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Original paper

α-Methyl tryptophanylphenylalanines and their arylethylamine "dipeptoid" analogues of the tetrapeptide cholecystokinin (30–33)

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Summary – A series of *N*-alkyl carbamate blocked α-Me-Trp-Phe and α-Me-Trp-phenethylamides has been identified as "dipeptoid" analogues of CCK–4 (CCK 30–33). These compounds have micromolar affinity for the type-B central CCK receptor, some of which increase the firing rate of CCK–B rich neurons in isolated slices containing the ventro-medial nucleus (VMN) of the hypothalamus from rat brain.

SAR studies indicate that the preferred *N*-substituents are bulky groups such as Boc–, Amoc–, Adoc and TcBoc–, and that D–α–Me–Trp and L–Phe configurations are preferred. The *C*-terminal phenyl group can be replaced by substituted phenyl and selected heteroaryl groups such as 2–thienyl and 2–pyridyl. The *C*-terminal amide group can be replaced by $-CH_2OH$, –CO–piperidide, and even –H without loss of binding affinity *e.g.* Boc–DL–α–Me–Trp–CH_2CH_2Ph as K_i of 11 μM.

These small non-peptide molecules have comparable receptor affinities to certain full tetrapeptide analogues of CCK–4 and compound **24**, TcBoc–DL-α-MeTrp–phenylethylamide, is the first CCK–dipeptoid with CCK–B like agonist properties so far described.

Résumé – α-Méthyltriptophanylphénylalamines et leurs analogues aryléthylamine "dipeptoïdes" du tétrapeptide CCK (30–33). *Une série d'α–Me–Trp–Phe et α–Me–Trp–phénéthylamides bloqués par des* N*-alkylcarbamates ont été identifiés comme des analogues "dipeptoïdes" du CCK–4 (CCK 30–33). Ces composés ont une affinité micromolaire pour le récepteur central CCK de type B, dont quelques-uns augmentent le taux de mise en jeu des neurones riches en CCK–B dans des couches isolées contenant les noyaux ventromédians (VMN) de l'hypothalamus du cerveau de rat.*

Les recherches de relations structure–activité montrent que les substituants de l'azote les plus favorables sont des groupes encombrants comme Boc–, Amoc–, Adoc et TcBoc– et que les configurations D–α–Me–Trp *et* L–Phe *sont les meilleures. Le groupe phényle* C*-terminal peut être remplacé par un phényle substitué ou des groupes hétéroaryles sélectionnés comme 2-thiényle et 2-pyridyle. Le groupe amide* C*-terminal peut être remplacé par* $-CH_2OH$, –CO–*pipéridide et même –H sans perte d'affinité de liaison, par exemple Boc–*DL–α–*Me–Trp–*CH_2–CH_2–*Ph a un* K_i *de 11 μM. Ces petites molécules non-peptidiques ont des affinités comparables à celles d'analogues tétrapeptidiques du CCK–4 et le composé* **24**, *TcBoc–*DL–α–*MeTrp–phényléthylamide est le premier "dipeptoïde" CCK décrit à ce jour et présentant des propriétés agonistes de type CCK–B.*

***N*-alkylcarbamate blocked α-methyl tryptophanylphenylalanines / α-methyl tryptophanylphenylethylamides / CCK (30–33) / CCK–B receptor affinities in micromolar range**

Introduction

We have previously shown by examination of the ability of fragments of cholecystokinin (26–33) (CCK8) to bind to the central CCK–B receptors from mouse cerebral cortex, that the simple dipeptide Boc–L–Trp–L–Phe–NH_2 retains pentamolar affinity [1].

This paper describes the synthesis and structure–activity relationships (SAR) of a novel series of α-methyl–Trp–Phe "dipeptoids" and their corresponding arylethyl-

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Abbreviations: **Boc:** *t*-butyloxycarbonyl, $(CH_3)_3C–O–CO$; **Amoc:** *t*-amyloxycarbonyl $CH_3CH_2C(CH_3)_2–O–CO$; **TcBoc:** trichloro-*t*-butyloxycarbonyl $Cl_3C–C(CH_3)_2–O–CO$; **Fmoc:** 9-fluorenylmethyloxycarbonyl; **1 or 2 Adoc:** (1 or 2-adamantyloxycarbonyl); **iBoc:** isobutyloxycarbonyl, $(CH_3)_2CHCH_2–O–CO–$; **Meoc:** methyloxycarbonyl, $CH_3–O–CO–$; **Eoc:** ethyloxycarbonyl, $CH_3CH_2–O–CO–$; **nProc:** n-propyloxycarbonyl, $CH_3(CH_2)_2–O–CO–$; **nBOC:** n-butyloxycarbonyl, $CH_3(CH_2)_3–O–CO–$; **iProc:** isopropyloxycarbonyl, $(CH_3)_2CH–O–CO–$; **Z:** benzyloxy carbonyl, $PhCH_2–O–CO–$; **PheOC:** phenyloxycarbonyl, $Ph–O–CO–$; ***t*-BuAc:** *tert*-butylacetyl, $(CH_3)_3C–CH_2CO–$; ***t*-Boc:** Boc; **NC:** not crystalline.

amine analogues, **1**. The α-methyl-Trp-derivatives were chosen for this study because the α- substituent helps to stabilize the peptide bond against acid and enzymatic degradation *in vivo* [2].

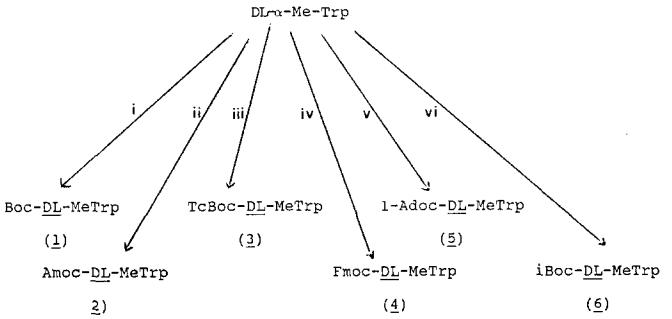
Several of the novel compounds from these series [3] show micromolar affinity for the CCK-B receptor, and increase the firing rate of neurones in the ventromedial nucleus (VMN) in isolated hypothalamic brain slices. These properties are consistent with CCK-B-like agonist activity.

Our approach to the design of CCK-like "peptoid" ligands with therapeutic potential is outlined in Figure 1. We consider this the optimum scheme to maximize SAR data on peptides in order to produce therapeutically credible drug candidates (peptoids). Stages 1–3 of the scheme (Fig. 1) have been illustrated by the identification of Boc-L-Trp-L-Phe-NH_2 from examination of continuous and non-continuous fragments of CCK (26–33) in a CCK-B binding assay [1]. Development of the scheme to stages 4 and 5 by investigation of the SAR of the novel series of α-methyl-Trp-derivatives [3] (Table I) is illustrated in this paper.

Chemistry

Compounds were prepared as outlined in Scheme 1. DL-α-Methyl-Trp-OH was *N*-blocked with the appropriate haloformates or dicarbonates under standard conditions [4]. The *N*-blocked acid was then coupled with the amino-acid amide or arylethylamine, using dicyclohexycarbodiimide (DCC) or active ester conditions to give the final dipeptoid products (Table I). When coupling was performed with L-phenylalanine amide or L-phenylalaninol a mixture of 2 diastereoisomers was produced which were separated by preparative HPLC. The D- and L- configuration of the α-methyl tryptophan residue in **7** and **8** respectively was confirmed by their independent synthesis from optically active D- and L- α-methyl tryptophan respectively prepared by chymotrypsin digest of DL-α-methyl tryptophan by the method of Anantharamaiah and Roeske [5].

DL-α-Me-Trp-OH → X-α-Me-Trp-OH → X-α-Me-Trp-NH-CH(Y)-CH_2Ar



i)$(Boc)_2O$, 1 N NaOH, Dioxan, room temperature (r.t.)
ii) $(Amoc)_2O$, 1 N NaOH, Dioxan, r.t.
iii) TcBoc-Cl, 1 N NaOH, $NaHCO_3$, Dioxan, r.t.
iv) Fmoc-Cl, $NaHCO_3$, Dioxan, r.t.
v) 1-Adoc-F, $NaHCO_3$, Dioxan, r.t.
vi) iBoc-Cl, 1 N NaOH, $NaHCO_3$, Dioxan, r.t.

1 Method A → **7–11**

2 Method A → **12–22**

3 Method A → **23–24**

4 Method A → **25**

6 Method A → **27**

25 DMF (Piperidine) → **28**

28 Method B → **29–35, 37**

28 Method C → **26**

Method A: X-MeTrp-OH, $NH_2CH(Y)CH_2Ar$, DCCI, HOBt, (pentafluorophenyl), solvent (EtOAc, CH_2Cl_2), r.t.
Method B: MeTrpNH-CH(Y)CH_2Ar, R-OCOCl (RCOCl), Et_3N, solvent, (THF, CH_2Cl_2, EtOAc), r.t.
Method C: MeTrpNHCH_2CH_2Ar, X-NCO, reflux, CH_2Cl_2.

Scheme 1. Synthetic scheme to compound **1–37**.

Biological results and discussion

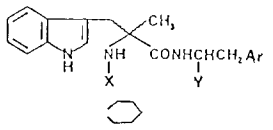
The binding affinities to the CCK-B receptors from mouse cerebral cortex of the α-Me-Trp-derivatives are given in Table I. These data on the *N*-Boc "dipeptoids" **7–10** shows that the D-α-Me-Trp-L-Phe configuration in **7** is preferred (K_i = 35 μM). The corresponding L-α-Me-Trp analogue appears to be marginally less active (K_i = 67 μM) and the D-Phe analogues **9** and **10** are inactive. Hence "dipeptoid" **7** retains pentamolar affinity comparable with some modified full tetrapeptide analogues of CCK (30–33) [1]. This novel "core molecule" has been subjected to chemical modification in particular at the *C*- and *N*-termini, in accord with the scheme to design "peptoids" (Fig. 1).

C-*Terminal modification*

The *C*-terminal binding SAR has been developed mainly using *N*-blocked Boc- and Amoc-α-methyl-Trp-derivatives. The Amoc-α-methyl-Trp-OH readily crystallized whereas the Boc-α-methyl-Trp-OH did so only with difficulty. The binding affinities of the phenethylamide derivatives **11** and **12** were of the same order (12 and 11 μM respectively). Hence the *N*-blocked Amoc- derivatives were preferred in the synthetic and SAR work.

Modifications of the C-terminal side chain. The primary amide group at the *C*-terminal of peptides is known to be labile to both acid and enzymatic degradation. In an attempt to stabilize the *C*-terminal amide moiety in **7** and increase lipophilicity, the piperidide **21** was synthesized

Table I. Physical data binding affinities for α-methyl Trp-Phe and α-methyl Trp-phenethylamide. Derivatives and analogues, compounds **1**–**37**.



No.	Mol. formula	X	Y	Configuration			Chemical Yield (%)	mp	Analysis	Synth. method	Purif. method	K_i (μMol) ($n = 3$)
				MeTrp	Phe	Ar						
1	$C_{17}H_{22}N_2O_4$	Boc	–	DL	–	–	58	163–166	C, H, N	–	E	–
2	$C_{18}H_{24}N_2O_4$	Amoc	–	DL	–	–	87	139–144	C, H, N	–	E	–
3	$C_{17}H_{19}N_2O_4Cl_3$	TcBoc	–	DL	–	–	54	190–205	C, H, N, Cl	–	E	–
4	$C_{27}H_{24}N_2O_4$	Fmoc	–	DL	–	–	71	175–182	C, N, H	–	E	–
5	$C_{23}H_{28}N_2O_4$	1-Adoc	–	DL	–	–	29	206–218	C, N, H	–	D	–
6	$C_{17}H_{22}N_2O_4$	iBoc	–	DL	–	–	64	NC	C, N, H	–	D	–
7	$C_{26}H_{32}N_4O_4$	Boc	$CONH_2$	D	L	Ph	44	NC	C, H, N	A	D	35
8	$C_{26}H_{22}N_4O_4$	Boc	$CONH_2$	L	L	Ph	37	182–182	C, H, N	A	D	67
9	$C_{26}H_{32}N_4O_4 \cdot 0.5\,H_2O$	Boc	$CONH_2$	L	D	Ph	26	NC	C, H, N	A	D	IA
10	$C_{26}H_{32}N_4O_4 \cdot 0.25\,H_2O$	Boc	$CONH_2$	D	D	Ph	30	182–187	C, H, N	A	D	IA
11	$C_{25}H_{31}N_3O_3$	Boc	H	DL	–	Ph	45	115–118	C, H, N	A	D	12
12	$C_{26}H_{33}N_3O_3$	Amoc	H	DL	–	Ph	49	129–130	C, H, N	A	D	11
13	$C_{27}H_{35}N_3O_4$	Amoc	H	DL	–	4(OMe)Ph	54	128–130	C, H, N	A	D	30
14	$C_{26}H_{32}N_3O_3Cl$	Amoc	H	DL	–	4(Cl)Ph	48	150–153	C, H, N, Cl	A	D	11
15	$C_{26}H_{31}N_3O_3Cl_2$	Amoc	H	DL	–	$3,4(Cl)_2Ph$	74	139–142	C, H, N, Cl	A	D	50
16	$C_{25}H_{32}N_4O_3$	Amoc	H	DL	–		27	151–152	C, H, N	A	D	47
17	$C_{25}H_{32}N_4O_3 \cdot 0.5\,H_2O$	Amoc	H	DL	–	3-Pyr	97	95–105	C, H, N	A	E	IA
18	$C_{25}H_{32}N_4O_3$	Amoc	H	DL	–	4-Pyr	58	138–141	C, H, N	A	E	IA
19	$C_{24}H_{31}N_3O_3S$	Amoc	H	DL	–	2-Thi	65	95– 98	C, H, N, S	A	D	14
20	$C_{27}H_{35}N_3O_3 \cdot 0.25\,H_2O$	Amoc	H	DL	–	4(Me)Ph	93	155–165	C, H, N	A	E	13
21	$C_{32}H_{42}N_4O_4 \cdot 0.25\,H_2O$	Amoc	CON ⬡	DL	L	Ph	54	NC*	C, H, N	A	D	23
22	$C_{27}H_{35}N_3O_4$	Amoc	CH_2OH	DL	L	Ph	52	NC*	C, H, N	A	D	9
23	$C_{25}H_{28}N_3O_3Cl_3$	TcBoc	H	DL	–	Ph	82	132–134	C, H, N, Cl	A	D	15
24	$C_{24}H_{27}N_4O_3Cl_3$	TcBoc	H	DL	–	2-Pyr	60	130–133	C, H, N, Cl**	A	D	10
25	$C_{35}H_{33}N_3O_3$	Fmoc	H	DL	–	Ph	8	179–181	C, H, N	A	D	IA
26	$C_{31}H_{37}N_3O_3 \cdot H_2O$	1-Adoc	H	DL	–	Ph	49	84– 86	C, H, N	A	D	5
27	$C_{25}H_{31}N_3O_3$	iBoc	H	DL	–	Ph	44	89– 91	C, H, N**	A	D	30
28	$C_{20}H_{32}N_3O$	H	H	DL	–	Ph	83	104–106	C, H, N	–	D	IA
29	$C_{23}H_{25}N_3O_3 \cdot 0.25\,H_2O$	Meoc	H	DL	–	Ph	26	62– 64	C, H, N	B	D	IA
30	$C_{23}H_{27}N_3O_3 \cdot O.5\,H_2O$	Eoc	H	DL	–	Ph	25	54– 57	C, H, N	B	D	IA
31	$C_{24}H_{29}N_3O_3$	nProc	H	DL	–	Ph	25	85– 61	C, H, N	B	D	IA
32	$C_{25}H_{31}N_3O_3$	nBoc	H	DL	–	Ph	24	93– 94	C, H, N	B	D	IA
33	$C_{24}H_{29}N_3O_3 \cdot 0.25\,H_2O$	iProc	H	DL	–	Ph	26	109–111	C, H, N	B	D	55
34	$C_{28}H_{29}N_3O_3$	Z	H	DL	–	Ph	66	130–133	C, H, N	B	D	IA
35	$C_{27}H_{27}N_3O_3$	Pheoc	H	DL	–	Ph	68	148–158	C, H, N	B	D	IA
36	$C_{25}H_{32}N_4O_2$	t-BuAc	H	DL	–	Ph	92	183–189	C, H, N	C	E	IA
37	$C_{26}H_{33}N_3O_2 \cdot 0.25\,H_2O$	t-BuAc	H	DL	–	Ph	23	104–109	C, H, N	B	D	IA
38	$C_{21}H_{29}N_3O_5 \cdot 0.25\,H_2O$	Amoc	H	DL	–	CO_2H	91	67– 70	C, H, N	A	D	IA
39	$C_{22}H_{33}N_3O_3S$	Amoc	H	DL	–	$CH_2CH_2SCH_3$	56	143–147	C, H, N	A	D	IA

[a]NC: not crystalline.
[b]See Scheme 1.
[c]Purification *Method D:* chromatography on silica gel; *Method E:* crystallize.
[d]IA: inactive at $>10^{-3}$ M.
*Free feeding, others palatable diet test.
**Calc: C, 54.82; H, 5.17; N, 10.65; Cl, 20.25; C, 54.60; H, 5.46; N, 10.51; Cl, 19.30.

and this mixture of diastereoisomers shown to have comparable binding affinity (23 μK) to **7** (K_i = 35 μM). The corresponding alcohol, **22** (K_i = 9 μM), shows micromolar K_i affinity. It was even found that complete removal of the *C*-terminal side chain, to give the simple phenethylamide analogue, **12**, also retained comparable affinity (K_i = 11 μM).

Modification of the C-terminal aryl group. Strategies to enhance biological activity by substitution of the benzene ring, or replace this ring by heteroaryl analogues are well known in medicinal chemistry [6]. It was surprising to find in this series of arylethylamides that a variety of substituted benzene ring analogues **12**–**15, 20,** a data set recommended by Topliss for developing this SAR, as well as the electron rich thiophene **19** and electron poor pyridine analogue **24** all had comparable binding affinities. (K_i = 11, 30, 11, 11, 13, 14, and 10 μM respectively).

The corresponding 3- and 4-pyridyl analogues **17** and **18** were inactive.

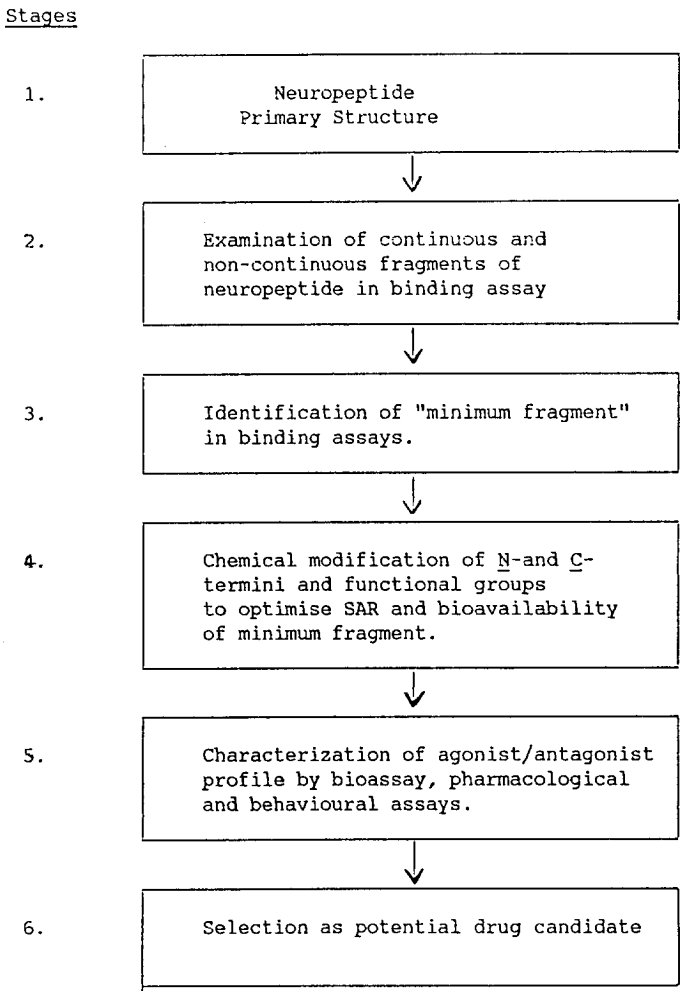


Fig. 1. Stepwise scheme to design therapeutically useful "peptoids" from putative endogenous neuropeptides.

Replacement of the arylethylamine group with the side chains of amino acids. Having established that the L-phenylalanine group can be replaced by the simple phenethylamine group, it was speculated that the CH_2Ph group may mimic the side chain of the L-Phe residue of CCK. Replacement of the CH_2Ph group with the side chains of the 20 naturally occurring mammalian amino acids was then investigated. This is considered a pertinent data set of chemico-physical information to probe for alternative binding sites on the receptor. Our first choice was replacement of the CH_2Ph group with CH_2CO_2H (the aspartic acid side chain), compound **38**, and $CH_2CH_2-S-CH_3$ (the methionine side chain), compound **39**. These particular side chains must be important as they are present in CCK (30–33) which has nanomolar binding affinity. However, both **38** and **39** had poor binding affinity ($IC_{50} > 100$ μM). Further representative members of the amino acid side chain analogues are under investigation.

N-*Terminal modifications*

We have previously shown that the nanomolar affinity of CCK (26–33 sulphated) for the CCK-B receptor can be retained when the whole *N*-terminal tetrapeptide fragment Asp-Tyr-(OSO_3H)-Met-Gly (CCK 26–29) is replaced by simpler groups such as Boc-β-alanyl (pentagastrin) the simple Boc-group [1] or even the free *N*-terminal. In the arylethylamine series (Table I) the SAR of replacement of the Boc-group itself in **11** by simple alkyl and aryl carbamates, amides and ureas was explored. Affinity is retained when the Boc- group is replaced by other bulky branched alkyl groups such as Amoc-, TcBoc-, 1-Adoc- and iBoc- compounds **12, 23, 26, 27** (K_i = 11, 15, 5 and 30 μM respectively). Binding affinity through the series **29–33, 11,** shows that branched, bulky alkyl chains are necessary (**11,** K_i= 12 μM) over straight chains ($IC_{50} > 100$ μM for **28–32,** 55 μM for **33**). Aryl groups do not appear to be well tolerated in the carbamate moieties in examples such as **25, 34** and **35.** When the carbamate linkage itself of **11** is replaced by the isosteric urea **36** or methylene amide **37,** activity is greatly diminished.

In order to improve the potential for oral bioavailability with these compounds, the acid labile Boc- group was replaced by the TcBoc- group, which is known to stabilize the carbamate linkage towards acidic cleavage [7] by virtue of the powerful negative inductive effect of the chlorine atom substituents. The TcBoc- analogues **23** and **24** retain good binding affinity (K_i = 15 and 10 μM respectively). Furthermore, compound **24** does show CCK-B (26–33)-like agonist activity by increasing the firing rate of neurons in the VMN in isolated hypothalamic slices. In this model, CCK (26–33 sulphated) (a non-selective but potent CCK-A and B agonist) consistently produced an increase in firing rate (EC_{50} = 0.3 μM) and the effect could be mimicked by **24** at a concentration of 80 μM (Fig. 2).

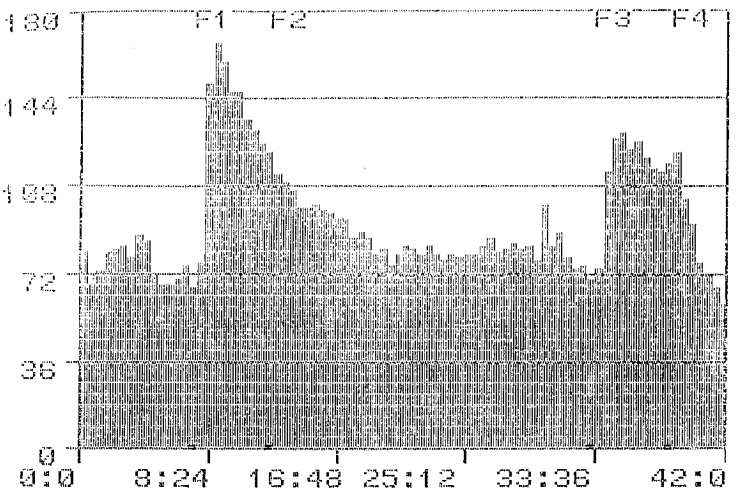


Fig. 2. The effects of CCK-8 sulphated and compound **24** on the spontaneous firing rate of a neurone in the ventromedial nucleus of a slice preparation of rat hypothalamus. The histogram shows the number of action potentials (ordinate) recorded in consecutive 30-s intervals during the time course of the experiment depicted in min *(abscissa)*. At the point F1 CCK-8 sulphated (500 nM) was applied and produced a large increase in firing rate. The firing rate returned to control levels on washing with drug-free saline (F2). Compound **24** (80 μM) applied at F3 also produced an increase in firing rate, reversible on washing (F4).

Despite CCK (26–33 sulphated) showing nanomolar affinity for the CCK-B receptor [1], this increase in firing rate was not reproducibly seen until these high (0.3 μM) concentrations were used. The affinity of **24** is 10 μM for the CCK-B receptor, *i.e.* approximately 10,000 × less than CCK (26–33 sulphated) and the reproducible and significant increase in firing rate was not seen until concentrations of 80 μM were used. Hence the intrinsic activity on this increase in firing rate appears to be much greater with **24** than with CCK (26–33 sulphated) itself. Further characterization of compound **24,** by antagonism of the increase in firing rate in VMN by selective CCK-A and CCK-B antagonists [8], is in progress.

Conclusions

Examination of fragments of CCK (30–33) in binding to CCK-B receptors has led to the design of novel α-methyl-Trp "dipeptoids" with micromolar affinity (*e.g.* compounds **24** and **26**). Electrophysiological data is consistent with CCK-B-like agonist activity, *e.g.* compound **24.** Our model suggests that this minimum fragment of CCK (30–33) can be read by the receptor as the continuous message Trp-Phe, corresponding to the non-continuous message of CCK (30–33) (Trp-Met-Asp-Phe-amide) (Fig. 3). In conclusion we have described the progression of our strategy to design biologically active "peptoids" from a putative peptide neurotransmitter. The development of these leads to produce analogues of therapeutic potential is undergoing further study.

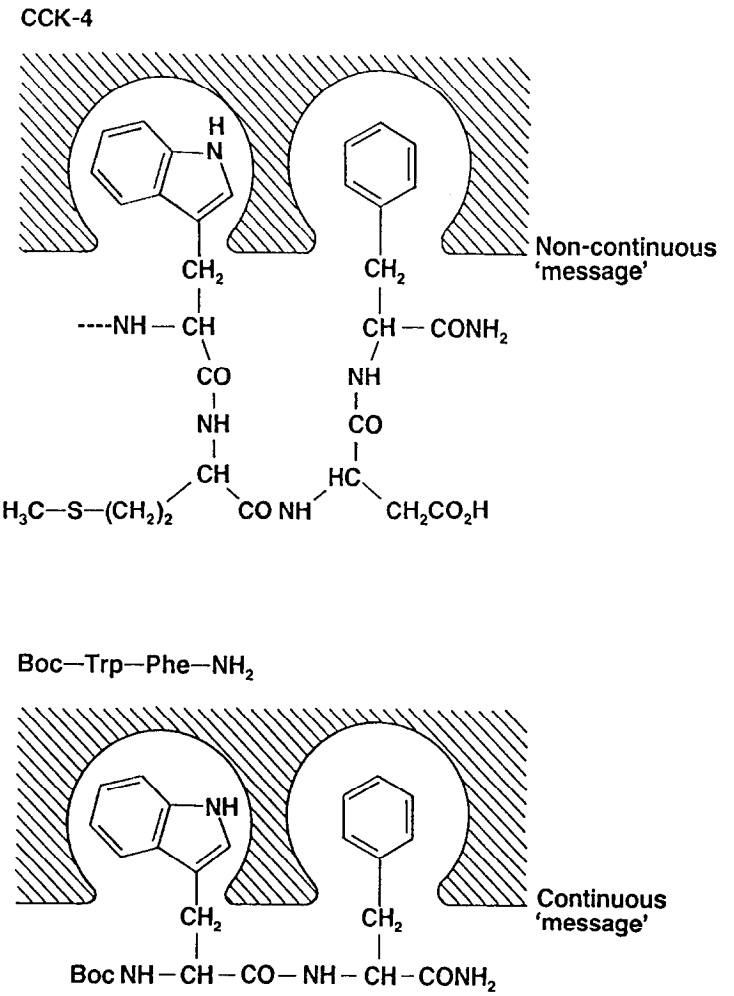


Fig. 3. Model of the non-continuous Trp-Phe-message of CCK-4 mimicked by the continuous Trp-Phe-message of the dipeptide Boc Trp-Phe-amide.

Experimental protocols

Chemistry

Melting points were determined using a Reichert Thermovar Hotstage and are uncorrected. Infra-red spectra were obtained from a Perkin–Elmer 1750 FT-IR spectrometer and ^{1}H NMR spectra from a Bruker AM300 spectrometer. Spectra are consistent with proposed structures. Elemental analyses were within 0.4% of all the calculated values unless stated otherwise. Solvents were stored over 4Å molecular sieves before use otherwise they were used as received. DL-α-Methyl tryptophan was obtained from Siber-Hegner. Optically active [D] and [L]-α-methyl tryptophan were obtained by chymotrypsin digest of D, L-α-methyl tryptophan methyl ester according to the method of Anantharamaiah and Roeske [5]. These were independently used to prepare **7** and **8** respectively by the coupling methods described below. Preparative HPLC was performed on the Jobin-Yvon modulprep using Kieselgel 60 (0.015–0.040 mm mesh) (E. Merck, Darmstadt.)

Synthesis of intermediates 1–6

Intermediates **1–6** were prepared from DL-α-methyl tryptophan and the corresponding alkyl chloroformate, fluoroformate or dialkyl dicarbonate according to published procedure [9–11].

General procedure

To a solution of DL-α-methyl tryptophan (7.00 g, 35.4 mmol) in 1 N sodium hydroxide solution (36 ml) was added sodium bicarbonate (3.50 g, 41.7 mmol) followed by dioxan (35 ml). To the stirred mixture was added a solution of TcBoc-Cl (8.5 g, 35.4 mmol) in dioxan (30 ml) slowly during 15 min. Stirring was continued overnight then the dioxan removed under reduced pressure. The residue was diluted with water and extracted once with ether (discarded). The aqueous layer was acidified with dilute citric acid solution to pH 3–4 then extracted with ethyl acetate (× 3). The extracts were dried ($MgSO_4$) and evaporated to an oil which crystallized from diethyl ether, giving the product **(3)** as a white solid; yield 8.54 g, 63%; mp: 190–205°C (Et_2O-petroleum ether); ^{1}H NMR (DMSO–CD_3OD), δ 1.50 (s, 3, α-CH_3), 1.93 (s, 3, CH_3–C–CCl_3), 1.95 (s, 3, CH_3–C–CCl_3), 3.4 (q, J = 7–8 Hz, indole–CH_2), 6.95–7.6 (m, 5, aromatic).

Similarly prepared were:

N-*Boc-DL-α-methyl tryptophan* **1.** Yield 58%, white crystalline solid; mp: 163–166°C (EtOAc); ^{1}H NMR (CD_3OD), δ 1.46 (s, 12, tBuO, α-CH_3), 3.3 (q, 2, indole–CH_2), 6.90–7.10 (m, 3, aromatic), 7.30 (d, J = 8 Hz, aromatic) 7.54 (d, J = 8 Hz, aromatic).

N-*Amoc-DL-α-methyl tryptophan* **2.** Yield 87%, white crystalline solid; mp: 139–144°C (EtOH); ^{1}H NMR (CD_3OD), δ 0.90 (t, J = 7.4 Hz, CH_2–CH_3), 1.43 (s, CH_3–C–CH_3), 1.47 (s, α-CH_3), 1.78 (q, J = 7.4 Hz, CH_2–CH_3), 3.36 (q, J = 8 Hz, indole–CH_2–C), 6.9–7.6 (m, aromatic).

N-*Fmoc-DL-α-methyl tryptophan* **4.** Yield 71%, white crystalline solid; mp: 175–182°C (Et_2O); ^{1}H NMR (CD_3OD), δ 1.47 (s, α-CH_3), 3.38 (m, indole–CH_2); 4.19 (t, Ar–CH–Ar), 4.36 (br.s, fluorenyl–CH_2), 6.9–7.75 (m, aromatic).

N-*1-Adoc-DL-α-methyl tryptophan* **5.** Yield 29%, off-white crystalline solid; mp: 206–218°C (EtOAc); ^{1}H NMR (CD_3OD), δ, 1.42 (s, adamantyl), 2.13 (s, adamantyl, bridgeheads), 3.33 (q, indole–CH_2), 6.95 –7.6 (m, aromatic).

N-*iBoc-DL-α-methyl tryptophan* **6.** Yield 64%, white non-cristalline solid ^{1}H NMR ($CDCl_3$), δ 0.90 (d, J = 6.7 Hz, $(CH_3)_2CH$–), 1.66 (s, α–H_3), 1.90 (m, $(CH_3)_2CH$–), 3.44 (q, 2, indole–CH_2), 3.88 (d, J = 6.7 Hz, CH_2–CH), 5.37 (br.s, OCO–*NH*), 6.97–7.6 (m, aromatic), 8.15 (s, indole–*NH*).

Coupling procedure

Standard peptide coupling procedures were employed in the synthesis of amides or dipeptides.

N-*Boc D- and L-α-methyl tryptophanyl-L-phenylalanine amides (compounds* **7** *and* **8**). A solution of *N*-Boc-DL-α-methyl tryptophan **(1)**, (0.13 g, 0.41 mmol) in dry ethyl acetate (2 ml) was treated successively with *N*-hydroxybenzotriazole hydrate (0.056 g, 0.41 mmol) then DCCI (0.085 g, 0.41 mmol). After stirring for 30 min L-phenylalanine amide (0.082 g, 0.50 mmol) was added in a single portion. The mixture was stirred overnight at room temperature then filtered to remove DCU. The filtrate was washed with dilute solutions of sodium bicarbonate, citric acid and finally sodium chloride. The organic phase was dried ($MgSO_4$) and evaporated to an oil which readily crystallized (169 mg, 89%).

Chromatography of the product yielded 2 diastereomeric compounds, using silica and eluting with 4% iPrOH / CH_2Cl_2. The faster running material was isolated as a white non-crystalline foam, **(7)** (0.082 g, 44%) while the slower running material was isolated as a white crystalline solid, **(8)**, (0.068 g, 37%).

N-*Boc-D-α-methyl tryptophanyl-L-phenylalanineamide* **7.** Yielded 44%, non-crystalline solid; ^{1}H NMR ($CDCl_3$), δ, 1.16 (s, α–CH_3), 1.37 (s, tBu), 2.98–3.24 (m, CH_2–Ph), 3.3–3.44 (q, CH_2–indole), 4.7–4.77 (q, –N–*CH*–CO–), 4.85 (s, NH), 5.35 (s, NH), 6.23–6.26 (d, *NH*–CH–CO), 6.90–7.6 (m, aromatic), 8.75 (s, indole–*NH*).

N-*Boc-L-α-methyl tryptophanyl-L-phenylalanineamide* **8.** Yielded 37%, white crystalline solid; mp: 182–7°C; ^{1}H NMR ($CDCl_3$), δ 1.17 (s, tBu), 1.53 (s, α–CH_3), 2.72–3.28 (m, 2 × CH_2 Ar), 4.39 (m, N–*CH*–CO), 5.48 (s, NH), 5.67 (s, NH), 6.15 (d, *NH*–CH), 6.57 (br.s, NH_2), 7.02–7.55 (m, aromatic), 10.20 (s, indole *NH*).

Note: the structures of both **7** and **8** have been confirmed by independent synthesis from optically pure D or L-α-methyl tryptophan.

Similarly prepared were:

N-*Boc-L-α-methyl trytophanyl-D-phenylalanineamide* **9.** Yielded 26%, the NMR spectrum was identical to example **(7)**.

N-*Boc-D-α-methyl tryptophanyl-D-phenylalanineamide* **10.** Yielded 30%, the NMR spectrum was identical to example **(8)**.

N-*Boc-DL-α-methyl tryptophanyl-2-phenethylamide* **11.** Prepared from *N*-Boc-DL-α-methyl tryptophan **(1)** and L-phenylalanineamide in 45% yield; ^{1}H NMR ($CDCl_3$), δ 1.41 (s, tBuO), 1.52 (s, α–CH_3), 2.66 (t, CH_2Ph), 3.32 (m, NHCH_2 + CH_2 indole), 4.99 (s, NH), 6.23 (br.t, *NH*CH_2), 6.97–7.61 (m, aromatics), 8.21 (s, indole–*NH*).

N-*Amoc-DL-α-methyl tryptophan-2-phenethylamide* **12.** Prepared from *N*-Amoc-DL-α-methyl tryptophan **(2)** and L-phenylalanineamide in 49% yield, mp: 129–130°C; ^{1}H NMR ($CDCl_3$), δ 0.84 (t, CH_3CH_2–), 1.37 (d, CH_2C–O–), 1.52 (s, α–CH_3), 1.73 (m, C–CH_2–CH_3), 2.64 (t, CH_2Ph), 3.33 (m, NHCH_2 + CH_2 indole), 5.00 (s, NH), 6.21 (br.t, *NH*CH_2), 6.97–7.62 (m, aromatics), 8.24 (s, indole *NH*).

N-*Amoc-DL-α-methyl tryptophanyl-2-(4-methoxyphenyl) ethylamide* **13.** Prepared from *N*-Amoc-DL-α-methyl tryptophan **(2)** and 2-(4-methoxyphenyl)-ethylamide in 54% yield, mp: 128–130°C; ^{1}H NMR ($CDCl_3$), δ 0.84 (t, CH_3CH_2), 1.37 (d, Me_2C–O), 1.52 (5, α–CH_3), 1.73 (q, CH_2CH_3), 2.58 (t, CH_2AC_2), 3.30 (m, NHCH_2 + CH_2 indole), 3.76 (s, OCH_3), 5.01 (s, NH), 6.18 (br.t, *NH*CH_2), 7.74–7.60 (m, aromatics), 8.22 (s, indole–*NH*).

N-*Amoc-DL-α-methyl tryptophanyl-2-(4-chlorophenyl)ethylamide* **14.** Prepared from *N*-Amoc-DL-α-methyl tryptophan **(2)** and 2-(4-chlorophenyl)-ethylamide in 48% yield, mp: 150–153°C; ^{1}H NMR ($CDCl_3$), δ 0.84 (t, CH_3CH_2), 1.37 (d, Me_2C–O), 1.52 (s, α–CH_3), 1.72 (q, CH_2CH_3), 2.60 (t, CH_2Ar), 3.31 (m, NHCH_2 + CH_2 indole), 4.98 (s, NH), 6.23 (br.t, *NH*CH_2), 6.90–7.60 (m, aromatics), 8.22 (s, indole *NH*).

N-*Amoc-DL-α-methyl tryptophanyl-2-(3,4-dichlorophenyl)ethylamide* **15.** Prepared from *N*-Amoc-DL-α-methyl tryptophan **(2)** and 2-(3,4-dichlorophenyl)-ethylamide in 74% yield, mp: 139–142°C; ^{1}H NMR ($CDCl_3$), δ 0.83 (t, CH_3CH_2), 1.36 (d, Me_2C–O), 1.53 (s, α–CH_3), 1.70 (q, CH_2CH_3), 3.36 (NHCH_2 + CH_2 indole), 4.96 (s, NH), 6.27 (br.t, *NH*CH_2), 6.85–7.61 (m, aromatics), 8.21 (s, indole *NH*).

N-*Amoc-DL-α-methyl tryptophanyl-2(2-pyridyl)ethylamide* **16.** Prepared from *N*-Amoc-DL-α-methyl tryptophan **(2)** and 2-(2-pyridyl)ethylamide in 27% yield, mp: 151–152°C; ^{1}H NMR ($CDCl_3$), δ 0.84 (t, CH_3CH_2), 1.35 (s, Me_2C–O), 1.54 (s, α–CH_3), 1.70 (q, CH_2CH_3), 2.85 (t, CH_2Ar), 3.33 (q, indole CH_2), 3.61 (q, NHCH_2), 5.14 (s, NH), 6.97–7.58 (m, aromatics), 8.21 (s, *NH*CH_2), 8.43 (d, indole *NH*).

N-*Amoc-DL-α-methyl tryptophanyl-2-(3-pyridyl)ethylamide* **17.** Prepared from *N*-Amoc-DL-α-methyl tryptophan **(2)** and 2-(3-pyridyl)ethylamide in 97% yield, mp: 95–105°C; ^{1}H NMR ($CDCl_3$), δ 0.84 (t, CH_3CH_2), 1.38 (d, Me_2C–O), 1.44 (s, α–CH_3), 1.71 (q, CH_2,CH_3), 2.64 (t, CH_2Ph), 3.32 (NHCH_2 + CH_2Ar), 4.98 (s, NH), 6.30 (br.t, *NH*CH_2), 6.97–7.60 (m, aromatics), 8.24 (s, indole NH), 8.30 (s, aromatic).

N-*Amoc-DL-α-methyl tryptophanyl-2-(4-pyridyl)ethylamide* **18.** Prepared from *N*-Amoc-DL-α-methyl tryptophan **(2)** and 2-(4-pyridyl)ethylamide in 58% yield, mp: 138–141°C; ^{1}H NMR (DMSO), δ 0.83 (t, CH_3CH_2), 1.25 (s, α–CH_3), 1.36 (s, Me_2C), 1.70 (q, CH_2CH_3), 2.68 (t, CH_2Ar), 3.3 (m, NHCH_2 + CH_2 indole), 6.46 (s, NH), 6.93–7.48 (m, aromatics), 7.83 (br.t, *NH*CH_2), 8.40 (d, aromatic).

N-*Amoc-DL-α-methyl tryptophanyl-2-(2-thienyl)ethylamide* **19.** Prepared from *N*-Amoc-DL-α-methyl tryptophan **(2)** and 2-(2-thienyl)ethylamide in 65% yield, mp: 95–98°C; ^{1}H NMR ($CDCl_3$), δ 0.87 (t, CH_3CH_2–), 1.49 (d, Me_2C–O), 1.53 (s, α–CH_3), 1.76 (q, CH_2CH_3), 2.90 (t, CH_2Ar), 3.36 (m, NHCH_2 + CH_2 indole), 5.03 (s, NH), 6.35–7.60 (m, aromatics), 8.26 (indole NH).

N-*Amoc-DL-α-methyl tryptophanyl-2-(4-methylphenyl)ethylamide* **20.** Prepared from *N*-Amoc-DL-α-methyl tryptophan **(2)** and 2-(4-methylphenyl)ethylamide in 93% yield, mp: 155–165°C; ^{1}H NMR ($CDCl_3$), δ 0.84 (t, CH_3CH_2–), 1.37 (d, Me_2C–O), 1.51 (s, α–CH_3), 1.72 (q, CH_2CH_3), 2.29 (s, ArCH_3), 2.59 (t, CH_2Ar), 3.30 (m, 4, NHCH_2 + CH_2 indole), 5.03 (s, NH), 6.20 (br.t, *NH*CH_2), 6.91–7.59 (m, aromatics), 8.25 (s, indole NH).

N-*Amoc-DL-α-methyl tryptophanyl-L-phenylalaninepiperidineamide* **21.** Prepared from *N*-Amoc-DL-α-methyl tryptophan **(2)** and L-phenyl-alanine piperidineamide in 54% yield, non-crystalline solid; ^{1}H NMR ($CDCl_3$), δ 0.84 (d.t, CH_3CH_2), 1.45 (m, Me_2C + β and α piperidine H's), 1.66 (s, α–CH_3), 1.75 (q, CH_2CH_3), 3.2 (m, indole CH_2 + CH_2Ph + α–piperidine H's), 5.06 (m, *NH* + Phe α–*CH*), 6.99–7.60 (m, aromatics), 8.20 (s, indole *NH*).

N-*Amoc-DL-α-methyl tryptophanyl-L-(1-hydroxymethyl-2-phenyl)ethylamide* **22.** Prepared from *N*-Amoc-DL-α-methyl tryptophan **(2)** and L-phenylalaninol in 52% yield, non-crystalline solid; ^{1}H NMR ($CDCl_3$), δ 0.83 (m, CH_3CH_2), 1.36 (dd, Me_2CH–O), 1.43 (d, α–CH_3), 1.72 (m, CH_2CH_3), 2.63 (m, indole CH_2 + CH_2OH), 4.16 (2br.s, α–*CH*), 5.05 (2s, NH), 6.15 (*br*.t, NHCH), 6.88–7.58 (m, aromatics), 9.02 (2s, indole *NH*) (mixture of diastereoisomers).

N-*TcBoc-DL-α-methyl tryptophanyl-2-phenethylamide* **23.** Prepared from *N-TcBoc-DL-α*-methyl tryptophan **(3)** and 2-phenethylamine in 82% yield, mp: 132–134°C; ^{1}H NMR ($CDCl_3$), δ 1.56 (s, α–CH_3), 1.86 (s, Me_2CH–O–), 2.62 (t, CH_2Ph), 3.34 (m, NHCH_2 + CH_2 indole), 5.48 (s, NH), 5.98 (br.t., *NH*CH_2), 6.96–7.58 (m, aromatics), 8.14 (s, indole *NH*).

N-*TcBoc-DL-α-methyl tryptophanyl-2-(2-pyridyl)ethylamide* ***24.*** Prepared from *N*-TcBoc-DL-α-methyl tryptophan **(3)** and 2-(2-pyridyl)ethylamide in 60% yield, mp: 130–133°C; ^{1}H NMR ($CDCl_3$), δ 1.60 (s, α–CH_3), 1.86 (d, Me_2CH–O–), 2.82 (t, CH_2Ar), 3.39 (s, CH_2 indole), 3.61 (m, $NHCH_2$), 5.68 (s, NH), 6.97–7.57 (m, aromatics), 8.12 (s, indole $NHCH_2$), 8.39 (d, indole *NH*).

N-*Fmoc-DL-α-methyl tryptophanyl-2-phenethylamide* ***25.*** Prepared from *N*-Fmoc-DL-α-methyl tryptophan **(4)** and 2-phenethylamine in 58% yield, mp: 179–181°C; ^{1}H NMR ($CDCl_3$–DMSO–d_6), δ 1.53 (s, α–CH_3), 2.65 (m, CH_2Ph), 3.40 (m, $NHCH_2$ + CH_2 indole), 4.13 (t, $CHAr_2$), 4.33 (d, fluorenyl-CH_2–), 5.85 (s, NH), 6.50 (br.t, $NHCH_2$), 6.88–7.80 (m, aromatics), 9.53 (s, indole *NH*).

N-*Adoc-DL-α-methyl tryptophanyl-2-phenethylamide* ***26.*** Prepared from *N*-Adoc-DL-α-methyl tryptophan **(5)** and 2-phenethylamine in 49% yield, mp: 84–86°C; ^{1}H NMR ($CDCl_3$), δ 1.50 (s, α–CH_3), 1.63 (s, adamantyl), 2.04 (s, adamantyl), 2.14 (s, adamantyl bridgehead H's), 2.65 (t, d, CH_2Ph), 3.30 (m, $NHCH_2$– + CH_2 indole), 4.93 (s, NH), 6.30 (br.t, $NHCH_2$), 6.98–7.60 (m, aromatics), 8.24 (s, indole–*NH*).

N-*iBoc-DL-α-methyl tryptophanyl-2-phenylethylamide* ***27.*** Prepared from *N*-iBoc-DL-αmethyl tryptophan **(6)** and 2-phenethylamine in 44% yield, mp: 88–91°C; ^{1}H NMR ($CDCl_3$), δ 0.88 (d, Me_2CH–), 1.55 (s, α–CH_3), 1.85 (m, Me_2CHCH_2–), 2.63 (t, CH_2Ph), 3.33 (m, $NHCH_2$ + CH_2 indole), 3.79 (d, CH_2CHMe_2), 5.25 (s, NH), 6.17 (br.t, $NHCH_2$), 6.93–7.57 (m, aromatics), 8.47 (s, indole–*NH*).

The remaining compounds **(29–37)** were prepared by acylation of the intermediate, DL-α-methyl tryptophanyl-2-phenylethylamide, **(28)**, prepared from the corresponding Fmoc-derivative **(25)**.

DL-α-Methyl tryptophanyl-2-phenethylamide ***28.*** A solution of **(25)** (2.00 g, 3.68 mmol) in dry DMF (5 ml), was treated with a 20% solution of piperidine in DMF (20 ml) at room temperature for 30 min. Removal of solvent and excess piperidine left a semi-solid residue which was first triturated with hexane to leave a tan coloured powder, which was recrystallized from ethyl acetate. Yield 0.996 g: 83%, mp: 104–106°C (EtOAc); ^{1}H NMR ($CDCl_3$), δ 1.39 (s, α–CH_3), 1.47 (s, α–NH_2), 2.67 (m, Ph–CH_2), 2.815 (d, J = 14 Hz, indole–CH–*H*), 3.37 (n, Ph–CH–*H*), 3.50 (m, PhCH–*H* + indole–CH–*H*), 6.95–7.67 (m, aromatic), 7.53 (br.t, CON*H*), 8.14 (s, indole–NH).

In a typical acylation procedure, **(28)** (0.321 g, 1 mmol) was dissolved in ethyl acetate (or THF) (10 ml) and triethylamine (0.11 g, 1.1 mmol) added with stirring. A solution of phenyl chloroformate (0.10 g, 1.2 mmol) in ethyl acetate (1 ml) was added dropwise and stirring continued overnight. Water was added to the mixture and the organic phase washed successively with dilute aqueous solutions of sodium bicarbonate, citric acid and sodium chloride then dried, ($MgSO_4$), and evaporated. The residue was chromatographed on silica, eluted with 2% methanol in dichloromethane and finally isolated as a white crystalline solid, **(35)**; yield 0.301 g, 68%, mp: 148–158°C; ^{1}H NMR (DMSO–d_6), δ 1.36 (s, α–CH_3), 2.69 (t, CH_2Ph), 3.31 (m, $NHCH_2$ + CH_2 indole), 6.95–7.52 (m, aromatics), 7.94 (t, $NHCH_2$), 10.90 (s, indole *NH*).

Similarly prepared were:

N-*Methyloxycarbonyl-DL-α-methyl tryptophanyl-2-phenethylamide* ***29.*** Yield 26%, mp: 62–64°C; ^{1}H NMR ($CDCl_3$), δ 1.58 (s, α–CH_3), 2.58 (t, CH_2–Ph), 3.34 (m, $NHCH_2$ + CH_2 indole), 3.60 (s, OCH_3), 5.25 (s, $NHCH_2$), 6.96–7.58 (m, aromatics), 8.01 (s, indole–*NH*).

N-*Ethyloxycarbonyl-DL-α-methyl tryptophanyl-2-phenethylamide* ***30.*** Yield 25%, mp: 54–57°C; ^{1}H NMR ($CDCl_3$), δ 1.19 (t, OCH_2CH_3), 1.56 (s, α–CH_3), 2.66 (t, CH_2–Ph), 3.31 (m, $NHCH_2$ + CH_2 indole), 4.03 (q, OCH_2CH_3), 5.17 (s, NH), 6.14 (br.t, $NHCH_2$), 6.96–7.59 (m, aromatics), 8.11 (s, indole–*NH*).

N-n-*Propyloxycarbonyl-DL-α-methyl tryptophanyl-2-phenylethylamide* ***31.*** Yield 25%, mp: 85–86°C; ^{1}H NMR ($CDCl_3$), δ 0.90 (t, CH_2CH_3), 1.6 (m, CH_2CH_3), 2.65 (t, CH_2Ph), 3.33 (m, $NHCH_2$ + CH_2 indole), 3.96 (t, OCH_2CH_2), 5.20 (s, $NHCH_2$), 6.14 (br.t, $NHCH_2$–), 6.95–7.60 (m, aromatics), 8.08 (s, indole–*NH*).

N-n-*Butyloxycarbonyl-DL-α-methyl tryptophanyl-2-phenethylamide* ***32.*** Yield 24%, mp: 93–94°C; ^{1}H NMR ($CDCl_3$), δ 0.90 (t, CH_2CH_3), 1.33 (m, $CH_3CH_2CH_2$), 1.52 (m, $CH_3CH_2CH_2C$), 2.66 (t, CH_2Ph), 3.35 (m, $NHCH_2$ + CH_2 indole), 4.00 (t, OCH_2CH_2–), 5.17 (s, NH), 6.14 (br.t., $NHCH_2$–), 6.95–7.59 (m, aromatics), 8.06 (s, indole NH).

N-*Isopropyloxycarbonyl-DL-α-methyl tryptophanyl-2-phenethylamide* ***33.*** Yield 26%, mp: 109–111°C, ^{1}H NMR ($CDCl_3$), δ 1.19 (d, Me_2CH–), 1.55 (s, α–CH_3), 2.66 (t, *CH*Ph), 3.33 (m, $NHCH_2$–, CH_2 indole), 4.86 (h, $CHMe_2$), 5.08 (s, NH), 6.13 (br.t, $NHCH_2$–), 6.95–7.59 (m, aromatics), 8.09 (s, indole *NH*).

N-*Benzyloxycarbonyl-DL-α-methyl tryptophanyl-2-phenethylamide* ***34.*** Yield 66%, mp: 130–133°C; ^{1}H NMR ($CDCl_3$), δ 1.55 (s, α–CH_3), 2.63 (t, CH_2Ph), 3.30 (m, $NHCH_2$ + CH_2 indole), 5.05 (s, OCH_2Ph), 5.29 (s, NH), 6.08 (br.t, $NHCH_2$–), 6.81–7.56 (m, aromatics), 8.07 (s, indole *NH*).

N-t-*Butylaminocarbonyl-DL-α-methyl tryptophanyl-2-phenethylamide* ***36.*** A solution of **(28)** (0.25 g, 0.78 mmol) in dry dichloromethane (1.5 ml) was treated at room temperature with *tert*-butyl isocyanate (0.09 ml, 0.78 mmol). After stirring for 1 h the mixture was heated to reflux and kept thus overnight and the crystalline precipitate collected and washed with a little dichloromethane and petroleum ether then dried. Yield 0.302 g, 92%, mp: 183–189°C; ^{1}H NMR (DMSO), δ 1.27 (s, tBvN), 2.68 (m, CH_2Ph), 3.3. (α–CH_3 + $NHCH_2$ + CH_2 indole), 5.73 (s, NH), 5.90 (s, NH), 6.89–7.65 (m, aromatics), 10.76 (s, indole *NH*).

N-tert-*Butylacetyl-DL-α-methyl tryptophanyl-2-phenylethylamide* ***37.*** Prepared by acylation of **(28)** with *tert*-butylacetyl chloride in 23% yield after chromatography. mp: 104–109°C; ^{1}H NMR ($CDCl_3$), δ 0.90 (s, *tBu*–CH_2), 1.64 (s, α–CH_3), 1.89 (q, tBuCH_2), 2.65 (m, CH_2Ph), 3.36 (abq, CH_2 indole), 3.48 (m, $NHCH_2$), 6.14 (s, NH), 6.47 (br.t, $NHCH_2$), 6.98–7.59 (m, aromatics), 8.15 (s, indole *NH*).

The compounds **(7–27)** were synthesized from the corresponding protected α-methyl tryptophan derivative and phenethylamine derivative using standard peptide coupling procedures. Each product was isolated pure following flash chromatography on silica and, where possible, diastereoisomers were separated in this step. Compounds **(1–6)** from which the coupled products **(7–27)** were produced were, in turn, synthesized by standard peptide methodology. Compounds **(29–37)** were synthesized by acylation of the intermediate amine **(28)** produced by the action of 20% piperidine in DMF on the Fmoc-protected compound **(25)**. The final products were isolated pure following flash chromatography on silica.

N-*Amoc-α-Me-Trp-β-alanine, methyl ester.* *N*-Amoc-α-methyltryptophan (0.664 g, 2 mmol) was coupled with β-alanine methyl ester (0.206 g, 2 mmol) using *Method A*. The title compound was isolated after column chromatography using ethyl acetate as eluent (0.554 g, 67%): mp: 118.8–122.6°C. Anal. ($C_{22}H_{31}N_3O_5$) C, H, N.

N-*Amoc-α-Me-Trp-β-alanine* ***38.*** *N*-Amoc-α-Me-Trp-β-alanine, methyl ester (0.95 g, 2.28 mmol) was dissolved in EtOH (25 ml) and NaOH (1 N, 5 ml) added to the solution. The reaction mixture was allowed to stir at room temperature for 3 h before dilution with water (50 ml) and acidification with HCL 10% (5 ml). The aqueous phase was extracted with ethyl acetate (2 × 50 ml) and the 2 layers separated. The organic solvent was dried, filtered and evaporated to yield a white crystalline solid, (0.835 g, 91%). mp: 66.7–70.2°C. Anal. ($C_{21}H_{29}N_3O_5$ 0.25 H_2O) C, H, N.

N-*Amoc-α-Me-Trp-1-[methylthio]propylamide* ***39.*** *N*-Amoc-α-methyltryptophan (0.664 g, 2 mmol) was coupled with 3-(methylthio)propylamine (0.209 g, 2.3 mmol) by *Method A*. The title compound was isolated after column chromatography using ethyl acetate as the eluting solvent (0.468 g, 56%). mp: 142.9–147.2°C. Anal. ($C_{22}H_{33}N_3O_3S$) C, H, N.

Biological assays

CCK-B receptor binding assay were performed using mouse cerebral cortex as previously described [1].

Effects on spontaneous firing rate in slices containing VMN of rat were performed as follows: rat brain slices 450 μM thick containing ventromedial nucleus (VMN) were cut using a Lanier vibratome and placed in

a Perspex recording chamber where they were superfused with artificial cerebrospinal fluid (ACSF) at 37°C, 4 ml/min. All drugs were applied dissolved in the superfusion saline. Extracellular recordings were made using glass micropipettes containing 2 M NaCl which were placed in the VMN. Spontaneous firing was recorded digitally and analyzed using a series of programmes to produce bar histograms of firing rate throughout the course of the experiment. CCK (26–33 sulphated) consistently produced an increase in firing rate in this model (EC_{50} = 0.3 μM) and this effect could be mimicked by compound **24** at a concentration of 80 μM.

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