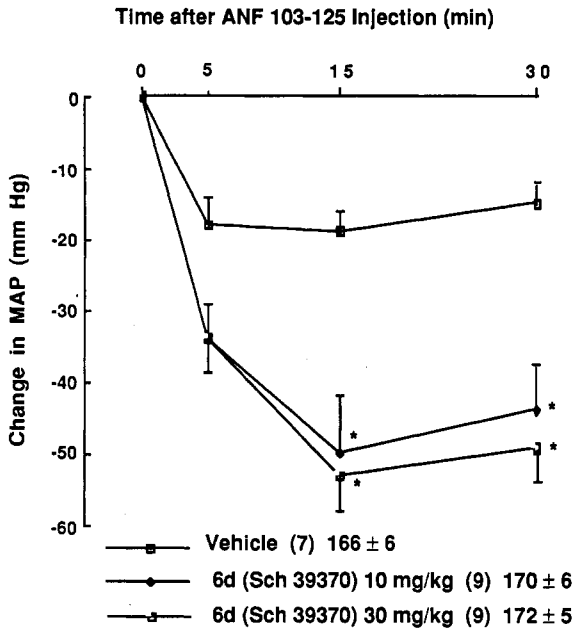

Figure 1. Potentiation of the antihypertensive effect of ANF 103–125, in SHR, by **6d** (Sch 39370); numbers in parentheses are number of animals in group, followed by baseline MAP ± SE.

results show that selective NEP inhibitors can enhance the magnitude and duration of the hypotensive activity of ANF.

Additional evidence that an NEP inhibitor prolongs the half-life of ANF was obtained by studying the plasma half-life of immunoreactive ANF in conscious rats following ANF 99–126 infusion (0.1 and 1 μg/kg per min for 30 min). The disappearance of immunoreactive ANF from the plasma was significantly prolonged in animals pretreated with compound **6d**.[14] Having several pieces of evidence that NEP inhibitors could potentiate exogenous ANF, experiments were next performed to determine if NEP inhibition could elicit a hypotensive response consistent with potentiation of endogenous ANF. The effects of the isoserine analogue **6d** were evaluated in rats treated with deoxycorticosterone acetate and salt (DOC salt rats), a volume-dependent model of hypertension in which endogenous levels of ANF are known to be elevated.[15] Treatment of these hypertensive rats with isoserine compound **6d** (30 mg/kg subcutaneously) resulted in a significant reduction in arterial pressure (Figure 2). The onset of hypotensive response was within 1–2 h of injection and the effect was sustained for the 4-h duration of the study. In spite of the fall in blood pressure, the heart rate was not altered significantly by **6d**. A high dose of the ACE inhibitor captopril (30 mg/kg subcutaneously) did not alter the blood pressure significantly in these DOC salt rats.[14]

The results of these studies provide evidence that NEP inhibition prevents degradation of ANF, enhances its hypotensive activity, and lowers blood pressure in a model of volume-dependent hypertension, the DOC salt rat. Although the precise role of ANF in the antihypertensive action of compound **6d** (Sch 39370) remains to be established, these results suggest that inhibition of NEP represents a novel mechanism by which to reduce arterial

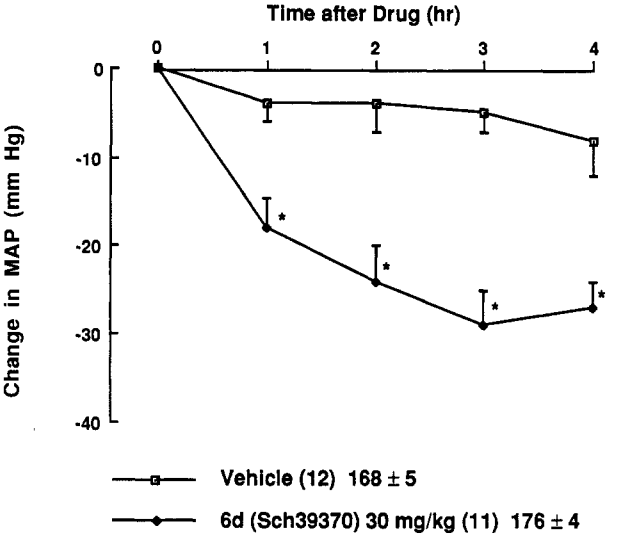

Figure 2. Hypotensive effect of **6d** (Sch 39370) as compared to vehicle in DOC salt rats; numbers in parentheses are number of animals in group, followed by baseline MAP ± SE.

blood pressure. The clinical significance of this action remains to be established.

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Hybrid Cholecystokinin (CCK) Antagonists: New Implications in the Design and Modification of CCK Antagonists†

Sir:

Recently two potent cholecystokinin (CCK) antagonists have been disclosed, CR 1409 (**1**), a proglumide derivative, which possesses enhanced potency over its progenitor,[1] and L-364,718 (**2**), a novel benzodiazepine[2a] with increased potency over CR 1409 and good selectivity[3] for peripheral CCK receptors[4] (CCK type A receptors). These disclosures have prompted considerable interest in understanding the structural interrelationships among the various classes of nonpeptide CCK antagonists as well as their relationship to CCK peptide counterparts. We report here our own efforts directed toward establishing a link between the proglumide and benzodiazepine series which has resulted

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(15) Snajdar, T.; Rapp, J. P. *Biochem. Biophys. Res. Commun.* **1986**, *137*, 876.

(1) Makovec, R.; Chiste, R.; Bani, M.; Pacini, M. A.; Setnikar, I.; Rovati, L. A. *Arzneim.-Forsch./Drug Res.* **1985**, *35*(II), 1048–51. Makovec, F.; Bani, M.; Chiste, R.; Revel, L.; Rovati, L. C.; Rovati, L. A. *Ibid.* **1986**, *36*(I), 98–102.
(2) (a) Evans, B. E.; Bock, M. G.; Rittle, K. E.; DiPardo, R. M.; Whitter, W. L.; Veber, D. F.; Anderson, P. S.; Freidinger, R. M. *Proc. Natl. Acad. Sci. U.S.A.* **1986**, *83*, 4918–22. (b) Parsons, W. H.; Patchett, A. A.; Davidson, J. L.; Chang, R. S. L.; Lotti, V. J.; Chen, T. B.; Smith, G. M.; Holloway, M. K. 20th National Medicinal Chemistry Symposium, June 15–19, 1986, Chapel Hill, NC, Abstract 30.
(3) Chang, R. S. L.; Lotti, V. J. *Proc. Natl. Acad. Sci. U.S.A.* **1986**, *83*, 4923–6.
(4) Moran, T. H.; Robinson, P. H.; Goldrich, M. S.; McHugh, P. R. *Brain Res.* **1986**, *362*, 175. Innis, R. B.; Snyder, S. H. *Proc. Natl. Acad. Sci. U.S.A.* **1980**, *77*, 6917. Hill, D. R.; Campbell, N. J.; Shaw, T. M.; Woodruff, G. N. *J. Neurosci.* **1987**, *7*, 2967.

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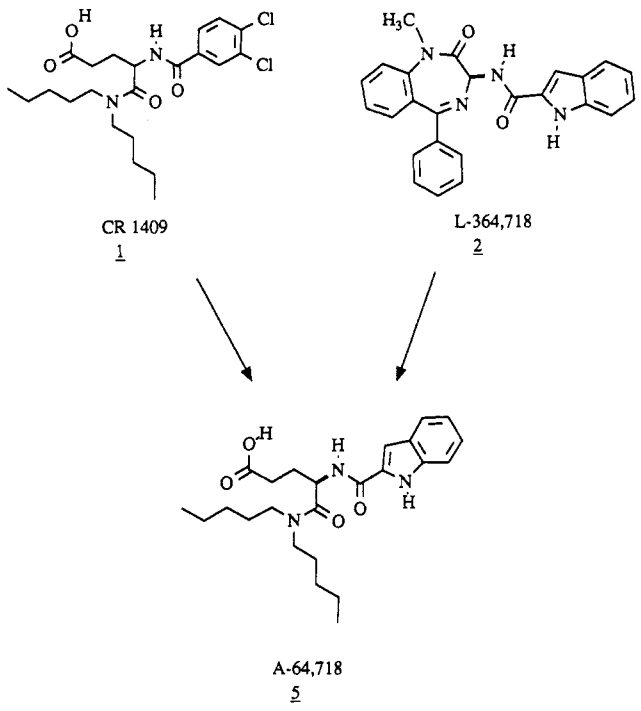


Figure 1.

in hybrid CCK antagonists exhibiting improved activity over the current members of the proglumide class.

Our initial studies focused on established stereoselectivities for the enantiomers of CR 1409, since this issue had not been addressed previously.[5] The optically pure enantiomers were prepared in a four-step sequence from the *S*(L) and *R*(D) forms of *N*-(*tert*-butyloxycarbonyl)glutamic acid γ-benzyl ester. First, the acids were coupled with dipentylamine with BOPCl [bis(2-oxo-3-oxazolidinyl)-phosphinic chloride][6] at −20 to 0 °C in CH_2Cl_2. The resultant amides were deprotected (HCl in dioxane at 0–5 °C) and then coupled with 3,4-dichlorobenzoyl chloride in THF. Finally, removal of the benzyl group was accomplished via transfer hydrogenolysis utilizing cyclohexadiene as the reductant.[7,8] Surprisingly, comparison of the enantiomers, **3** (*R*) and **4** (*S*), demonstrated that the unnatural *R*(D) isomer had a 70-fold higher binding affinity for pancreatic acinar cells and a 48-fold increased potency in inhibiting CCK_8-stimulated amylase release over that of the natural isomer.[9] Previously, Evans et al.[2a] had demonstrated that the *S* enantiomer of the 3-amido-substituted benzodiazepine **2** was more potent than its *R* isomer. We attempted to relate these two structurally different enantiomers (**2** and **3**) because of their similar selectivities for the type A CCK receptor. We perceived that the dichlorobenzoyl residue of **3** may be related to the indolyl-2-carbonyl function of L-364,718 (Figure 1). Since Evans and co-workers had shown that the indolyl-2-carbonyl moiety was responsible for significantly enhancing affinity and improving potency, we reasoned that introduction of this moiety in the proglumide-like series should provide a more potent compound. Accordingly, we then prepared compound **5** (A-64,718) in a manner similar to compound **3**, utilizing indole-2-carboxylic acid and standard carbodiimide methods in the coupling step.[8] Compound **5** proved to be a more potent CCK antagonist than **3** (4-fold increase in binding affinity and 5-fold increase in inhibition of amylase release), thus adding some credence to our hypothesis.

In an effort to further elucidate the relationship between these two antagonist series, we examined L-364,718 (**2**) and the hybrid A-64,718 (**5**) by using molecular modeling techniques[10] (Figure 2). Both compounds were first subjected to molecular dynamics simulations (800 K) for 30 ps and the conformations generated every picosecond were minimized to their low-energy forms. The low-energy conformations thus generated were used for comparison. Superimposition of the indolyl-2-carbonyl moieties in low-energy conformations of **2** and **5** results in the further overlapping of the pentyl side chains in **5** with the 5-phenyl and fused aromatic rings of L-364,718. The extensive overlapping of hydrophobic residues and the heteroaromatic nuclei suggested a functional homology between these two antagonists. If this functional homology between **2** and **5** was valid, then the SAR reported for the benzodiazepine series should be paralleled in the glutamate series. We examined this possibility further by preparing several analogues of A-64,718 suggested by the initial work on the L-364,718 series and related compounds (Table I). As expected, the *S* enantiomer **6** was less potent by 10-fold. The indolyl-3-carbonyl analogue **7** also proved less potent in binding and amylase inhibition, following the trend reported by Evans.[2a] Parsons[2b] has described a series of benzolactam-based antagonists (Figure 3) which has been correlated with L-364,718. Within this series the 2-naphthyl (**13**) and 2-indolyl (**14**) substitutions were of comparable potency. Thus, substitution of the 2-naphthyl group in place of the 2-indolyl in compound **5** was examined. The resultant compound (**8**) was found to be equipotent with the lead **5**, while the 1-naphthyl substitution **9** demonstrated marked decreases in affinity.

Reasoning that reintroduction of an aromatic nitrogen may impart higher affinity, various quinoline isomers were prepared. Although all of these analogues were fairly potent, the 3-quinolinyl isomer **10** (A-65,186) proved even more potent than the 2-naphthyl analogue **8**. Since compounds **8**, **10**, **11**, and **12** are topologically identical with

(5) Preliminary reports of our work were presented at the Cold Spring Harbor Workshop on "CCK Antagonists", Nadzan, A. M.; Kerwin, J. F., Jr.; Kopecka, H.; Lin, C. W.; Miller, T.; Witte, D.; Burt, S. *Cholecystokinin Antagonists, Neurology and Neurobiology, Vol. 47.* Wang, R. Y., Schoenfeld, R., Eds.; Alan R. Liss, Inc.: New York, 1988; pp 93–103, and at the 21st National Medicinal Chemistry Symposium, June 19–23, 1988, Minneapolis, MN. Since these presentations, reports addressing this point have appeared: Makovec, F.; Chiste, R.; Rovati, L. C.; Setnikar, I. *Gastroenterology* **1988**, *94*(5), A279. Freidinger, R. M. European Patent Application 87305088.4.

(6) Tung, R. D.; Rich, D. H. *J. Am. Chem. Soc.* **1985**, *107*, 4342–43.

(7) Hydrogenolyses were run in ethanol with 10% palladium in carbon as the catalyst. Hydrogenolyses performed with ammonium formate as reductant or coreductant led to partial dechlorination of the aromatic ring.

(8) All compounds gave satisfactory NMR, MS, and C, H, N analyses (±0.4%). Enantiomeric compounds possessed equal and opposite rotations throughout their synthetic sequences. Additionally, the enantiomeric purity of the (*R*)- and (*S*)-N^{α},N^{α}-di-*n*-pentylglutamate γ-benzyl ester hydrochlorides was assessed by preparing (*S*)-α-methoxy-α-(trifluoromethyl)-phenylacetamides. ^1H and ^{19}F NMR spectroscopy indicated less than 0.5% cross contamination by the other enantiomer.

(9) The radioligand used in the binding assays was ^{125}I-Bolton-Hunter-CCK_8. The antagonism of amylase release is measured against a 0.3 nM concentration of CCK_8. Protocols for these assays are provided in: Lin, C. W.; Bianchi, B.; Grant, D.; Miller, T.; Danaher, E. A.; Tufano, M. D.; Kopecka, H.; Nadzan, A. M. *J. Pharmacol. Exp. Ther.* **1986**, *236*, 729–34.

(10) The program Discover was used for the energy-minimization study. Structures generated every picosecond were minimized (gradients within 0.01 kcal/mol). The structures that were compared do not necessarily represent the global minima. Cf. Dauber, P.; Osguthorpe, D.; Hagler, A. T. *Biochem. Soc. Trans.* **1982**, *10*, 312–18.

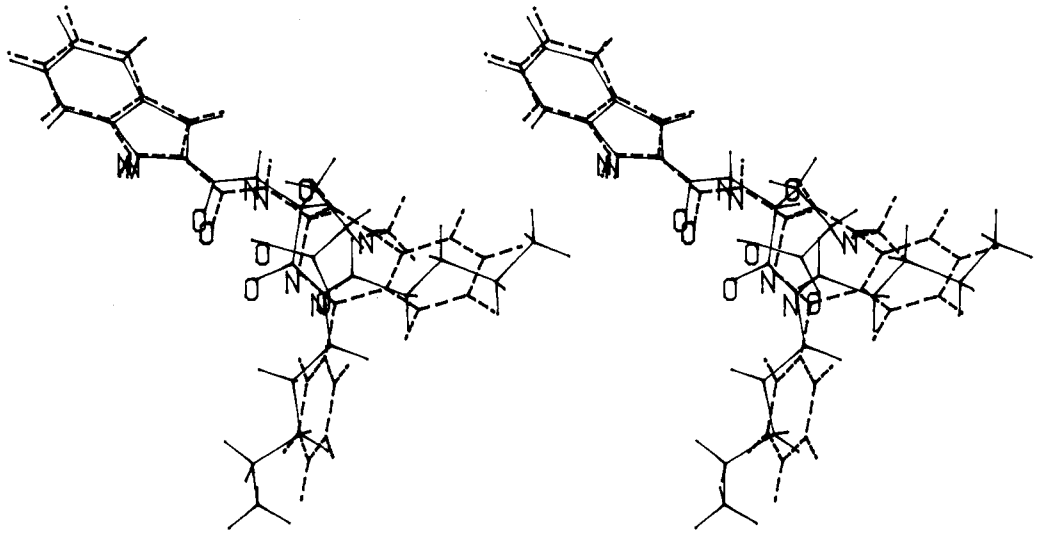
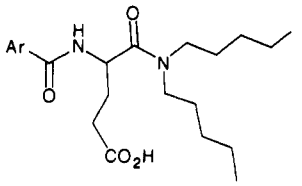
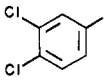
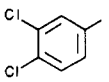
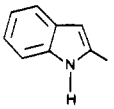
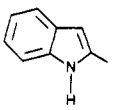
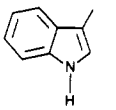
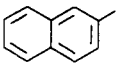
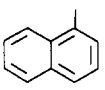
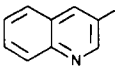
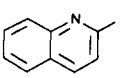
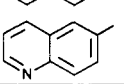


Figure 2.

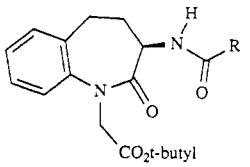
Table I. Structure and Biological Activities for CCK Antagonists



compd	Ar	enantiomer	binding affinity: IC_{50},[a] nM		inhibn of amylase release: IC_{50},[a] nM
			pancreas	cortex	
3		*R*	66 ± 30 (5)	12400 ± 1300 (3)	180 ± 10 (3)
4		*S*	4900 ± 2100 (5)	48000 (2)	11000 ± 3100 (3)
5		*R*	19 ± 2.6 (4)	1300 ± 88 (4)	36 (2)
6		*S*	200 ± 32 (5)	19000 ± 210 (3)	590 ± 170 (5)
7		*R*	260 ± 46 (5)	32000 ± 4400 (3)	830 ± 170 (3)
8		*R*	12 ± 6.6 (4)	4200 ± 600 (3)	36 ± 9 (3)
9		*R*	320 ± 260 (3)	11000 ± 1300 (3)	280 ± 50 (3)
10		*R*	5.1 ± 1.7 (7)	3500 ± 770 (6)	16 ± 3.6 (3)
11		*R*	63 ± 31 (5)	4200 ± 710 (4)	110 ± 3.7 (3)
12		*R*	31 ± 2.2 (3)	11000 (2)	65 ± 14 (3)

[a] Standard deviations are shown where available. The number of determinations are in parentheses.

one another, direct comparison of these compounds indicated a preferred orientation of the aromatic nitrogen atom. Whether this preference results from a direct interaction of the nitrogen atom at the receptor binding site



13 R = 2-naphthyl

14 R = 2-indolyl

Figure 3.

or from an electronic perturbation of the aromatic ring is currently being investigated.

In conclusion, we have determined the stereochemical preferences of the CCK receptor for the *R*(D) enantiomer of CR 1409 and related analogues and have proposed a functional identity between glutamate-derived CCK antagonists and the 3-amidobenzodiazepine series represented by L-364,718. Our initial efforts have resulted in the compound A-65,186 (**10**), which possesses both good potency at CCK type A receptors and high selectivity (700-fold) for type A over type B CCK receptors. Currently A-65,186 is being utilized for additional pharmacological and chemical investigations, the results of which will be forthcoming.[11]

(11) Vickroy, T. W.; Bianchi, B.; Kerwin, J. F., Jr.; Kopecka, H.; Nadzan, A. M. *Eur. J. Pharmacol.* **1988**, *152*, 371–72. Britton, D. R.; Yahiro, L.; Cullen, M. J.; Kerwin, J. F., Jr.; Kopecka, H.; Nadzan, A. *Pharmacol., Biochem., Behav.* Submitted.

† This paper is dedicated to Professor Kenneth L. Rinehart, Jr. in honor of his 60th birthday.

‡ Computer Assisted Molecular Design Group.

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Received October 3, 1988

Articles

Analogues of Cisplatin Derived from Diaminodideoxytetritols. Synthesis and Activity against the ADJ/PC6 Plasmacytoma in Mice

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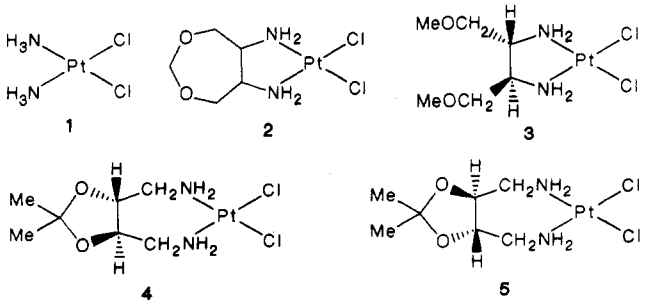
Four new analogues of the anticancer drug cisplatin have been prepared that contain a diaminodideoxytetritol derivative as the amine ligand moiety, and their activities have been measured against the ADJ/PC6 plasmacytoma in mice. Two of these compounds, the enantiomers of *cis*-dichloro(1,4-diamino-1,4-dideoxy-2,3-*O*-isopropylidenethreitol)-platinum(II), show a higher TI value than cisplatin when administered by intraperitoneal injection and, importantly, show significant antitumour activity when administered orally.

Continuing interest[1-4] in the development of analogues of the anticancer drug cisplatin (**1**), which, for example, are less toxic, have increased clinical efficacy and have a broader spectrum of activity than the parent compound, and recent reports[5,6] of analogues containing diamino carbohydrate derivatives, led us to prepare four new complexes **2–5**, each of which contain a diaminodideoxytetritol derivative in place of the amine ligands present in **1**. Our reasoning for this line of investigation hinged on the concept that subtle changes in the lipophilic–hydrophilic nature of such platinum complexes might lead to useful differences in their chemotherapeutic properties. Further, we reasoned that complexes that contained protecting groups on the organodiamine moiety that might be removed under particular physiological conditions, for example the acetal residues of **2**, **4**, and **5**, could have a novel type of anticancer action, especially if release were to be triggered within or close to the cancer cell. We report the synthesis of these platinum complexes and results of tests on the ADJ/PC6 plasmacytoma in mice.



1 **2** **3**

4 **5**

† University of East Anglia.

‡ Johnson Matthey Technology Centre.

(1) *Cisplatin: Current Status and New Developments*; Prestayko, A. W., Crooke, S. T., Carter, S. K., Eds.; Academic Press: New York, 1980.

(2) Cleare, M. J. In *Structure–activity Relationships of Antitumour Agents*; Reinhoudt, D. N., Connors, T. A., Pinedo, H. M., van de Poll, K. W., Eds.; Martinus Nijhoff, The Hague, 1983; pp 59–91.

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