Bioorganic & Medicinal Chemistry Letters, Vol.3, No.5, pp. 875-880, 1993
Printed in Great Britain

0960-894X/93 $6.00 + .00
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DIPHENYLPYRAZOLIDINONE AND BENZODIAZEPINE CHOLECYSTOKININ ANTAGONISTS: A CASE OF CONVERGENT EVOLUTION IN MEDICINAL CHEMISTRY

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(*Received* 9 *June* 1992; *accepted* 4 *September* 1992)

Abstract: *Two recently described classes of nonpeptide cholecystokinin receptor antagonists contain diphenylpyrazolidinone and benzodiazepine substructures, respectively. Although the origins and development of these series were completely independent, the low energy conformations predicted by modeling showed remarkable three-dimensional homology between the structures.*

While there were no examples prior to 1985 of *de novo* development of potent non-peptide ligands for peptide receptors, the years since have witnessed remarkable progress in this area. The earliest successes were antagonists for the cholecystokinin (CCK) receptor, and this receptor family continues to enjoy the richest structural diversity among its antagonists, with compounds selective for both CCK-A (pancreatic) and CCK-B (brain) subtypes of the receptor now available. The major structural types described to date include benzodiazepine derivatives such as MK-329[1] (A-selective) and L-365,260[2] (B-selective), glutamic amides such as loxiglumide[3] and A-65186[4] (both A-selective), "dipeptoid" compounds such as CI-988[5] (B), diphenylpyrazolidinones[6] such as LY262691[7] (B) and LY219057[8] (A), and quinazolinones[9] (B).

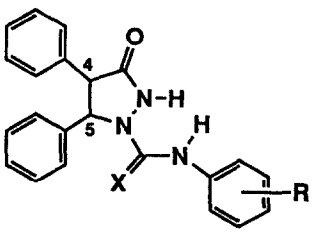
In several of these series, receptor subtype selectivity is dependent on the stereochemistry within the molecule. In the diphenylpyrazolidinone series, selectivity for the CCK-B receptor appears to associate most strongly with 4*S*,5*R* stereochemistry at the phenyl groups[10]. Thus, in the B-selective racemate LY262691 (**1**), the high affinity for the CCK-B receptor is almost entirely due to the 4*S*,5*R* enantiomer, LY288513 (**3**) (Table 1). Conversely, selectivity for the CCK-A receptor appears to be conferred by the antipodal 4*R*,5*S* stereochemistry, as seen for the A-selective racemate, LY219057 (**4**), wherein the 4*R*,5*S* isomer, LY294290 (**5**), is nearly 50 times as potent at CCK-A as its enantiomer (**6**). For benzodiazepines which contain a phenylurea side-chain, such as L-365,260, a similar reversal of selectivity between enantiomers is seen, with CCK-B selectivity being associated with *R* stereochemistry, and CCK-A selectivity with *S* (**7** - **8**)[2]. The pattern is different, however, for benzodiazepines having an arylamido side-chain (**9** - **12**)[11]. Both enantiomers appear to be strongly A-selective, although the *S* isomer has greater absolute potency at CCK-A, consistent with the selectivity expressed in the phenylurea side-chain analogs.

The CCK-B-selective compounds in the diphenylpyrazolidinone and benzodiazepine series contain several common substructures, despite independent origins and developments of their structure-activity relationships. The phenylurea side-chain seen in both is a particularly striking homology, and both also contain two other phenyl platforms, either pendant from or fused to their respective central heterocyclic ring systems. The reversal of selectivity between enantiomers in both

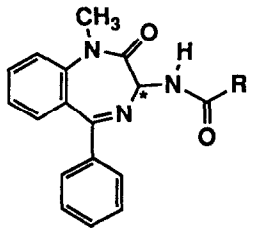
series further reinforces the sense of commonality. The common features of CCK-A-selective compounds in the two series appear less clear-cut. In the present study, representative CCK-B antagonists from each series, LY288513 and L-365,260, were subjected to a thorough conformational search to establish the preferred conformations of each structural type. The resulting low energy conformations were then compared to assess whether these homologous domains also have a three-dimensional correspondence. Similar studies have previously explored possible pharmacophoric relationships among the glutamic amides, the benzodiazepines, and a series of benzolactams related to the latter, using molecular modeling, as well as synthesis of structural hybrids between the series, and demonstrated a plausible mapping of certain domains.[4,12,13,14]

Compounds were modeled using MacroModel Vers. 3.0. After initial minimization of LY288513, the MULTIC utility was used to vary the indicated dihedral angles 1, 2, and 3 in increments of 30°, producing 1343 trial conformations (Figure 1). The dihedral angles of the 4- and

Table 1 Binding to CCK receptors



compounds **1 - 6**



compounds **7 - 12**

no.	X	R	stereochem.	compound	binding IC50, μM CCK-B [a,b]	CCK-A [a,c]
1	O	4-Br	racemic *trans*	LY262691	0.031	11.6
2			4*R*,5*S*	LY288512	0.37	6.4
3			4*S*,5*R*	LY288513	0.019	20.5
4	S	3-CF_3, 4-Cl	racemic *trans*	LY219057	0.88	0.042
5			4*R*,5*S*	LY294920	1.9	0.017
6			4*S*,5*R*	LY294919	0.55	0.81
7 [d]	--	3-Me-phenyl-NH	*S*		0.15	0.003
8 [d]	--	3-Me-phenyl-NH	*R*	L-365,260	0.002	0.28
9 [e]	--	2-indolyl	*S*	MK-329	0.27	0.00008
10 [e]	--	2-indolyl	*R*		3.7	0.0083
11 [e]	--	4-Cl-phenyl	*S*	[f]	2.9	0.00039
12 [e]	--	4-Cl-phenyl	*R*	[f]	11.0	0.0029

[a] average of 2 to 5 determinations for **1 - 6**
[b] inhibition of binding of [^{125}I]CCK-8 to mouse brain at pH 6.5 (**1 - 3**) or pH 7.4 (**4 - 6**), or to guinea pig brain (**7 - 12**)
[c] inhibition of binding of [^{3}H]L-364,718 (**1 - 6**) or of [^{125}I]CCK-33 or [^{125}I]CCK-8 (**7 - 12**) to rat pancreas
[d] data from reference 2
[e] data from reference 11
[f] 2-F on phenyl pendant from diazepine ring

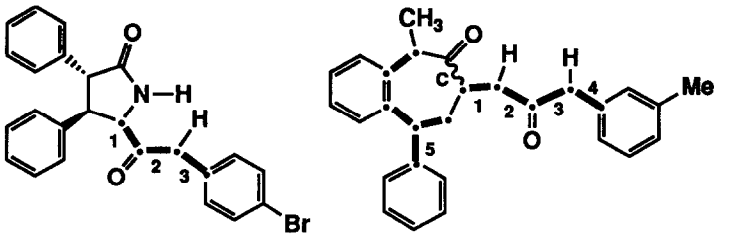


Figure 1

Dihedral angles varied during MULTIC conformer generation with LY288513 (left) and L-365,260

5-phenyl groups were not varied, since preliminary work indicated a strong preference for each to be oriented perpendicular to the plane of the pyrazolidinone ring, as seen in the initially minimized structure. The trial conformations were completely minimized (RMS < 0.050 Å) using the MM2 force field in BATCHMIN Vers. 3.1d, to give, after elimination of high energy and duplicate structures, four conformations within 20 kJ/mol of the global minimum. L-365,260 was similarly processed. In addition to varying the five indicated non-ring dihedral angles in increments of 60°, alternate conformations of the benzodiazepine ring were generated by varying three ring dihedral angles, using the indicated bond (**C**) for closure (Figure 1). Of 1404 trial conformations submitted, 11 remained within 20 kJ/mol of the global minimum after complete minimization.

In all the low energy conformations found for LY288513, the portion of the structure encompassing dihedral angles 1 and 2 was essentially planar, as would be expected from the amide-like linkages in this urea substructure. Conformations 1-4 represent the four possible combinations of cisoid and transoid configurations about each amide bond (Table 2). The two lowest energy conformations contain a "virtual" ring defined by a hydrogen bond between the N2 hydrogen of the pyrazolidinone and the carbonyl of the urea side-chain.

The low energy conformations of L-365,260 clustered into four groups that were closely related in structure and energy (conformations 1-3, 4-6, 7-10, and 11) (Table 3). Two distinct, essentially enantiomeric boat-like conformations of the benzodiazepine ring system were seen, one which forced the phenylurea side-chain into an axial orientation (groups 1 and 2), and the other resulting in an equatorial orientation (groups 3 and 4). The phenylurea substructure was very nearly planar in all cases, as found for LY288513. Dihedral angle 2 was transoid in groups 1 and 3, and cisoid in groups 2 and 4, while dihedral angle 3 was transoid throughout all conformations. Within each group the only significant differences were variations in dihedral angle 4. The orientation of the 3-methyl group relative to the urea had no apparent affect on conformational energies (compare conformations 2 and 3; 4 and 5; 7 and 8; etc.).

Table 2 Lowest energy conformations of LY288513

conform.	energy, kJ/mol	dihedral angle, degrees 1 [a]	2	3	comment
1	87.34	5.6	12.3	55.4	H-bond: N2-H --> O=C
2	91.48	-5.9	-179.7	-29.0	H-bond: N2-H --> O=C
3	95.51	175.6	-15.5	-41.5	no H-bond
4	100.93	171.6	177.0	-30.5	no H-bond

[a] defined relative to C-5 of the pyrazolidinone ring

Table 3 Lowest energy conformations of L-365,260

conform. group	conform. no.	energy, kJ/mol	side-chain	dihedral angle, degrees C [a]	1 [b]	2	4 [c]
1	1	53.63	axial	-67.9	162.0	178.1	-29.9
	2	53.99	axial	-68.0	163.9	177.2	154.9
	3	54.37	axial	-68.0	167.7	177.3	-155.0
2	4	56.96	axial	-58.0	-177.5	-2.7	-154.6
	5	56.99	axial	-56.8	-172.9	-4.0	153.2
	6	57.04	axial	-56.8	-172.7	-4.1	-29.6
3	7	59.17	equat.	64.1	-160.4	-176.7	25.5
	8	59.18	equat.	64.1	-161.0	-177.5	-25.4
	9	59.22	equat.	64.0	-160.6	-176.6	-157.5
	10	59.23	equat.	64.0	-160.7	-177.6	157.9
4	11	63.78	equat.	60.0	-57.0	3.2	25.3

Dihedral angle 3 was 180±5.7° for all conformations. Dihedral angle 5 was -30.4° to -32.3° for conformations 1-6, and 27.6° to 32.5° for conformations 7-11.

[a] defined between the two adjacent N's of the diazepine ring

[b] defined relative to adjacent N of the diazepine ring

[c] defined relative to C-2 of the phenyl ring (i.e. adjacent to the 3-Me group)

The finding that axial conformations were lowest in energy was surprising, given that previously reported X-ray and solution studies of 3-alkyl-,[15,16,17] 3-hydroxy-,[18] and 3-amido-substituted[19] benzodiazepines consistently indicated a preference for equatorial disposition of the 3-substituent. In order to assess whether the force field used in the present exercise was inherently biased toward axial conformations of benzodiazepines, the simple 3-methyl-substituted derivative (i.e. 1,3-dimethyl-5-phenyl-1,3-dihydro-2*H*-1,4-benzodiazepin-2-one) was modeled similarly to L-365,260. In agreement with literature reports on very similar structures,[15,17] the equatorial conformation was considerably lower in energy than axial (149.84 vs. 155.12 kJ/mol). The reversal of this preference in L-365,260 may reflect influences on the ring conformation from the phenylurea side-chain, perhaps dipolar in nature, which are absent in previously studied types of 3-substituents.

The assessment of three-dimensional overlap between conformations of LY288513 and L-365,260 was guided by the observations on likely homologous domains mentioned above. Thus, various pairs of conformations were rigidly superimposed, using matched pairs of atoms at the 1- and 4-positions of the three phenyl rings in each structure. It was immediately apparent that the separations among aromatic domains in the equatorial conformations of L-365,260 were much greater than any possible for LY288513, so these were excluded from further consideration. In comparing the axial conformations of L-365,260, the distances among the aromatic rings consistently suggested an overlap between the 4-phenyl group of LY288513 and the pendant phenyl of L-365,260, and between the 5-phenyl group of LY288513 and the fused benzo ring of L-365,260. However, no single superposition produced an obvious alignment of the molecules. It was therefore decided to retest the overlap using the enantiomers of the conformations of LY288513. In this manner there was discovered a remarkable three-dimensional congruence between the enantiomer of conformation 2 of LY288513 and the lowest energy conformation of L-365,260 (Figure 2). The spatial disposition of the three aromatic domains was very comparable between the two molecules,

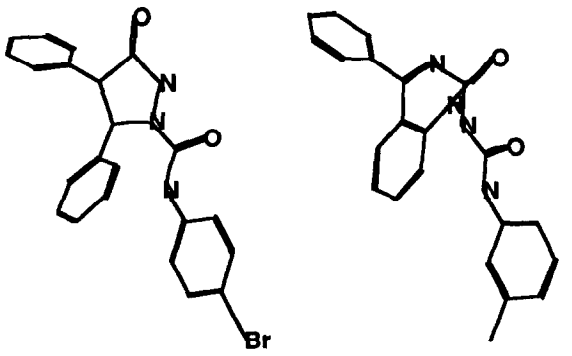


Figure 2

Comparison of enantiomer of conformation 2 of LY288513 (left) with conformation 1 of L-365,260

and, in addition, the planar orientations of each corresponding pair of rings were quite similar. The urea substructures also showed good spatial correspondence and alignment with one another. Such a high degree of overlap between very low energy conformations of the two structures strongly suggests that they represent the common mode of their binding to the CCK receptor. It is noteworthy that the pyrazolidinone and diazepine rings did not overlap or otherwise appear to map to one another; the heterocyclic rings thus appear to function primarily as scaffolding for the proper disposition of the aromatic and urea domains.

This result is at first puzzling in one respect, because it appears to correlate the CCK-B-selective isomer of L-365,260 with the isomer of LY262691 (LY288512, **2**) which has the least affinity for the CCK-B receptor. The selectivity within these racemates is only relative, however, and the CCK-B affinities of all the isomers of LY262691 and L-365,260 are well into the nanomolar range. Stereochemistry therefore appears not to be an absolute determinant of selectivity, despite the suggestion of this independently in certain portions of each series. (This is borne out in the related arylamidobenzodiazepines, which show a strongly biased pattern of preference.) Our current working model holds that the diphenylpyrazolidinone and benzodiazepine nuclei are in fact different manifestations of a common pharmacophore, either enantiomer of which can provide a basis for high-affinity interaction with either the CCK-A or CCK-B receptor. The actual affinity at and relative selectivity between CCK-A and CCK-B would then be a subtle function of all the features of the side-chain, including the type of linkage (urea, thiourea, or amide), the nature of the aromatic group, and its substitution pattern, along with the stereochemistry. Dependence on both stereochemistry and side-chain structure may explain, for instance, why LY288513 and L-365,260 show substantial divergence in the aromatic substituent structure-activity relationships on their homologous urea phenyl groups[2,6], since they also have apparently opposing absolute stereochemistry. The notion that a single nucleus, through attachment of different side-chains, can serve as a platform for ligands for multiple peptide receptors is already precedented for the benzodiazepines, certain of which are potent agonists for κ-opioid receptors[20]. Further modeling work, utilizing CCK-A selective compounds from these two series, should help determine the true generality of this model. It will also be of interest to extend the comparison to compounds from other series (e.g. the glutamic amides), to see whether it is possible to define a universal pharmacophore for CCK antagonists.

In conclusion, this modeling study has strongly substantiated the correspondence of several domains within these two series of CCK antagonists at the three-dimensional level, and has led to the proposal of a common pharmacophore for their binding to the CCK family of receptors. Because the discovery and elaboration of these series were completely unconnected, this would appear to represent a case of convergent evolution in the realm of medicinal chemistry.

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