Pergamon

Bioorganic & Medicinal Chemistry Letters 11 (2001) 705–709

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

Design of Non-Peptide CCK_2 and NK_1 Peptidomimetics Using 1-(2-Nitrophenyl)thiosemicarbazide as a Novel Common Scaffold

Edward K. Dziadulewicz,* Christopher S. Walpole,† Christopher R. Snell, Roger Wrigglesworth,‡ Glyn A. Hughes, David Beattie,§ John N. Wood,‖ Margaret M. Beech and Paul R. Coote

Novartis Institute for Medical Sciences, 5 Gower Place, London WC1E 6BN, UK

Received 6 September 2000; revised 29 December 2000; accepted 12 January 2001

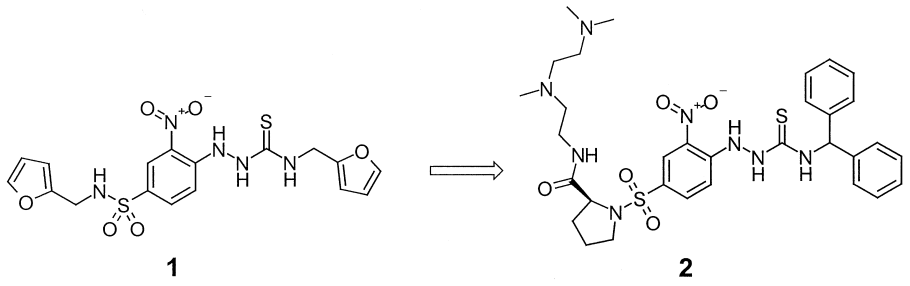
Abstract—A β-turn overlay hypothesis has been used to transform the core scaffold of a selective non-peptide bradykinin B_2 receptor antagonist into ligands specifically recognized by the CCK_2 or NK_1 receptors.

Introduction

Recently we disclosed a novel, potent, orally-active non-peptide bradykinin (BK) B_2 receptor antagonist (**2**).[1,2] The compound was developed from a high-throughput screening lead (**1**) by optimizing the binding determinants projecting from a nitrophenylthiosemicarbazide (TSC) framework (Fig. 1). The drastic loss of binding potency incurred upon alteration of this framework[1] suggested a unique role in its presentation of pharmacophoric information to the B_2 receptor subtype. If the TSC is somehow acting as a surrogate for the C-terminal type II′ β-turn in what is believed to be the biologically-relevant conformation of BK,[3] then it should be possible to extrapolate this model (Fig. 2) to mimic other peptides in whose biologically-active forms a β-turn is a postulated structural motif.[4] In this communication we report our results in the de novo design of novel TSC-based lead compounds for the central cholecystokinin (CCK_2) and neurokinin-1 (NK_1) receptors.

Peptidomimetic Design Strategy

In extending the aforementioned overlay model to other peptides, a number of assumptions were made: (1) the β-turn is the most important structural feature for receptor recognition;[4,5] (2) there is a high degree of peptide/non-peptide spatial coincidence at the binding site;[6] (3) the TSC functions purely as a scaffold without



1 **2**

Figure 1. Optimization of BK B_2 receptor screening lead.

*Corresponding author. Tel.: +44-20-7333-2167; fax: +44-20-7387-4116; e-mail: ed.dziadulewicz@pharma.novartis.com
†Present address: AstraZeneca R&D Montréal, 7171 Frédérick-Banting, St-Laurent, Québec, Canada, H4S 1Z9
‡Present address: Institut de Recherche Jouveinal, Parke-Davis, 3–9 Rue de la Loge, B.P. 100, 94265 Fresnes Cedex, France.
§Present address: Novartis Horsham Research Centre, Wimblehurst Road, Horsham, West Sussex, RH12 5AB, UK.
‖Present address: Department of Biology, University College London, Darwin Building, Gower Street, London WC1E 6BT, UK.

0960-894X/01/$ - see front matter
PII: S0960-894X(01)00044-0

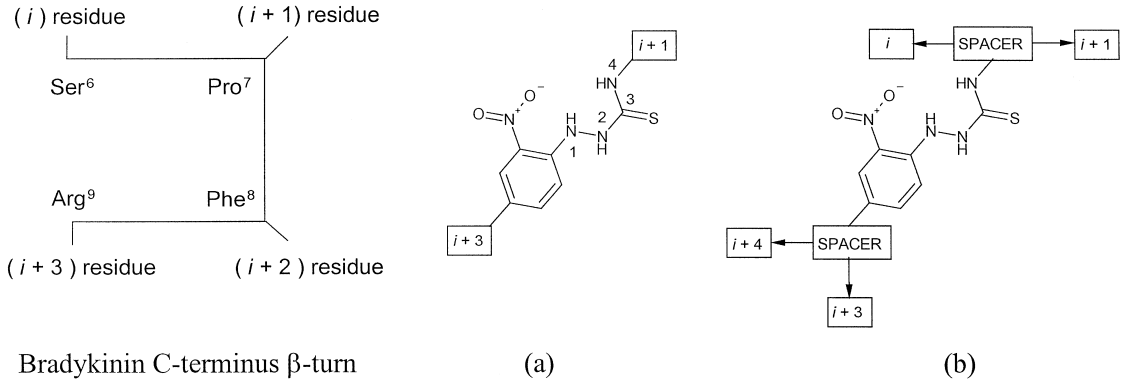


Figure 2. Schematic illustration of β-turn overlay model.

any specific receptor interaction;[7] (4) the TSC spans the β-turn from the $i+1$ position to the $i+3$ position with the same directionality as indicated in Figure 2a.[8]

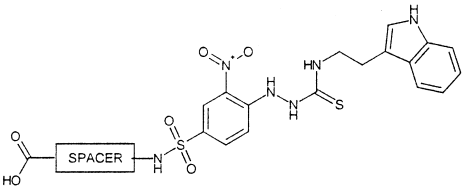
Where residues of the turn which are known to be important for activity are not aligned along the $i+1$-to-$i+3$ axis, the intention was to use spacer groups to access, for example, positions i or $i+4$ from the central core (Fig. 2b). We defined as first generation those compounds mimicking two of the β-turn side chains of the target peptide.[9] In each case optimization of the spacer groups would be guided by biological data from the receptor binding assays, and rationalized by modeling studies. Any structural leads would then be further elaborated in an attempt to mimic a third side chain (second generation compounds). The overlay would be supported, albeit circumstantially, if the second generation compounds were more active than the first, in accordance with the three-point binding model for small molecule interactions with peptide receptors.[10]

CCK_2 Peptidomimetics

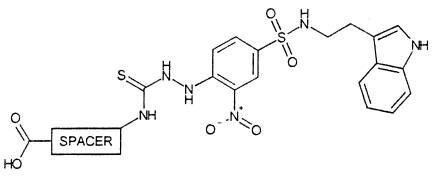
Several biologically active forms of the polypeptide hormone CCK exist, with CCK-33, CCK-8, and CCK-4 predominating in the periphery and CCK-8 (CCK-8S; Asp^{26}-$Tyr[SO_3H]^{27}$-Met^{28}-Gly^{29}-Trp^{30}-Met^{31}-Asp^{32}-Phe^{33}-NH_2) being the prevailing form in the CNS.[11] CCK mediates its diverse biological effects through two receptor subtypes, CCK_1 (the peripheral or alimentary receptor; formerly designated CCK_A) and CCK_2 (the central or brain receptor; formerly designated CCK_B). The CCK_2 receptor was regarded as an interesting therapeutic target, especially in light of its possible role in pain.[12]

Conformational analysis of native CCK-8 using high resolution ^1H NMR spectroscopy, suggested the existence of a β-turn for the Gly^{29}-Trp^{30}-Met^{31}-Asp^{32} sequence.[13] The TSC backbone was overlaid onto Ac-CCK-4 (Ac-Trp^{30}-Met^{31}-Asp^{32}-Phe^{33}-NH_2), which can adopt a β-turn orientation in an accessible low energy conformation. Using established protocols,[1] a series of

Table 1. In vitro CCK_2 and BK B_2 receptor affinities (IC_{50}) of compounds **3**–**10**



3-8

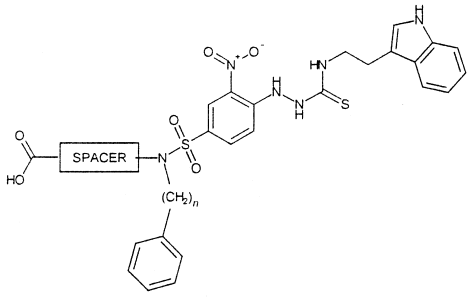


9-10

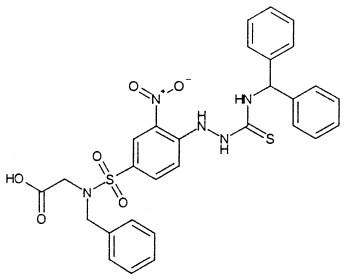
Compd[a]	Spacer	CCK_2 IC_{50} (μM)[b]	B_2 IC_{50} (μM)[c]
3	CH_2	46	>100
4	$(CH_2)_2$	24	>100
5	$(CH_2)_3$	11	>100
6	1,2-Disubstituted Ph	4.5±0.86[d]	>100
7	1,3-Disubstituted Ph	3.6±0.75[d]	>100
8	1,4-Disubstituted Ph	10	>100
9	1,3-Disubstituted Ph	31	>100
10	1,4-Disubstituted Ph	24	>100
CCK-8S		0.0013±0.00025 [e]	

[a]All the compounds gave satisfactory ^1H NMR, mass spectra, and elemental microanalyses.
[b][^{125}I-$Tyr(SO_3H)^{27}$]-CCK-8 binding assay (see ref 15). IC_{50} values were determined from concentration–response curves at mouse cerebral cortical membranes expressing the CCK_2 receptor. Unless otherwise stated, the values shown are the average of duplicate determinations.
[c][^{3}H]-BK binding assay (see ref 2). IC_{50} value limits were determined from concentration–response curves at the rat B_2 BK receptor (expressed in NG108-15 neuroblastoma–glioma hybrid cell membranes).
[d]Mean IC_{50} values (±SEM) calculated from $n=3$ replicate determinations.
[e]K_i value from ref 15.

Table 2. In vitro CCK_2 and BK B_2 receptor affinities (IC_{50}) of compounds **11**–**17**



11-16



17

Compd[a]	Spacer	*n*	CCK_2 IC_{50} (μM)[b]	B_2 IC_{50} (μM)[c]
11	CH_2	1	1.8±0.49[d]	>100
12	CH_2	2	6	>100
13	CH_2	3	6	>100
14	$(CH_2)_2$	1	0.51±0.02[d]	>100
15	$(CH_2)_3$	1	2.43±0.07[d]	>100
16	1,3-Disubstituted Ph	1	4	>100
17			1.9±0.55[d]	0.41±0.13[d]
CCK-8S			0.0013±0.00025[e]	

[a–e]See corresponding footnotes in Table 1.

first generation compounds was then synthesized in which the side chains of positions $i+1$ (Trp^{30}) and $i+3$ (Asp^{32}) were mimicked (**3**–**10**). Table 1 summarizes the binding affinities of this initial set of compounds against the mouse CCK_2 receptor expressed as IC_{50} values.[14]

Table 1 shows that CCK_2-selectivity can be conferred upon the TSC core simply by appending the appropriate functionality, as dictated by the structure of the amino acid side chains in the design template, in this case, the putative CCK-8 β-turn. There is a gradual if modest increase in binding affinity at the CCK_2 receptor as the spacer chain length is increased (compounds **3**, **4** and **5**). Introducing conformational constraint in the spacer chain improves affinity (**6** vs **4**; **7** vs **5**) until a certain distance from the TSC core is reached, at which point potency begins to fall off again (**8**). Interestingly, compounds in which the TSC directionality is reversed bind less well to the CCK_2 receptor (**9** vs **7**; **10** vs **8**), supporting our overall design concept.

A series of second generation compounds was then pursued, based on structures **3**–**7**, in which a mimetic of the $i+4$ (Phe^{33}) side chain was sought from the sulphonamide terminus (Table 2). Simple *N*-benzylation of compounds **3**, **4** and **5** afforded the corresponding analogues **11**, **14** and **15** with attendant increases in CCK_2 affinities of 26-, 47- and 5-fold, respectively. However, the constrained compound **7** was not amenable to the same approach (**16** vs **7**), and no further benefit was derived from homologation of the arylalkyl chain, either singly (**12**, **13** vs **11**), or in tandem with the carboxyalkyl chain (data not shown). Given the proximity of the benzyl substituent to the sulphonamide moiety, the increase in affinities of **11**, **14** and **15** may originate from a reduction in the conformational mobility of the carboxyalkyl chains upon sulphonamide *N*-alkylation, rather than from occupation of an auxiliary binding site. Indeed, the degree of CCK_2 binding exhibited by **14**, the lead to emerge from this series, would suggest a two-point receptor interaction (Fig. 3). In comparison with other potent CCK_2 antagonists,[16] the separation between the $i+1$ and $i+3/i+4$ terminals may be too great.

The level of receptor specificity was governed by the extent of side-chain modulation. Changing from the

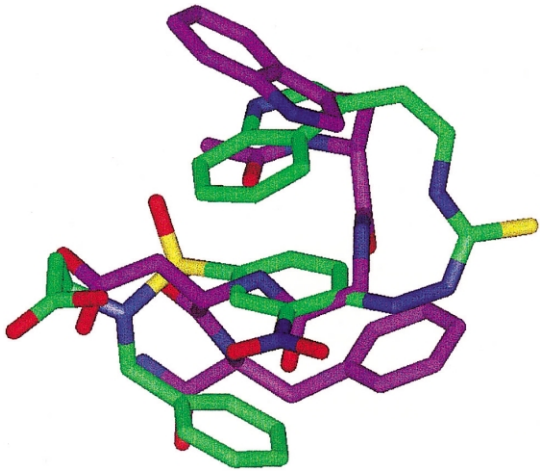
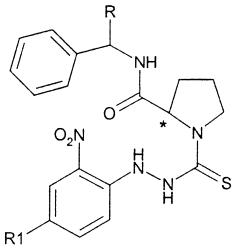


Figure 3. Overlay of a low energy conformation of compound **14** (green) with Ac-CCK-4 (purple) held in a Type I β-turn. The side chains were energy-minimized using a 'Conjugate Gradient' protocol, and the $i+2$ side chain Met^{31} was omitted for clarity. Compound **14** was subject to molecular dynamics for 250 picoseconds at 1000 K, and at every picosecond the conformation was sampled and exhaustively minimized to 300 K using the 'Conjugate Gradient' protocol to give 250 low energy structures. The lowest energy conformer was then overlaid with the peptide template (rms = 1.86). The molecular modeling was performed using the SYBYL suite (version 6.6) and the Discover energetics module with the 'cvff' force field (both Tripos Assoc., St. Louis, MO, USA).

Table 3. In vitro NK_1 and BK B_2 receptor affinities (IC_{50}) of compounds **18**–**23**



18-23

Compd[a]	R	R_1	(*)	NK_1 IC_{50} (μM)			B_2 IC_{50} (μM)[e]
				Rat[b]	Rabbit[c]	GPI[d]	
18	H	$SO_2NH(CH_2)_2SCH_3$	(*S*)	>100	>100	ND	>100
19	H	$SO_2NH(CH_2)_2SCH_3$	(*R*)	>100	>100	ND	>100
20	Ph	H	(*S*)	>100	0.83±0.23	1.67±0.15	>100
21	Ph	H	(*R*)	>100	~50	2.31±0.57	>100
22	Ph	$SO_2NH(CH_2)_2SCH_3$	(*S*)	ND	0.59±0.24	0.98±0.16	>100
23	Ph	$SO_2NH(CH_2)_2SCH_3$	(*R*)	ND	>50	7.93±0.07	>100
CP-99,994[f]						0.0029±0.0007	

[a]All the compounds gave satisfactory ^1H NMR, mass spectra, and elemental microanalyses.
[b]Rat NK_1 binding assay: displacement of [^{3}H][Sar^{9}Met(O$_2$)^{11}]SP binding to rat whole forebrain membranes (see ref 22). ND = Not determined.
[c]Rabbit NK_1 binding assay: displacement of [^{3}H][Sar^{9}Met(O$_2$)^{11}]SP binding to rabbit whole brain membranes (see ref 22). Data represent the mean IC_{50} values ± SEM calculated from 3 separate experiments.
[d]Guinea pig ileum organ bath bioassay: antagonism of smooth muscle contractions evoked by [Sar^{9}Met(O$_2$)^{11}]SP (see ref 22). Data represent the mean IC_{50} values ± SEM calculated from 3 or 4 separate experiments. ND = Not determined.
[e][^{3}H]-BK binding assay (see ref 2). IC_{50} value limits were determined from concentration–response curves at the rat B_2 BK receptor (expressed in NG108-15 neuroblastoma–glioma hybrid cell membranes).
[f](+)-(2*S*,3*S*)-3-(2-Methoxybenzylamino)-2-phenylpiperidine.

indolylethyl TSC **11** to the benzhydryl analogue (**17**) restores B_2 affinity without altering the CCK_2 binding profile. Hirschmann et al.[17] have proposed that such polyvalency is indicative of significant similarities in the topologies of the receptor binding domains; similarities which are not revealed by the endogenous peptide ligands.

NK_1 Peptidomimetics

The preferred endogenous ligand for the NK_1 receptor is the undecapeptide neurotransmitter Substance P (SP; Arg^1-Pro^2-Lys^3-Pro^4-Gln^5-Gln^6-Phe^7-Phe^8-Gly^9-Leu^{10}-Met^{11}-NH_2).[18] The therapeutic potential of NK_1 antagonists arises from the involvement of SP in the pathogenesis of a number of diseases, and in processes such as pain transmission and neurogenic inflammation.[19]

As with BK, a β-turn has been observed in the final four C-terminal residues of SP, Phe^8-Gly^9-Leu^{10}-Met^{11}, by ^1H NMR experiments. It has also been suggested that the Gly^9-Leu^{10} dipeptide portion plays a structural role, helping to orient the Phe^8 (*i*) and Met^{11} (*i*+3) side chains.[20] In the absence of an *i*+1 side chain to target in this instance, the *i* and *i*+3 side chains therefore became the focus for first generation strategy. In order to avoid using too many freely rotatable bonds from the TSC N(4) atom to access position *i* (Phe^8) and risking a loss in binding through entropic factors, N(4) was incorporated as part of a proline ring, with a view to branching out toward position *i* via the C-2 carboxyl group. Since proline is found almost 30% of the time in position *i*+1 of β-turns, its use as an extension of the TSC scaffold in this quadrant seemed reasonable. The fact that we chose to conduct molecular modeling studies on the potent NK_1 agonist WS-septide (Ac-Arg^6-Phe^7-Phe^8-Pro^9-Leu^{10}-Met^{11}-NH_2) further reinforced this viewpoint: a proline residue is located at the *i*+1 position in the secondary structure of this peptide.[21] The binding affinities of a selected number of compounds prepared as NK_1 ligands are presented in Table 3.

Given the marked species differences in receptor affinity noted for the NK_1 ligand class, a number of primary assays were performed at the outset of the lead finding process. Compounds **18**–**21** were inactive at the rat NK_1 receptor, a simple benzyl substituent on the proline amide being equally insufficient for rabbit NK_1 receptor affinity in the first generation compounds **18** and **19**. However, upon incorporation of a second aryl ring, submicromolar affinity was observed at the rabbit NK_1 receptor for the (*S*)-enantiomers (**20** and **22**), again highlighting the potency-enhancing effect of the benzhydryl moiety previously noted in the BK series of TSCs.[1] As with CCK_2, no affinity for the BK B_2 receptor was recorded for these NK_1-designed proline amides. The compounds with activity in the rabbit assay retained comparable potency in the GPI, the eudismic ratios being smaller in the latter assay than in the former. Compounds **22** and **23** can be regarded as second generation if one hypothesizes that the benzhydryl moiety mimics the Phe^7-Phe^8 side chains of SP, i.e. one aryl ring accesses the *i*–1 position (Figure 4 shows that this is possible for the eutomer **22**). However, with no significant difference in activity between **20** and

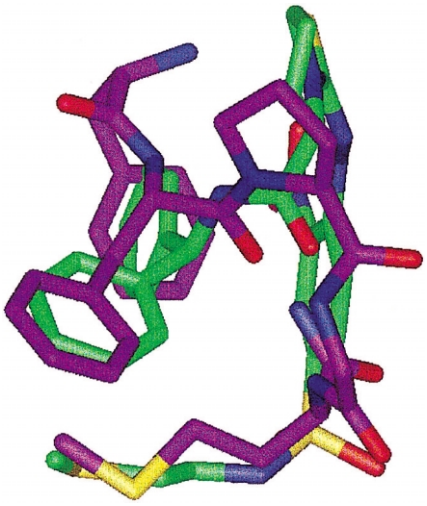


Figure 4. Overlay of a low energy conformation of compound **22** (green) with the Type I β-turn of WS-septide (purple; Ac-Arg[6] and the $i+2$ side chain Leu[10] have been omitted for clarity; rms = 2.40). For molecular modeling method see Figure 3.

22, it was the former compound which provided the impetus for a directed screening approach, based on the proline amide sub-structure, which culminated in the discovery of the clinical candidate, SDZ NKT 343, as already described.[22,23]

Summary

The present communication describes how a series of novel, potent non-peptide bradykinin B_2 receptor antagonists was transformed into ligand classes selectively recognized by the CCK_2 and NK_1 receptors. Ac-CCK-4 and WS-septide were used as surrogate peptide templates for CCK-8 and Substance P, respectively, and a β-turn overlay hypothesis was employed as a guiding principle in choosing which side chains to append to a designated core scaffold. In this exploratory work, lead molecules were discovered in both areas (compounds **14** and **20**), although we chose only to advance the latter; compound **20** facilitating a directed screening exercise which led to the identification of the NK_1 antagonist NKT 343. Previous studies on the NK_1 and CCK_2 receptors have concluded that the receptor residues conferring peptide agonist specificity differ from those underlying non-peptide antagonist affinity.[24,25] In these cases, the non-peptide ligands did not chemically resemble the native peptides. Our findings demonstrate that a structure-based design approach,[26] in which peptide side-chain functional groups are used to decorate a scaffold clearly compatible with another G-protein coupled receptor system (bradykinin B_2), can still be useful in complementing and directing established lead-finding practices.

References and Notes

1. Dziadulewicz, E. K.; Ritchie, T. J.; Hallett, A.; Snell, C. R.; Ko, S. Y.; Wrigglesworth, R.; Hughes, G. A.; Dunstan, A. R.; Bloomfield, G. C.; Drake, G. S.; Brown, M. C.; Lee, W.; Burgess, G. M.; Davis, C.; Yaqoob, M.; Perkins, M. N.; Campbell, E. A.; Davis, A. J.; Rang, H. P. *J. Med. Chem.* **2000**, *43*, 769.
2. Burgess, G. M.; Perkins, M. N.; Rang, H. P.; Campbell, E. A.; Brown, M. C.; McIntyre, P.; Urban, L.; Dziadulewicz, E. K.; Ritchie, T. J.; Hallett, A.; Snell, C. R.; Wrigglesworth, R.; Lee, W.; Davis, C.; Phagoo, S. B.; Davis, A. J.; Phillips, E.; Drake, G. S.; Hughes, G. A.; Dunstan, A. R.; Bloomfield, G. C. *Br. J. Pharmacol.* **2000**, *129*, 77.
3. Lee, S. C.; Russell, A. F.; Laidig, W. D. *Int. J. Pept. Protein Res.* **1990**, *35*, 367.
4. Rose, G. D.; Gierasch, L. M.; Smith, J. A. *Adv. Protein Chem.* **1985**, *37*, 1.
5. Stanfield, R. L.; Fieser, T. M.; Lerner, R. A.; Wilson, I. A. *Science* **1990**, *248*, 712.
6. In the absence of site-directed mutagenesis studies, competition binding experiments with chimeric receptors, or photoaffinity labeling studies, this statement remains speculative for the majority of examples.
7. This parallels the view that the amide backbone components of peptides do not necessarily participate directly in receptor occupation. Walter, R. *Federation Proc* **1977**, *36*, 1872.
8. This is not meant to imply a planar TSC backbone. X-ray crystallographic analyses of several simple 4-substituted-1-(2-nitrophenyl)thiosemicarbazides revealed the mean C(Ar)–N(1)–N(2)–C(3) torsion angle to be 108.3°. A possible intramolecular H-bond between the nitro group and N(1)H was also observed. The N(2)H–CS and N(4)H–CS bonds were seen to adopt *cis* and *trans* conformations, respectively. Dziadulewicz, E. Unpublished results.
9. Although the $i+2$ position can theoretically be accessed from the N(2) atom, it was synthetically expedient to omit $i+2$ side-chain mimics in formulating first generation strategy.
10. Farmer, P. S.; Ariens, E. J. *Trends Pharmacol. Sci.* **1982**, *3*, 362.
11. Nadzan, A. M.; Kerwin, J. F., Jr. *Annu. Rep. Med. Chem.* **1991**, *26*, 191.
12. Noble, F.; Derrien, M.; Roques, B. P. *Br. J. Pharmacol.* **1993**, *109*, 1064.
13. Loomis, R. E.; Lee, P.-C.; Tseng, C.-C. *Biochim. Biophys. Acta* **1987**, *911*, 168.
14. First generation compounds in which positions $i+1$ (Trp^{30}) and $i+4$ (Phe^{33}) were reproduced with 3-indolylethyl and ω-phenyl-(C_1–C_3)alkyl, respectively, were inactive at the CCK_2 receptor.
15. Clark, C. R.; Daum, P.; Hughes, J. *J. Neurochem.* **1986**, *46*, 1094.
16. Higginbottom, M.; Horwell, D. C.; Roberts, E. *Bioorg. Med. Chem. Lett.* **1993**, *3*, 881.
17. Hirschmann, R.; Yao, W.; Cascieri, M. A.; Strader, C. D.; Maechler, L.; Cichy-Knight, M. A.; Hynes, J., Jr.; van Rijn, R. D.; Sprengeler, P. A.; Smith, A. B., III *J. Med. Chem.* **1996**, *39*, 2441.
18. Maggi, C. A.; Patacchini, R.; Rovero, P.; Giachetti, A. *J. Auton. Pharmacol.* **1993**, *13*, 23.
19. Nakanishi, S. *A. Rev. Neurosci.* **1991**, *14*, 123.
20. Fink, B. E.; Kym, P. R.; Katzenellenbogen, J. A. *J. Am. Chem. Soc.* **1998**, *120*, 4334.
21. Byk, G.; Halle, D.; Zeltser, I.; Bitan, G.; Selinger, Z.; Gilon, C. *J. Med. Chem.* **1996**, *39*, 3174.
22. Walpole, C.; Ko, S. Y.; Brown, M.; Beattie, D.; Campbell, E.; Dickenson, F.; Ewan, S.; Hughes, G. A.; Lemaire, M.; Lerpiniere, J.; Patel, S.; Urban, L. *J. Med. Chem.* **1998**, *41*, 3159.
23. Walpole, C. S. J.; Brown, M. C. S.; James, I. F.; Campbell, E. A.; McIntyre, P.; Docherty, R.; Ko, S.; Hedley, L.; Ewan, S.; Buchheit, K.-H.; Urban, L. A. *Br. J. Pharmacol.* **1998**, *124*, 83.
24. Gether, U.; Johansen, T. E.; Snider, R. M.; Lowe, J. A., III; Nakanishi, S.; Schwartz, T. W. *Nature* **1993**, *362*, 345.
25. Beinborn, M.; Lee, Y.-M.; McBride, E. W.; Quinn, S. M.; Kopin, A. S. *Nature* **1993**, *362*, 348.
26. Hirschmann, R.; Hynes, J., Jr.; Cichy-Knight, M. A.; van Rijn, R. D.; Sprengeler, P. A.; Spoors, P. G.; Shakespeare, W. C.; Pietranico-Cole, S.; Barbosa, J.; Liu, J.; Yao, W.; Rohrer, S.; Smith, A. B., III. *J. Med. Chem.* **1998**, *41*, 1382.