4, 138354-38-8; 5, 138354-39-9; 6, 138354-40-2; 7, 138354-41-3; 8, 138354-42-4; 9, 138354-43-5; 10, 138384-12-0; 11, 138354-44-6; 12, 138354-46-8; 13, 138354-46-8; 14, 138354-47-9; 15, 138354-48-0; 16, 138354-49-1; 17, 138354-50-4; 18, 138354-51-5; 19, 138354-52-6; 20, 138354-53-7; 21, 138354-54-8; 22, 138384-13-1; 23, 138384-14-2; II, 3531-24-6; III, 5666-18-2; IV, 5785-66-0; V, 6078-95-1; VI·HCl, 93007-57-9; VII, 122-39-4; VIII, 42393-65-7; IX, 1140-29-0; ClC-H_2-COOH, 79-11-8; diethyl (cyanomethyl)phosphonate, 2537-48-6; benzophenone, 119-61-9; indole-2-carboxylic acid, 1477-50-5; 4-chlorophenyl isocyanate, 104-12-1; potassium phthalimide, 1074-82-4; 4,4-diphenyl-3-butenyl-1-phthalimide, 95958-02-4.

(H^+,K^+)-ATPase Inhibiting 2-[(2-Pyridylmethyl)sulfinyl]benzimidazoles. 4.[1] A Novel Series of Dimethoxypyridyl-Substituted Inhibitors with Enhanced Selectivity. The Selection of Pantoprazole as a Clinical Candidate

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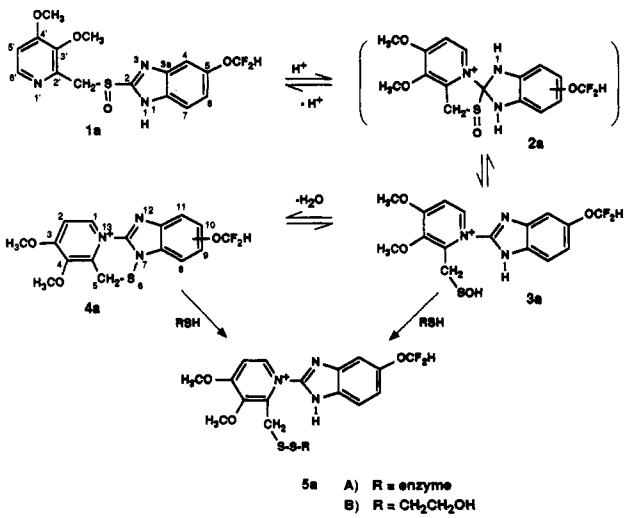
[(Pyridylmethyl)sulfinyl]benzimidazoles 1 (PSBs) are a class of highly potent antisecretory (H^+,K^+)-ATPase inhibitors which need to be activated by acid to form their active principle, the cyclic sulfenamide 4. Selective inhibitors of the (H^+,K^+)-ATPase in vivo give rise to the nonselective thiophile 4 solely at low pH, thus avoiding interaction with other thiol groups in the body. The propensity to undergo the acid-catalyzed transformation is dependent on the nucleophilic/electrophilic properties of the functional groups involved in the formation of 2 since this step is both rate-determining and pH-dependent. The aim of this study was to identify compounds with high (H^+,K^+)-ATPase inhibitory activity in stimulated gastric glands possessing acidic pH, but low reactivity (high chemical stability) at neutral pH as reflected by in vitro (Na^+,K^+)-ATPase inhibitory activity. The critical influence of substituents flanking the pyridine 4-methoxy substituent present in all derivatives was carefully studied. The introduction of a 3-methoxy group gave inhibitors possessing a combination of high potency, similar to omeprazole and lansoprazole, but increased stability. As a result of these studies, compound 1a (INN pantoprazole) was selected as a candidate drug and is currently undergoing phase III clinical studies.

Introduction

Control of gastric pH by means of antisecretory drugs has proven to be a valuable principle in the treatment of peptic ulcers.[2] The identification of the gastric (H^+,K^+)-ATPase,[3,4] located in the apical membrane of parietal cells, as the gastric proton pump has stimulated considerable interest in the inhibitors of this enzyme as antiulcer therapeutics. Its unique environment[5] in the

Scheme I



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