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Quantitative Structure–Activity Relationship Study on Some Nonpeptidal Cholecystokinin Antagonists

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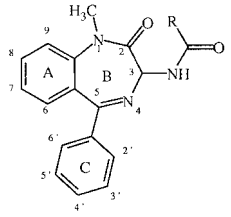
Abstract—A quantitative structure–activity relationship (QSAR) analysis has been performed on a series of 1,4-benzodiazepine derivatives, which were found to act as antagonists of cholecystokinin (CCK), a gastrointestinal peptide hormone. The CCK acts with three different receptor subtypes termed as CCK-A, CCK-B, and gastrin receptor, which can be found in peripheral system, brain, and stomach, respectively. With all the three subtypes, the binding of the compounds is found to significantly depend on the lipophilicity of the compounds and their ability to form the hydrogen bonds with the receptor. However, the binding sites in CCK-A receptor seem to be slightly rigid as compared to those in CCK-B or gastrin receptor. The latter two appear to have similar binding features.

Introduction

The cholecystokinin (CCK) is a gastrointestinal polypeptide hormone, closely related chemically to gastrin. It was supposed primarily to be implicated in the control of pancreatic and biliary secretion, gall bladder contraction, and gut motility,[1–4] but recent studies have pointed out a key role of it in the central nervous system as well, perhaps as a neurotransmitter or neurohormone.[3–7] In the central nervous system, it has been found to induce a variety of pharmacological effects such as hypothermia, analgesia, hyperglycemia, stimulation of pituitary hormone release, and decrease in exploratory behaviour, etc.[8]

The peripheral actions of CCK are mediated by a receptor subtype termed as CCK-A, while the central actions are mediated by a receptor subtype termed as CCK-B, for which the minimum agonist ligand required is CCK-4, i.e., Try-Met-Asp-Phe-NH_2,[9] which is identical to tetragastrin (G-4). A third receptor subtype, which appears to be closely related to CCK-B subtype, is the stomach gastrin receptor.[10]

The major naturally occurring molecular forms of CCK are polypeptides, but there have been found some nonpeptides to act as antagonist of CCK. A 1,4-benzodiazepine derivative (**1**), designated as MK-329 (formerly as L-364, 718), was found to be a highly potent and selective antagonist of CCK-A with receptor affinity comparable to that of the native peptide.[11–13] Similarly, another derivative of 1,4-benzodiazepine (**2**), designated as L-365, 260, has been recently found to possess high potency and selectivity for CCK-B and gastrin receptors.[14,15]



1, R= 2-indolyl (*S* – Configuration)

2, R= *m* – Me – phenylamino (*R* – configuration)

These nonpeptidal antagonists of CCK created great hope to find the substitutes of peptidal analogues, with which there remained always the problem of achieving good oral bioavailability. Consequently, variety of nonpeptidal ligands were studied, among which benzodiazepine derivatives were more widely investigated.[11–19] In order to find out the mechanisms of interaction of these ligands with CCK receptors and to rationalize the substituents selection, we undertook a QSAR study on them. Our two previous communications[19,20] have thrown enough light on the binding of some nonpeptidal CCK-antagonists. In this communication, we report a QSAR analysis on a series of benzodiazepine derivatives studied by Evans group.[11,14]

Materials and Methods

The series of compounds that were subjected to QSAR analysis are given in Table 1. These compounds were

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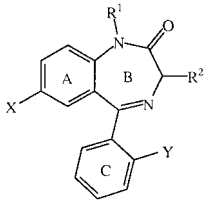
Table 1. The nonpeptidal CCK-antagonists (**3**) studied by Evans et al.[11,14]

Compound	X	Y	R^1	R^2	Stereo.
1	H	F	CH_3	NCHO-*p*-chlorophenyl	*S*
2	H	F	CH_3	NCHO-*p*-chlorophenyl	*R*
3	H	F	CH_2COOEt	NCHO-*p*-chlorophenyl	*RS*
4	H	F	H	NHCONH-*p*-Cl-Phe	*RS*
5	H	F	CH_2COOEt	NHCONH-*p*-Cl-Phe	*RS*
6	H	H	CH_2COOEt	NHCONH-*p*-Cl-Phe	*RS*
7	H	H	$CH_2CON(CH_2)_4$	NHCONH-*p*-Cl-Phe	*RS*
8	H	H	CH_3	NHCONH-*p*-Cl-Phe	*RS*
9	H	H	CH_3	NHCONH-*p*-Cl-Phe	*S*
10	H	H	CH_3	NHCONH-*p*-Cl-Phe	*R*
11	H	H	CH_3	NHCONH-*m*-Me-Phe	*RS*
12	H	H	CH_3	NHCONH-*m*-Me-Phe	*S*
13	H	H	CH_3	NHCONH-*m*-Me-Phe	*R*
14	Cl	H	H	CH_2-3-indolyl	*R*
15	Cl	H	H	CH_2-3-indolyl	*S*
16	Cl	H	CH_3	CH_2-3-indolyl	*R*
17	H	H	H	CH_2-3-indolyl	*R*
18	H	F	H	CH_2-3-indolyl	*R*
19	H	F	H	CH_2-3-indolyl	*S*
20	H	F	CH_3	CH_2-3-indolyl	*R*
21	H	F	CH_2COOH	CH_2-3-indolyl	*R*
22	H	H	H	NHCO-2-indolyl	*RS*
23	H	H	H	NHCO-3-indolyl	*RS*
24	H	F	H	$NHCOCH_3$	*RS*
25	H	H	CH_3	NHCO-2-indolyl	*RS*
26	H	H	CH_3	NHCO-2-indolyl	*S*
27	H	H	CH_3	NHCO-2-indolyl	*R*
28	H	H	CH_2COOH	NHCO-2-indolyl	*RS*
29	H	F	H	NHCO-2-indolyl	*RS*
30	H	F	CH_3	NHCO-2-indolyl	*RS*

studied by Evans et al.[11,14] for the binding with all the three subtypes of CCK receptors. Table 2 lists all the binding data reported by Evans et al.[11,14] along with logP values of the compounds (P = octanol/water partition coefficient). The logP values were calculated as suggested by Hansch and Leo.[21]

The binding data are in terms of IC_{50}, the molar concentrations leading to half maximal inhibitions of bindings of [^{125}I]CCK-8 to receptors in rat pancreatic tissues (CCK-A) and in guinea pig cerebral cortex (CCK-B) and the binding of ^{125}I-labelled gastrin to guinea pig gastric glands. On these binding data, a Hansch type analysis has been performed to correlate them with various physicochemical and structural properties of the compounds. Only ClogP (calculated logP) has been found to be an important parameter.



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Results and Discussion

A multiple regression analysis performed on all the binding data revealed that CCK-A binding data can be significantly correlated with ClogP and various indicator variables as

$$\begin{aligned}\log(1/IC_{50})_{CCK\text{-}A} &= 2.642(\pm1.896)\mathrm{ClogP} \\ &\quad - 0.337(\pm0.265)(\mathrm{ClogP})^2 \\ &\quad + 0.842(\pm0.556)I_{R1} + 1.464(\pm0.626)I_{R2} \\ &\quad - 1.093(\pm0.955)I_X - 0.750(\pm0.620)I_R \\ &\quad + 1.531 \\ n = 30,\ r = 0.901,\ s = 0.69,\ &F_{6,23} = 16.54(3.71), \\ (\mathrm{ClogP})_o &= 3.92 \end{aligned} \quad (1)$$

The indicator variables I_{R1}, I_{R2}, and I_X stand, with a value of unity each, for the substituents $R^1 = CH_3$, R^2 = NHCO-*p*-chlorophenyl or NHCO-2-indolyl, and X = Cl, respectively, and are zero in each case for the other substituents. The variable I_R is meant for the stereospecificity of R^2-substituents. Compounds are present in *R*, *S*, or *RS* configuration. To account for the configuration effect, we defined two parameters I_R and I_S for *R* and *S* configurations, respectively, with a value of 1 each relative to *RS* taken equal to zero. Statistically significant effect was found to be associated with only I_R. Among the statistical parameters shown below the equation, n is the number of data points used to derive the equation, r is the correlation coefficient, s is the standard deviation, and F is the F-ratio between the variances of calculated and observed activities (the parenthesis enclose the F-value of 99% level). The data

Table 2. The nonpeptidal CCK-antagonists (**3**) and their binding affinities for three different receptors

Compound	ClogP	log(1/IC$_{50}$)					
		CCK-A		CCK-B		Gastrin	
		Obsd[a]	Calcd eqn (2)	Obsd[b]	Calcd eqn (3)	Obsd[b]	Calcd eqn (4)
1	3.938	9.40	8.99	5.54	5.19	5.61	5.34
2	3.938	8.54	8.08	4.96	5.19	5.12	5.34
3	4.638	6.96	7.96	5.73	5.85	6.17	6.23
4	3.775	6.96	7.02	6.53	7.81	6.20	7.04
5	4.896	6.66	6.47	8.92	8.86	8.39	8.47
6	5.103	6.44	6.25	9.00	9.05	8.48	8.74
7	5.340	6.29	5.95	9.55	9.27	9.30	9.04
8	4.409	7.30	7.56	7.64	8.41	7.66	7.85
9	4.409	7.60	7.56	6.39	8.40	6.27	7.85
10	4.409	5.96	6.65	8.26	8.41	7.92	7.85
11	3.893	8.10	7.73	8.15	7.92	8.51	7.19
12	3.893	8.53	7.73	8.83	7.92	6.89	7.19
13	3.893	6.56	6.82	8.70	7.92	8.96	7.19
14	4.525	5.47	4.74				
15	4.525	4.50	5.65				
16	4.551	5.86	5.44				
17	3.651	5.93	6.10				
18	3.444	6.30	6.06				
19	3.444	4.98	6.96				
20	3.631	6.56	6.82				
21	3.443	6.52	6.06				
22	3.354	8.32	8.21				
23	3.254	5.96	6.85				
24	1.310	4.40	4.43				
25	3.704	8.95	9.00				
26	3.704	9.09	9.00				
27	3.704	7.18	8.10				
28	3.517	8.85	8.26				
29	3.417	8.67	8.23				
30	3.497	8.85	8.98				

[a]For compounds **1**–**14** taken from ref 14 and for the rest from ref 11.
[b]Taken from ref 14.

with ± sign within the parentheses in the equation are 95% confidence intervals.

Equation (1) represents a highly significant correlation, accounting for 81% of the variance in the activity ($r^2 = 0.81$). This equation, however, predicts a very high activity for compound **19** (6.96) as compared to its observed activity (4.98). Therefore, if this compound, behaving as an outlier, is removed, a more significant correlation is obtained (eqn (2)), which accounts for 86% of the variance in the activity.

$$\begin{aligned}\log(1/IC_{50})_{CCK\text{-}A} &= 3.229(\pm 1.669)ClogP \\ &\quad - 0.429(\pm 0.235)(ClogP)^2 \\ &\quad + 0.723(\pm 0.491)I_{R1} + 1.268(\pm 0.552)I_{R2} \\ &\quad - 1.121(\pm 0.817)I_X - 0.909(\pm 0.541)I_R \\ &\quad + 0.932 \\ n = 29,\ r = 0.926,\ s = 0.59,\ &F_{6,22} = 22.16(3.76), \\ (ClogP)_o &= 3.76 \end{aligned} \quad (2)$$

However, both eqns (1) and (2) suggest that the CCK-binding will be largely governed by the hydrophobic property of the molecule, but since they represent parabolic correlation in ClogP, the ClogP will have an optimum value, essentially the same in both the equations (3.92 and 3.76). Since the binding data are of in vitro system, where the compounds were not to cross any lipophilic–hydrophilic barrier, the dependence of activity on hydrophobicity means the molecule can be involved in hydrophobic interaction with the receptor and the optimization of ClogP will mean the hydrophobic site at the receptor has the limited bulk tolerance.

The positive coefficients of variables I_{R1} and I_{R2} in the equations suggest that the binding affinity of the compounds for CCK-A receptor can be further enhanced by $R^1 = CH_3$ and $R^2 =$ NHCO-2-indolyl or NHCO-*p*-chlorophenyl. The CH_3 at N1 can be assumed to completely fit in the active site of the receptor, and in the NHCO-2-indolyl or NHCO-*p*-chlorophenyl group at 3-position, the NHCO moiety may be assumed to have optimum hydrogen bond interaction and 2-indolyl or *p*-chlorophenyl moiety to have optimum polar interaction with the receptor (Fig. 1). However, the interaction of any R^2 substituent in *R* configuration seems to be less advantageous than the racemate, as in both eqns (1) and (2) I_R has the negative coefficient. The variable I_S used for *S* configuration was not found to be statistically significant, hence the *S* configuration was assumed to produce little effect as compared to the racemate. The less advantageous role of *R* configuration may be due to its effect on optimal orientation of the R^2 substituent towards the receptor sites.

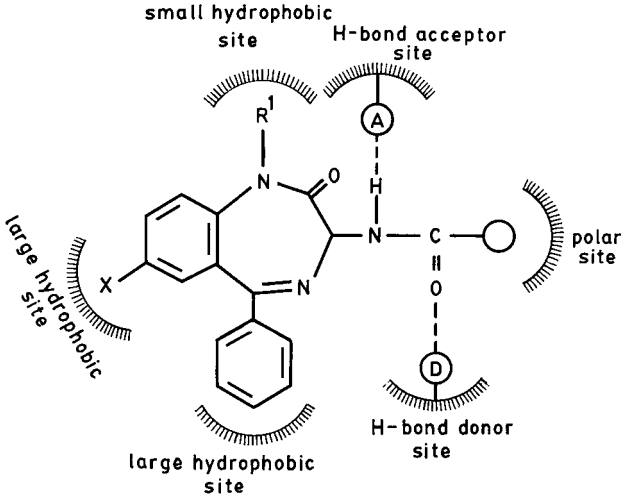

Figure 1. A model of interaction of a benzodiazepine derivative with CCK-A receptor. Only active sites of the receptor are shown.

In the case of CCK-B and gastrin receptor bindings, the best correlations that we could obtain were

$$\log(1/IC_{50})_{CCK\text{-}B} = 0.932(\pm 0.855)ClogP + 2.773(\pm 1.024)D_{R2} + 1.521 \quad (3)$$
$$n = 12,\ r = 0.922,\ s = 0.67,\ F_{2,9} = 25.64$$

$$\log(1/IC_{50})_{gastrin} = 1.281(\pm 0.820)ClogP + 1.912(\pm 0.963)D_{R2} + 0.292 \quad (4)$$
$$n = 11,\ r = 0.921,\ s = 0.60,\ F_{2,8} = 22.49$$

where the indicator variable D_{R2} has been used for R^2 substituents. It is equal to 1 for an R^2 substituent that contains NHCONH moiety and zero for the one that contains NHCO moiety. The large positive coefficients of this variable in both eqns (3) and (4) suggest that in both CCK-B and gastrin bindings, the NHCONH moiety would be more advantageous than NHCO moiety, while in CCK-A binding the latter has been shown to be better. Thus, the CCK-B and gastrin receptors seem to be slightly different from the CCK-A receptor. The former appear to offer more possibilities of hydrogen bonding than the latter, so that all the three hydrogen bonding sites of NHCONH, i.e. both NH moieties and CO, can be involved in hydrogen bonding with them and strengthen the drug-receptor interaction, while in the binding with CCK-A the additional NH group may produce some steric effect.

Additionally, since no other substituents except R^2 were found to play any role in CCK-B or gastrin binding, in both of them only the hydrogen bonding sites seem to play the effective role. However, the significant linear dependence of both CCK-B and gastrin bindings on logP indicates that there must be a large flexible lipophilic site in both CCK-B and gastrin receptors, unlike the one of limited bulk tolerance in CCK-A receptor, for hydrophobic interaction with the molecule.

In CCK-B and gastrin bindings the configurational parameters were also not found to be significant. Thus, all these studies lead to suggest that CCK-B and gastrin receptors have almost similar binding features, which are different from those in CCK-A receptor. In the derivation of eqn (3), however, compound **9** was not included and in the derivation of eqn (4) compounds **9** and **13** were not included. They had exhibited aberrant behaviour, the reasons for which were not apparent.

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