32, 126053-70-1; 33, 126053-69-8; 34, 126053-79-0; 35, 126053-71-2; 36, 126053-75-6; 37, 135284-71-8; 38, 135284-72-9; 39, 135284-73-0; 40, 135284-74-1; 41, 135284-75-2; 42, 135284-76-3; 47, 108279-08-9; 48, 126054-20-4; 49, 126054-21-5; 50, 126054-22-6; 51, 126054-00-0; 52, 126054-01-1; 53, 126054-02-2; 54, 74071-58-2; 55, 74071-59-3; 56, 74071-60-6; 57, 108695-16-5; BOC-Asp(Bzl)-OH, 7536-58-5; $PhCH_2CH_2NH_2$, 64-04-0; BOC-Gly-OH, 4530-20-5; BOC-Arg(Tos)-OH, 13836-37-8; BOC-Ser(Bzl)-OH, 23680-31-1; $EtNH_2$, 75-04-7; $MeOC(NH_2)=NH\cdot H_2SO_4$, 52328-05-9; H-Arg(Tos)-OH, 4353-32-6; PhCHO, 100-52-7.

Conformational Restriction of the Phenylalanine Residue in a Cyclic Opioid Peptide Analogue: Effects on Receptor Selectivity and Stereospecificity†

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In an effort to determine the effect of side chain conformational restriction on opioid receptor selectivity, the cyclic phenylalanine analogues 2-aminoindan-2-carboxylic acid (Aic), 2-aminotetralin-2-carboxylic acid (Atc), and tetrahydroisoquinoline-3-carboxylic acid (Tic) were substituted for Phe in the potent cyclic opioid peptide analogue H-Tyr-D-Orn-Phe-Glu-NH_2, which lacks significant opioid receptor selectivity. Compounds were tested in μ- and δ-opioid receptor representative binding assays and bioassays in vitro. The analogue H-Tyr-D-Orn-Aic-Glu-NH_2 was found to be a potent agonist with high preference of μ receptors over δ receptors. Opening of the five-membered ring of Aic in the latter peptide, as achieved through substitution of C^{α}-methylphenylalanine or o-methylphenylalanine, resulted in only slightly selective compounds, indicating that the high μ selectivity of the Aic analogue is exclusively the consequence of the imposed side chain conformational restriction. Both diastereoisomers of H-Tyr-D-Orn-(D,L)-Atc-Glu-NH_2 were highly μ-selective and, in contrast to the weak affinity observed with the D-Phe^3 analogue as compared to the L-Phe^3 analogue, both had similar potency. Thus, stereospecificity was lost as a consequence of side chain conformational restriction. Further structure–activity data obtained with analogues containing L- or D-homophenylalanine (Hfe) or 3-(1′-naphthyl)alanine (Nap) in place of Phe^3 and consideration of geometric interrelationships between Nap and the L and D isomers of Atc, Hfe, and Phe led to the proposal that the D-Phe^3 and the D-Atc^3 analogue may have different modes of binding to the receptor. The very low potency observed with H-Tyr-D-Orn-N^{α}MePhe-Glu-NH_2 (N^{α}MePhe = N^{α}-methylphenylalanine) and H-Tyr-D-Orn-Tic-Glu-NH_2 indicated that N^{α}-alkylation at the 3-position is detrimental to activity.

Most of the small linear opioid peptides isolated to date are known to be structurally flexible molecules whose conformations are strongly dependent on the environment (for a review, see ref 2). This structural flexibility not only prevents meaningful conformational studies aimed at elucidating the bioactive (receptor-bound) conformation(s) but also may be one of the major reasons for the lack of significant selectivity of most of these peptides toward one or the other of the different opioid receptor types (μ, δ, κ). In efforts to obtain better conformational integrity and to improve receptor selectivity, various conformationally restricted opioid peptide analogues have been developed. In particular, peptide cyclizations via side chains led to cyclic opioid peptide analogues displaying quite high μ-receptor selectivity (e.g. H-Tyr-cyclo[-D-A_2bu-Gly-Phe-Leu-][3] or H-Tyr-D-Orn-Phe-Asp-NH_2[4]) or greatly improved δ-receptor selectivity (e.g. H-Tyr-D-Pen-Gly-Phe-D(or L)-Pen-OH[5] or H-Tyr-D-Cys-Phe-D-Pen-OH).[6] Molecular mechanics studies recently performed with the cyclic analogues H-Tyr-D-Orn-Phe-Asp-NH_2 and H-Tyr-D-Pen-Gly-Phe-D-Pen-OH revealed that the exocyclic Tyr^1 residue and the Phe^3 (Phe^4) side chain in these compounds still enjoy considerable orientational freedom.[7,8] Since the latter moieties are crucial for opioid activity, it is obvious that they also need to be conformationally restricted in order to obtain more definitive insight into the distinct bioactive conformations of these cyclic opioid peptide analogues at the μ and δ receptor.

In the present paper we describe a series of analogues

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(1) Symbols and abbreviations are in accordance with recommendations of the IUPAC–IUB Joint Commission on Biochemical Nomenclature: Nomenclature and Symbolism for Amino Acids and Peptides. *Biochem. J.* 1984, *219*, 345–373. The following other abbreviations were used: A_2bu, α,γ-diaminobutyric acid; Aic, 2-aminoindan-2-carboxylic acid; Atc, 2-aminotetralin-2-carboxylic acid; Boc, tert-butoxycarbonyl; BOP, benzotriazol-1-yl-oxytris(dimethylamino)phosphonium hexafluorophosphate; C^{α}MePhe, C^{α}-methylphenylalanine; DAGO, H-Tyr-D-Ala-Gly-N^{α}MePhe-Gly-ol; DCC, dicyclohexylcarbodiimide; DIEA, diisopropylethylamine; DSLET, H-Tyr-D-Ser-Gly-Phe-Leu-Thr-OH; FAB, fast atom bombardment; Fmoc, (fluoren-9-ylmethoxy)carbonyl; GPI, guinea pig ileum; Hfe, homophenylalanine; MVD, mouse vas deferens; Nap, 3-(1′-naphthyl)alanine; N^{α}MePhe, N^{α}-methylphenylalanine; oMePhe, o-methylphenylalanine; Pen, penicillamine; TFA, trifluoroacetic acid; Tic, 1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid; U69,593, (5α,7α,8β)-(−)-*N*-methyl-[7-(1-pyrrolidinyl)-1-oxaspiro[4.5]dec-8-yl]benzeneacetamide.

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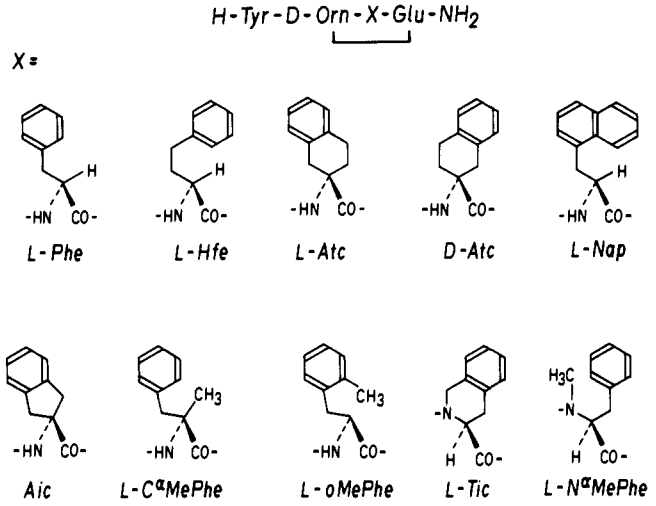

Figure 1. Structural formulas of phenylalanine analogues.

of the cyclic opioid peptide analogue H-Tyr-D-Orn-Phe-Glu-NH_2 that contain various conformationally restricted analogues of phenylalanine in 3-position of the peptide sequence (Figure 1). H-Tyr-D-Orn-Phe-Glu-NH_2 is potent but only slightly μ-receptor-selective and, therefore, represents an ideal parent peptide to study the effect of conformational constraints introduced at the Phe[3] residue on receptor affinity and receptor selectivity. Local conformational restriction of the peptide backbone around the 3-position residue was achieved by replacement of Phe[3] with C^{α}-methylphenylalanine (C^{α}MePhe) or N^{α}-methylphenylalanine (N^{α}MePhe), whereas substitution of 2-aminoindan-2-carboxylic acid (Aic), 2-aminotetralin-2-carboxylic acid (Atc), or tetrahydroisoquinoline-3-carboxylic acid (Tic) resulted in conformational restriction of both the side chain and the peptide backbone. In order to distinguish whether the effect on the opioid activity profile upon substituting Aic or Atc for Phe[3] was due to the introduced conformational constraints per se or to the presence of a substituent in the ortho position of the aromatic ring, a cyclic analogue containing *o*-methylphenylalanine (oMePhe) in the 3-position was also synthesized and tested. Since Atc represents a conformationally restricted analogue not only of Phe but also of homophenylalanine (Hfe), the D- and L-Hfe[3] analogues of H-Tyr-D-Orn-Phe-Glu-NH_2 were prepared and characterized as well. Finally, the ability of the opioid receptor subsite interacting with the Phe[3] side chain of the parent peptide to accommodate a more extended aromatic moiety (naphthyl ring) was examined through preparation and characterization of the analogue H-Tyr-D-Orn-Nap-Glu-NH_2 (Nap = 3-(1'-naphthyl)alanine).

Chemistry. Aic and Atc were prepared by a modified version of the Strecker synthesis[9,10] through conversion of 2-indanone or 2-tetralone to the corresponding spirohydantoins, followed by hydrolysis with 40% alkali. Atc was obtained in racemic form and incorporated as such into the cyclic peptide structure. L-C^{α}MePhe was obtained in optically pure form through digestion of the *N*-trifluoroacetyl derivative of the commercially available racemate with carboxypeptidase A, as described in the literature.[11] D,L-oMePhe was synthesized by condensation of *o*-methylbenzyl chloride with ethyl acetamidocyanoacetate in the presence of sodium ethoxide and subsequent hydrolysis with 6 N HCl.[12] Racemic oMePhe was used in the preparation of the peptide analogue.

The cyclic peptide analogues were prepared by the solid-phase method on a *p*-methylbenzhydrylamine resin according to a scheme described elsewhere.[13] The C-terminal tripeptide segment to be cyclized was assembled by using N^{α}-Fmoc amino acids with Boc and *tert*-butyl protection for the side chains of Orn and Glu, respectively. The side chain protecting groups of the C-terminal tripeptide were removed by treatment with TFA, and side chain to side chain cyclization of the still-resin-bound peptide was then performed by reaction with the BOP [benzotriazol-1-yl-oxytris(dimethylamino)phosphonium hexafluorophosphate] reagent[14] in DMF containing 1.5% (v/v) diisopropylethylamine (DIEA). The time required for amide bond formation to be complete varied from 12 to 48 h. After the cyclization step, the N-terminal Fmoc protecting group was removed and the peptide chains were completed by coupling Boc-Tyr(Boc)-OH. Following the removal of the Boc groups, the cyclic peptide amides were cleaved from the resin by HF/anisole treatment in the usual manner. Crude products were purified by gel filtration on Sephadex G-25 and by reversed-phase chromatography. In all cases the desired cyclic monomer was the predominant component (60–70%) in the crude reaction product, but cyclodimerization[13] and cyclopolymerization did occur to some extent. In the case of H-Tyr-D-Orn-(D,L)-Atc-Glu-NH_2 and H-Tyr-D-Orn-(D,L)-oMePhe-Glu-NH_2 the two diastereoisomers were isolated separately by semipreparative HPLC.

Bioassays and Receptor Binding Assays. Affinities for μ-, δ-, and κ-opioid receptors were determined by displacement of [^{3}H]DAGO, [^{3}H]DSLET, and [^{3}H]U69,593 from rat brain membrane binding sites. For the determination of their in vitro opioid activities, analogues were tested in bioassays based on inhibition of electrically evoked contractions of the guinea pig ileum (GPI) and of the mouse vas deferens (MVD). In the GPI preparation opioid effects are primarily mediated by μ receptors; however, κ receptors are also present in this tissue. μ-Receptor interactions in the GPI are characterized by relatively low K_e values for naloxone as antagonist (1–2 nM),[15] in contrast to the considerably higher values (20–30

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nM)[16] observed with κ-receptor ligands. The MVD assay is usually taken as being representative for δ receptor interactions, even though the vas also contains μ and κ receptors.

Results and Discussion

The cyclic parent peptide H-Tyr-D-Orn-Phe-Glu-NH_2 (1) displays very high affinity for μ receptors but also binds quite strongly to δ receptors and, therefore, is only moderately μ-selective (Table I). Configurational inversion at the 3-position residue (analogue 2) resulted in a more than 1000-fold drop in affinity for both μ and δ receptors. The latter finding confirms the strict requirement for L configuration in the 3-position of both linear and cyclic opioid peptide analogues that are structurally related to the dermorphin family.[17] Methylation of the α-carbon of the Phe^3 residue (analogue 3) produced a 7-fold decrease in μ affinity and a similar decrease in δ affinity, thus resulting in a compound with still only modest preference of μ receptors over δ receptors. It is of interest to point out that C^α-methylation of an amino acid residue in a peptide drastically reduces the conformational space available to the peptide backbone around that residue, insofar as the torsion angles at the position of the methylated residues are limited to values around $\phi = -50°$, $\psi = -50°$ and $\phi = +50°$, $\psi = +50°$.[18] The slight reduction in affinity observed with analogue 3 could be due either to the backbone conformational constraints introduced at the 3-position or to steric interference of the C^α-methyl group. Introduction of a methyl group in the ortho position of the Phe^3 aromatic ring (analogue 4) had little effect on μ-receptor affinity and receptor selectivity.

More extensive conformational restriction at the 3-position was achieved through substitution of the conformationally restricted phenylalanine analogue Aic (Figure 1). In comparison with parent peptide 1, the cyclic peptide analogue H-Tyr-D-Orn-Aic-Glu-NH_2 (5) showed only 4 times lower μ-receptor affinity but 65 times lower affinity for δ receptors and, consequently, greatly improved μ selectivity ($K_i^\delta/K_i^\mu = 49.6$). Since analogues 3 and 4 are distinguished from analogue 5 merely by the opening of one or the other of two adjacent bonds in the five-membered-ring structure of Aic^3 (see Figure 1), comparison of the receptor binding activity profiles of these three analogues permits the unambiguous conclusion that the high μ-receptor selectivity of the Aic^3 analogue is *exclusively* the consequence of the imposed side chain conformational restriction. Replacement of Phe^3 with Aic in the cyclic peptide structure produces conformational constraints both in the side chain and in the peptide backbone at the 3-position. Torsional angles of the 3-position side chain in the Aic^3 analogue are limited to values of $\chi_1 = -80°$, $\chi_2 = -20°$ and $\chi_1 = -160°$, $\chi_2 = +20°$, whereas the backbone torsional angles around the 3-position, as in the case of the $C^\alpha MePhe^3$ analogue, again can only assume values around

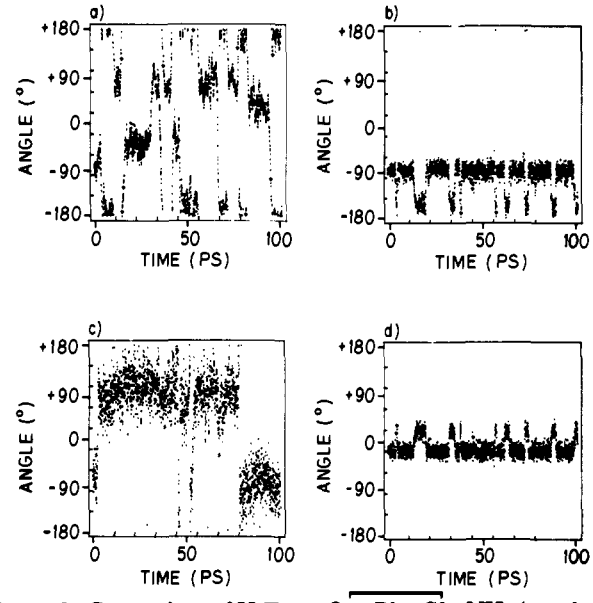

Figure 2. Comparison of H-Tyr-D-Orn-Phe-Glu-NH_2 (panels on the left) and H-Tyr-D-Orn-Aic-Glu-NH_2 (panels on the right) in a molecular dynamics simulation: (a) χ_1^3 (Phe), (b) χ_1^3 (Aic), (c) χ_2^3 (Phe), (d) χ_2^3 (Aic).

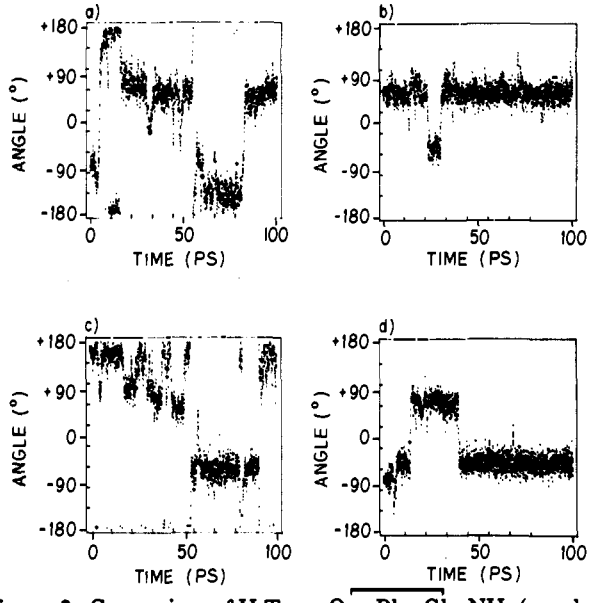

Figure 3. Comparison of H-Tyr-D-Orn-Phe-Glu-NH_2 (panels on the left) and H-Tyr-D-Orn-Aic-Glu-NH_2 (panels on the right) in a molecular dynamics simulation: (a) ϕ_3 (Phe), (b) ϕ_3 (Aic), (c) ψ_3 (Phe), (d) ψ_3 (Aic).

$\phi = -50°$, $\psi = -50°$ and $\phi = +50°$, $\psi = +50°$. These torsional angle values were indeed observed in molecular dynamics simulations that were carried out at 600 K and lasted for 100 ps (Figures 2 and 3). The computer simulations confirm the severe conformational constraints in the Aic^3 side chain and show its conformational transitions between the two conformational states characterized by the χ_1, χ_2 angles indicated above. The limited conformational space available to the Aic^3 side chain and its conformational transitions are also evident in Figure 4, which shows superpositions of 20 snapshots of the Phe^3 and Aic^3 analogues taken at 5-ps intervals along the dynamics trajectory.

The two diastereoisomers of the Atc^3 analogue (compounds 6 and 7) show even higher μ selectivity (K_i^δ/K_i^μ values of 190 and 133, respectively) due to very weak af-

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Figure 4. Twenty snapshots of H-Tyr-D-Orn-Phe-Glu-NH_2 (left) and H-Tyr-D-Orn-Aic-Glu-NH_2 (right) taken at 5-ps intervals along the dynamics trajectory.

Table I. Receptor Binding Assays of Cyclic Opioid Peptide Analogues

		[^{3}H]DAGO		[^{3}H]DSLET		
no.	compd	K_i^μ,[a] nM	rel potency[b]	K_i^δ,[a] nM	rel potency[b]	K_i^δ/K_i^μ
1	H-Tyr-D-Orn-Phe-Glu-NH_2	0.981 ± 0.013	9.63 ± 0.10	3.21 ± 0.32	0.790 ± 0.078	3.27
2	H-Tyr-D-Orn-D-Phe-Glu-NH_2	1660 ± 50	0.00568 ± 0.00018	14000 ± 1600	0.000181 ± 0.000021	8.43
3	H-Tyr-D-Orn-C$^\alpha$MePhe-Glu-NH_2	7.17 ± 0.26	1.31 ± 0.05	54.6 ± 15.0	0.0464 ± 0.0128	7.62
4	H-Tyr-D-Orn-oMePhe-Glu-NH_2	1.92 ± 0.06	4.91 ± 0.15	9.22 ± 0.73	0.275 ± 0.021	4.80
5	H-Tyr-D-Orn-Aic-Glu-NH_2	4.21 ± 0.39	2.24 ± 0.21	209 ± 2	0.0121 ± 0.0001	49.6
6	H-Tyr-D-Orn-(D or L)-Atc-Glu-NH_2 (I)	8.26 ± 1.94	1.14 ± 0.27	1570 ± 9	0.00161 ± 0.00001	190
7	H-Tyr-D-Orn-(D or L)-Atc-Glu-NH_2 (II)	26.3 ± 0.6	0.358 ± 0.008	3510 ± 15	0.000721 ± 0.000003	133
8	H-Tyr-D-Orn-Hfe-Glu-NH_2	1.17 ± 0.08	8.04 ± 0.57	15.4 ± 0.9	0.165 ± 0.009	13.2
9	H-Tyr-D-Orn-D-Hfe-Glu-NH_2	258 ± 6	0.0365 ± 0.0008	2600 ± 10	0.000975 ± 0.000004	10.1
10	H-Tyr-D-Orn-Nap-Glu-NH_2	2.56 ± 0.15	3.68 ± 0.22	50.7 ± 2.3	0.0500 ± 0.0023	19.8
11	H-Tyr-D-Orn-N$^\alpha$MePhe-Glu-NH_2	1000 ± 130	0.00941 ± 0.00119	12800 ± 1500	0.000198 ± 0.000022	12.8
12	H-Tyr-D-Orn-Tic-Glu-NH_2	2410 ± 90	0.00391 ± 0.00015	50100 ± 2700	0.0000505 ± 0.0000027	20.8
13	[Leu^5]enkephalin	9.43 ± 2.07	1	2.53 ± 0.35	1	0.268

[a] Mean of three determinations ± SEM. [b] Potency relative to that of [Leu^5]enkephalin.

finity for the δ receptor, while μ-receptor affinity comparable to that of [Leu^5]enkephalin is maintained (Table I). As in the case of the Aic^3 analogue, it is the side chain conformational restriction per se which is directly responsible for the drastic selectivity enhancement. Again, the Atc residue essentially is able to adopt only two side chain conformations which are characterized by the torsional angles $\chi_1 = 180°$ (t), $\chi_2 = +25°$ and $\chi_1 = -60°$ (g^-), $\chi_2 = -25°$ for L-Atc, and $\chi_1 = 180°$ (t), $\chi_2 = -25°$ and $\chi_1 = +60°$ (g^+), $\chi_2 = +25°$ for D-Atc. Obviously, these side chain conformational constraints are even more detrimental to δ-receptor affinity than those present in the Aic^3 analogue which has about 10 times higher δ affinity than the Atc^3 analogues. Interestingly, the computer simulations showed that in the cyclic opioid peptide analogues described here both the L- and the D-Atc side chain underwent fewer conformational transitions than the Aic side chain (Wilkes, B. C.; Schiller, P. W., manuscript in preparation).

As indicated above, the μ-receptor affinity of the cyclic dermorphin analogue H-Tyr-D-Orn-Phe-Glu-NH_2 is more than a 1000 times higher than that of the corresponding D-Phe^3 analogue, whereas the μ receptor affinities of both diastereoisomers of H-Tyr-D-Orn-(D,L)-Atc-Glu-NH_2 are high and differ from one another by a factor of only 3.18. Careful examination by HPLC revealed that each diastereoisomer of the Atc^3 analogue contained less than 2% of the other and, thus, the similar affinities could not be explained by insufficient separation of the diastereoisomers. Even cross-contamination of each diastereoisomer to the extent of 5% would still result in a diastereomeric potency ratio of only 3.76, and cross-contamination of around 23.5% would have to occur if the potency ratio of the two Atc^3 diastereoisomers were assumed to be similar to that of the two Phe^3 diastereoisomers. The loss of stereospecificity as a consequence of the side chain conformational constraint may be due to the fact that the D-Atc^3 analogue binds to the receptor in a manner different from that of the D-Phe^3 analogue. In the case of the D-Phe^3 analogue a stepwise process of binding according to the "zipper"-type model[19] may occur such that the D-Phe^3 side chain never has a chance to bind to the hydrophobic receptor subsite with which the L-Phe^3 aromatic ring interacts. The observation that in the case of a corresponding cyclic analogue with homophenylalanine (Hfe) substituted in position 3 the L-Hfe^3 analogue (8) has again over 200 times higher μ-receptor affinity than the D-Hfe^3 analogue (9) (Table I) is of interest in view of the fact that Atc represents a conformationally restricted analogue of both Phe and Hfe (with reversed configurational relationships). The receptor binding site with which the 3-position aromatic rings of the L-Phe^3 and L-Hfe^3 analogues interact appears to be fairly large, since it is also able to

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Table II. Guinea Pig Ileum (GPI) and Mouse Vas Deferens (MVD) Assay of Cyclic Opioid Peptide Analogues

no.	compd	GPI		MVD		MVD/GPI
		IC_{50},[a] nM	rel potency[b]	IC_{50},[a] nM	rel potency[b]	IC_{50} ratio
1	H-Tyr-D-Orn-Phe-Glu-NH_2	1.17 ± 0.07	210 ± 13	1.11 ± 0.21	10.3 ± 2.0	0.949
2	H-Tyr-D-Orn-D-Phe-Glu-NH_2	879 ± 97	0.280 ± 0.031	3860 ± 240	0.00295 ± 0.00018	4.39
3	H-Tyr-D-Orn-C$^{\alpha}$MePhe-Glu-NH_2	3.31 ± 0.45	74.3 ± 10.2	5.51 ± 0.05	2.07 ± 0.20	1.66
4	H-Tyr-D-Orn-oMePhe-Glu-NH_2	0.543 ± 0.056	453 ± 47	1.11 ± 0.18	10.3 ± 1.7	2.04
5	H-Tyr-D-Orn-Aic-Glu-NH_2	7.21 ± 0.83	34.1 ± 3.9	36.5 ± 6.7	0.312 ± 0.057	5.06
6	H-Tyr-D-Orn-(D or L)-Atc-Glu-NH_2 (I)	31.6 ± 3.1	7.78 ± 0.76	254 ± 33	0.0449 ± 0.0058	8.04
7	H-Tyr-D-Orn-(D or L)-Atc-Glu-NH_2 (II)	285 ± 47	0.863 ± 0.143	3070 ± 330	0.00371 ± 0.00040	10.8
8	H-Tyr-D-Orn-Hfe-Glu-NH_2	5.82 ± 0.58	42.3 ± 4.2	34.3 ± 5.8	0.332 ± 0.056	5.89
9	H-Tyr-D-Orn-D-Hfe-Glu-NH_2	728 ± 86	0.338 ± 0.040	4160 ± 330	0.00274 ± 0.00022	5.71
10	H-Tyr-D-Orn-D-Nap-Glu-NH_2	4.07 ± 0.89	60.5 ± 13.2	10.9 ± 1.2	1.05 ± 0.12	2.68
11	H-Tyr-D-Orn-N$^{\alpha}$MePhe-Glu-NH_2	707 ± 150	0.348 ± 0.074	2070 ± 260	0.00551 ± 0.00069	2.93
12	H-Tyr-D-Orn-Tic-Glu-NH_2	3060 ± 270	0.0803 ± 0.0071	7310 ± 420	0.00156 ± 0.00009	2.39
13	[Leu^5]enkephalin	246 ± 39	1	11.4 ± 1.1	1	0.0463

[a] Mean of three determinations ± SEM. [b] Potency relative to that of [Leu^5]enkephalin.

accommodate the naphthyl ring of the cyclic analogue H-Tyr-D-Orn-Nap-Glu-NH_2 (**10**) which retains high μ receptor affinity. Configurational inversion of Atc produces a change in the way the aromatic ring is fused to the cyclohexane structure by shifting it from one position to an adjacent one (see Figure 1). This shift in the positioning of the aromatic ring of the 3-position residue is tolerated by the "large" hydrophobic binding site on the receptor and, due to the side chain conformational constraints, the process of binding of not only the L-Atc^3 but also the D-Atc^3 analogue is such that the Atc aromatic ring is forced to interact with this binding site. Thus, the low activity of the D-Phe^3 analogue as compared to that of the D-Atc^3 analogue may be explained with a different process and mode of receptor binding.

Methylation of the α-nitrogen in position 3 of the peptide sequence of H-Tyr-D-Orn-Phe-Glu-NH_2 resulted in a weakly active analogue (**11**) (Table I). The more than 1000-fold decrease in μ-receptor affinity observed with this compound could be due to the introduced local conformational restriction of the peptide backbone (ϕ angle), the unavailability of the amide proton in the 3-position for the formation of an important hydrogen bond, or the bulk of the N-methyl group which might not be tolerated in this position. In view of the low affinity of analogue **11**, it is not surprising that the Tic^3 analogue **12** is also very weakly active. Interestingly, replacement of the N-terminal exocyclic D-Phe residue in somatostatin-derived opioid antagonists with D-Tic has recently been shown to produce an increase in μ-receptor affinity and selectivity.[20]

The determined K_i^{μ} values of all analogues were higher than 2×10^{-5} M, indicating that none of them had appreciable affinity for κ receptors.

The results of the GPI and MVD bioassays (Table II) were found to be in qualitative but not always quantitative agreement with the receptor binding data. In general, the obtained $IC_{50}(MVD)/IC_{50}(GPI)$ ratios were much lower than the corresponding K_i^{δ}/K_i^{μ} ratios determined in the receptor binding assays, due to the fact that the compounds examined showed relatively higher potency in the MVD assay than in the [^{3}H]DSLET receptor binding assay. The unexpectedly high potencies of the analogues in the MVD assay are due to the fact that they produce the opioid effect through interaction with both δ- and μ-opioid receptors present in the vas preparation. To some extent, the discrepancies between binding assay and bioassay data may also be due to a different degree of nonspecific adsorption of these more or less hydrophobic molecules in the various tissues. Since, aside from being conformationally restricted, all examined analogues contain a D-amino acid residue in the 2-position of the peptide sequence and a C-terminal carboxamide function, they can be expected to be equally stable against enzymolysis under the conditions of the binding assays and bioassays and, thus, a different extent of peptide degradation in the various tissues can be ruled out as a factor explaining the differences between bioassay and binding assay data. In the GPI assay all analogues showed K_e values for naloxone as antagonist ranging from 1 to 3 nM. Such low K_e values are typical for μ-receptor interactions and rule out an additional interaction with κ receptors, since κ-receptor interactions would be characterized by much higher K_e values. These results are in agreement with the lack of κ-receptor affinity established in the receptor binding assay.

Conclusions

In the present paper we described a systematic effort to further rigidify the structure of the cyclic opioid peptide analogue H-Tyr-D-Orn-Phe-Glu-NH_2 through introduction of additional conformational constraints at the Phe^3 residue. This goal was successfully achieved through substitution of the Phe^3 residue with the conformationally restricted phenylalanine analogues Aic and Atc, which resulted in potent and highly μ-receptor-selective compounds. Furthermore, comparison of the opioid receptor affinity profile of the Aic^3 and Atc^3 analogues with those of corresponding C$^{\alpha}$MePhe and oMePhe analogues led to the first unambiguous demonstration that side chain conformational restriction per se may result in enhanced receptor selectivity. Another finding of fundamental importance was the loss of stereospecificity due to side chain conformational restriction observed with the Atc^3 analogues which may be explained with an altered mode of receptor binding. As in the case of the parent peptide (**1**), the exocyclic tyrosine residue in analogues **5–7** retains

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considerable orientational freedom and will also have to be conformationally restricted in order to obtain better insight into the μ-receptor-bound conformation of this type of cyclic opioid tetrapeptide.

Experimental Section

General Methods. Precoated plates (silica gel G, 250 μm, Analtech, Newark, DE) were used for ascending TLC in the following solvent systems (all v/v): (I) $CHCl_3$/MeOH/AcOH (90/8/2); (II) $CHCl_3$/MeOH/benzene/H_2O (8/8/8/1); (III) *n*-BuOH/AcOH/H_2O (4/1/5, organic phase), and (IV) *n*-BuOH/pyridine/AcOH/H_2O (15/10/3/12). Reversed-phase HPLC was performed on a Varian VISTA 5500 liquid chromatograph, utilizing a Waters column (30 × 0.78 cm) packed with C-18 Bondapak reversed-phase (10 μm) material. For amino acid analyses peptides (0.2 mg) were hydrolyzed in 6 N HCl (0.5 mL) containing a small amount of phenol for 24 h at 110 °C in deaerated tubes. Hydrolysates were analyzed on a Beckman Model 121C amino acid analyzer equipped with a Model 126 Data System integrator. Proton nuclear magnetic resonance spectra were recorded at 25 °C on a Varian VXR-400S spectrometer using 3-(trimethylsilyl)-1-propanesulfonic acid (sodium salt) as an internal standard. Molecular weights of peptides were determined by FAB mass spectrometry on an MS-50 HMTCTA mass spectrometer interfaced to a DS-90 data system (Drs. M. Evans and M. Bertrand, Department of Chemistry, University of Montreal).

Amino Acid Synthesis. **D,L-*o*-Methylphenylalanine (oMePhe).** Sodium metal (920 mg, 0.04 g-atom) was dissolved in 200 mL of absolute EtOH under nitrogen. When the sodium had dissolved, 6.8 g (40 mmol) of ethyl acetamidocyanoacetate and 4.4 g (40 mmol) of 2-methylbenzyl chloride were added. The solution was stirred at 60 °C for 24 h and then evaporated in vacuo. The residue was dissolved in 150 mL of $CHCl_3$ and extracted with water. The organic phase was dried (Na_2SO_4), filtered, and evaporated in vacuo. The obtained oily residue was dissolved in 200 mL of 6 N HCl and refluxed for 16 h. The solution was then treated with Norite, filtered, and allowed to stand in the cold. The solid which deposited was collected by filtration and dried in vacuo over KOH. The product was recrystallized from MeOH/Et_2O to afford 6.6 g (60%) of a white powder: mp 248–252 °C; TLC R_f 0.55 (III); ^{1}H NMR (D_2O) δ 2.56 (s, 3 H, CH_3), 3.25 (q, 1 H, CH_2), 3.58 (q, 1 H, CH_2), 4.13 (q, 1 H, CH), 7.48 (m, 4 H, ar); MS *m/e* 179 (M^+).

2-Aminoindan-2-carboxylic Acid (Aic). A mixture of 2.6 g (0.02 mol) of 2-indanone, 2.82 g (0.05 mol) of NaCN, 21 g of ammonium carbonate, and 90 mL of EtOH/H_2O (1:1, v/v) was heated at 55–60 °C for 6 h. The solid that precipitated on cooling was collected by filtration and recrystallized from EtOH to give 2.67 g (73%) of 2-spirohydantoinindan (mp 262 °C).

2-Spirohydantoinindan (3.8 g, 0.02 mol), 20 mL of a 40% (w/w) aqueous NaOH solution and 10 mL of propylene glycol were combined, stirred, and refluxed for 24 h. The reaction mixture was then cooled and diluted with 50 mL of water. After acidification with 1 N HCl to pH 2 and filtration of the precipitated solid, the pH was adjusted to 6 by careful addition of a 10% (w/w) aqueous $NaHCO_3$ solution. The amino acid then precipitated in crystalline form and was collected by filtration. Recrystallization from EtOH/H_2O gave 2.45 g (82%) of 2-aminoindan-2-carboxylic acid: mp 298 °C; TLC R_f 0.54 (III); ^{1}H NMR (D_2O) δ 3.37 (d, 2 H, H-1, H-3), 3.77 (d, 2 H, H-1, H-3), 7.34 (s, 4 H, ar); MS *m/e* 177 (M^+).

D,L-2-Aminotetralin-2-carboxylic Acid (Atc). Commercially available 2-spirohydantointetralin (Aldrich) was converted to D,L-2-aminotetralin-2-carboxylic acid in the same manner as that described for the preparation of Aic. The free amino acid precipitated upon adjustment of the pH to 5.5 and after recrystallization from EtOH/H_2O was obtained as a white powder in 60% yield: mp 315 °C; TLC R_f 0.56 (III); ^{1}H NMR (D_2O) δ 2.15 (m, 1 H, H-3), 2.42 (m, 1 H, H-3), 2.91 (m, 1 H, H-4), 3.02 (m, 1 H, H-4), 3.10 (d, 1 H, H-1), 3.52 (d, 1 H, H-1), 7.25 (m, 4 H, ar); MS *m/e* 191 (M^+).

L-1,2,3,4-Tetrahydroisoquinoline-3-carboxylic Acid (Tic). L-phenylalanine (2.5 g, 0.015 mol) was dissolved in 25 mL of concentrated HCl, and 2 g of paraformaldehyde and 0.5 mL of concentrated H_2SO_4 were added. The solution was then refluxed for 2 days. After cooling and filtration the resulting solid was dissolved in hot H_2O/EtOH (1/1, v/v) and aqueous NH_4OH (30%) was added until a pH of 7.0 was reached. The crystals which precipitated upon cooling of the solution were collected by filtration, washed with EtOH, and dried to yield 1.6 g (60%) of product: mp 315 °C; TLC R_f 0.51 (III); ^{1}H NMR (D_2O) δ 3.27 (q, 1 H, H-4), 3.49 (q, 1 H, H-4), 4.49 (m, 3 H, H-1, H-1, H-3), 7.32 (m, 4 H, ar); MS *m/e* 177 (M^+).

The Fmoc derivatives of L-C$^\alpha$MePhe, D,L-oMePhe, Aic, D,L-Atc, L-Hfe, D-Hfe, L-Nap, L-N$^\alpha$MePhe, and L-Tic were prepared by reaction with 9-fluorenylmethyl chloroformate in the usual manner.[21]

N$^\alpha$-Fmoc-C$^\alpha$-methylphenylalanine (N$^\alpha$Fmoc-C$^\alpha$MePhe): mp 124–128 °C; TLC R_f 0.58 (I), 0.60 (II); ^{1}H NMR ([$^2H_6$]DMSO) δ 1.17 (s, 3 H, CH_3), 2.90 (d, 1 H, C$^\beta$H), 3.25 (d, 1 H, C$^\beta$H), 4.23 (t, 1 H, CH), 4.31 (q, 1 H, CH_2), 4.50 (q, 1 H, CH_2), 7.24 (s, 1 H, NH), 6.99–7.95 (m, 13 H, ar), 12.52 (br, 1 H, COOH); $[\alpha]^{25}_D$ –6.8° (*c* 1.0, CH_3OH).

N$^\alpha$-Fmoc-(D,L)-*o*-methylphenylalanine (N$^\alpha$Fmoc-(D,L)-oMePhe): mp 125–128 °C; TLC R_f 0.56 (I), 0.58 (II); ^{1}H NMR ([$^2H_6$]DMSO) δ 2.29 (s, 3 H, CH_3), 2.85 (q, 1 H, C$^\beta$H), 3.11 (q, 1 H, C$^\beta$H), 4.03 (q, 1 H, C$^\alpha$H), 4.15 (m, 3 H, CH + CH_2), 7.77 (d, 1 H, NH), 7.05–7.88 (m, 12 H, ar), 12.74 (br, 1 H, COOH).

N$^\alpha$Fmoc-2-aminoindan-2-carboxylic acid (N$^\alpha$Fmoc-Aic): mp 195 °C; TLC R_f 0.80 (I), 0.75 (II); ^{1}H NMR ([$^2H_6$]DMSO) δ 3.23 (d, 2 H, H-1, H-3), 3.45 (d, 2 H, H-1, H-3), 4.20 (m, 1 H, CH), 4.28 (m, 2 H, CH_2), 7.14–7.88 (m, 12 H, ar), 7.90 (s, 1 H, NH), 12.54 (br, 1 H, COOH).

N$^\alpha$-Fmoc-(D,L)-2-aminotetralin-2-carboxylic acid (N$^\alpha$Fmoc-(D,L)-Atc): mp 173–175 °C; TLC R_f 0.80 (I), 0.74 (II); ^{1}H NMR ([$^2H_6$]DMSO) δ 1.95 (m, 1 H, H-3), 2.31 (m, 1 H, H-3), 2.72 (m, 2 H, H-4), 3.07 (d, 1 H, H-1), 3.16 (d, 1 H, H-1), 4.20 (m, 1 H, CH), 4.29 (m, 2 H, CH_2), 7.62 (s, 1 H, NH), 7.04–7.87 (m, 12 H, ar), 12.48 (br, 1 H, COOH).

N$^\alpha$-Fmoc-homophenylalanine (N$^\alpha$Fmoc-Hfe): mp 131 °C; TLC R_f 0.56 (I), 0.58 (II); ^{1}H NMR ([$^2H_6$]DMSO) δ 1.92 (m, 2 H, C$^\beta$H), 2.63 (m, 2 H, C$^\gamma$H), 3.90 (m, 1 H, C$^\alpha$H), 4.25 (m, 1 H, CH), 4.33 (m, 2 H, CH_2), 7.75 (s, 1 H, NH), 7.16–7.91 (m, 13 H, ar), 12.57 (br, 1 H, COOH); $[\alpha]^{25}_D$ –6.3° (*c* 1.0, CH_3OH).

N$^\alpha$-Fmoc-D-homophenylalanine (N$^\alpha$-Fmoc-D-Hfe): mp 125 °C; TLC R_f 0.56 (I), 0.58 (II); ^{1}H NMR ([$^2H_6$]DMSO) δ 1.94 (m, 2 H, C$^\beta$H), 2.63 (m, 2 H, C$^\gamma$H), 3.90 (m, 1 H, C$^\alpha$H), 4.25 (m, 1 H, CH), 4.32 (m, 2 H, CH_2), 7.74 (s, 1 H, NH), 7.16–7.90 (m, 13 H, ar), 12.58 (br, 1 H, COOH); $[\alpha]^{25}_D$ +5.8° (*c* 1.0, CH_3OH).

N$^\alpha$-Fmoc-3-(1'-naphthyl)alanine (N$^\alpha$-Fmoc-Nap): mp 130–133 °C; TLC R_f 0.56 (I), 0.56 (II); ^{1}H NMR ([$^2H_6$]DMSO) δ 3.26 (q, 1 H, C$^\beta$H), 3.65 (q, 1 H, C$^\beta$H), 4.14 (m, 3 H, CH + CH_2), 4.31 (m, 1 H, C$^\alpha$H), 7.84 (s, 1 H, NH), 7.23–8.14 (m, 15 H, ar), 12.86 (br, 1 H, COOH); $[\alpha]^{25}_D$ – 59.0° (*c* 1.0, CH_3OH).

N$^\alpha$-Fmoc-N$^\alpha$-methylphenylalanine (N$^\alpha$Fmoc-N$^\alpha$MePhe): mp 129–133 °C; TLC R_f 0.59 (I), 0.61 (II); ^{1}H NMR ($CDCl_3$) (due to nitrogen inversion two isomers are observed) (isomer I) δ 2.79 (s, 3 H, CH_3), 3.13 (q, 1 H, C$^\beta$H), 3.38 (q, 1 H, C$^\beta$H), 4.21 (t, 1 H, CH), 4.38 (m, 2 H, CH_2), 4.91 (q, 1 H, C$^\alpha$H), 6.95–7.76 (m, 13 H, ar); (isomer II) δ 2.74 (q, 1 H, C$^\beta$H), 2.78 (s, 3 H, CH_3), 3.13 (q, 1 H, C$^\beta$H), 4.15 (t, 1 H, CH), 4.39 (q, 1 H, CH_2), 4.56 (q, 1 H, CH_2), 4.58 (q, 1 H, C$^\alpha$H), 6.95–7.76 (m, 13 H, ar); $[\alpha]^{25}_D$ –60.7° (*c* 1.0, CH_3OH).

N$^\alpha$-Fmoc-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid (N$^\alpha$Fmoc-Tic): mp 129–132 °C; TLC R_f 0.58 (I), 0.60 (II); ^{1}H NMR ([$^2H_6$]DMSO) δ 3.15 (m, 2 H, H-4), 4.28 (m, 1 H, CH), 4.38 (m, 2 H, CH_2), 4.45 (d, 1 H, H-1), 4.61 (d, 1 H, H-1), 4.84 (q, 1 H, H-3), 7.14 (m, 12 H, ar), 12.72 (br, 1 H, COOH); $[\alpha]^{25}_D$ –3.6° (*c* 1.0, CH_3OH).

Other Fmoc- and Boc-amino acids were purchased from IAF BioChem International (Laval, Quebec, Canada) and from Bachem Bioscience, (Philadelphia, PA).

Solid-Phase Synthesis and Purification of Cyclic Peptide Analogues. Peptide synthesis was performed by the manual solid-phase technique using a *p*-methylbenzhydrylamine resin (1% cross-linked, 100-200 mesh, 0.2 mM/g of titratable amine) obtained from United States Biochemical Corp. (Cleveland, OH).

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Table III. Analytical Data of Cyclic Opioid Peptide Analogues

no.	compd	amino acid anal.	TLC, R_f III	TLC, R_f IV	HPLC[a] K' value	FAB-MS (MH^+), m/e
1	H-Tyr-D-Orn-Phe-Glu-NH_2	Tyr 0.91, Orn 1.05, Phe 0.91, Glu 1.00	0.56	0.76	0.85	553
2	H-Tyr-D-Orn-D-Phe-Glu-NH_2	Tyr 1.00, Orn 1.09, Phe 1.11, Glu 0.98	0.55	0.75	1.06	553
3	H-Tyr-D-Orn-C$^{\alpha}$MePhe-Glu-NH_2	Tyr 1.00, Orn 1.08, Glu 0.92	0.57	0.76	1.21	567
4	H-Tyr-D-Orn-oMePhe-Glu-NH_2	Tyr 1.08, Orn 0.89, Glu 1.00	0.59	0.77	1.26	567
5	H-Tyr-D-Orn-Aic-Glu-NH_2	Tyr 1.00, Orn 1.08, Glu 0.91	0.46	0.78	1.18	565
6	H-Tyr-D-Orn-(D or L)-Atc-Glu-NH_2 (I)	Tyr 0.94, Orn 1.06, Glu 1.00	0.43	0.80	1.37	579
7	H-Tyr-D-Orn-(D or L)-Atc-Glu-NH_2 (II)	Tyr 0.92, Orn 1.00, Glu 1.09	0.43	0.80	2.21	579
8	H-Tyr-D-Orn-Hfe-Glu-NH_2	Tyr 1.00, Orn 0.98, Glu 1.01	0.58	0.77	1.15	566
9	H-Tyr-D-Orn-D-Hfe-Glu-NH_2	Tyr 1.05, Orn 1.00, Glu 1.00	0.58	0.76	1.55	566
10	H-Tyr-D-Orn-Nap-Glu-NH_2	Tyr 0.99, Orn 1.07, Glu 0.99	0.53	0.77	3.81	603
11	H-Tyr-D-Orn-N$^{\alpha}$MePhe-Glu-NH_2	Tyr 1.00, Orn 0.96, Glu 0.90	0.56	0.77	1.21	567
12	H-Tyr-D-Orn-Tic-Glu-NH_2	Tyr 0.88, Orn 1.05, Glu 1.00	0.56	0.75	0.69	565

[a] 0.1% TFA/MeOH 80/10; flow rate 1.5 mL/min, monitored at λ = 280 nM.

The cyclic lactam analogues were synthesized according to a protection scheme described in detail elsewhere.[13] After neutralization of the resin with 10% (v/v) DIEA in CH_2Cl_2 (2 × 10 min) and washing with CH_2Cl_2 (3 × 1 min), the C-terminal tripeptide segment to be cyclized was assembled according to the following protocol: (1) addition of Fmoc-amino acid in CH_2Cl_2 (2.5 equiv), (2) addition of DCC (2.5 equiv) and mixing for 4–24 h (completeness of the reaction was monitored by the ninhydrin test[22]), (3) Fmoc deprotection with 50% piperidine in CH_2Cl_2 (30 min), (4) washing with DMF (3 × 1 min) and EtOH (3 × 1 min). After coupling of Fmoc-D-Orn(Boc)-OH, Fmoc protection of the N-terminal amino group was retained and the side chains of the Orn and Glu residues to be linked were deprotected by treatment with 50% (v/v) TFA in CH_2Cl_2 (30 min). Following neutralization with 10% (v/v) DIEA in CH_2Cl_2 (2 × 10 min) and washing with CH_2Cl_2 (3 × 1 min) and DMF (3 × 1 min), cyclization was carried out in DMF containing 1.5% (v/v) DIEA at room temperature by addition of the BOP reagent (3.0 equiv). Monitoring of the ring-closure reaction with the ninhydrin test revealed that cyclization was usually complete after 12–48 h. After performance of the cyclization step, the N-terminal Fmoc group was removed as usual and washing of the resin was carried out as described above. Subsequently, Boc-Tyr(Boc)-OH (2.5 equiv) in CH_2Cl_2 and DCC (2.5 equiv) were added, and the resin suspension was mixed for 24 h. After final deprotection with 50% (v/v) TFA in CH_2Cl_2 (30 min), the resin was washed with CH_2Cl_2 (3 × 1 min) and EtOH (3 × 1 min) and was dried in a desiccator. Peptides were cleaved from the resin by treatment with HF for 90 min at 0 °C and for 15 min at room temperature (20 mL of HF plus 1 mL of anisole/g of resin). After evaporation of HF, the resin was extracted three times with Et_2O and, subsequently, three times with 7% AcOH. The crude peptide was then obtained in solid form through lyophilization of the acetic acid extract.

Peptides were purified by gel filtration on a Sephadex-G-25 column in 0.5 N AcOH, followed by reversed-phase chromatography on an octadecasilyl silica column,[23] with a linear gradient of 0–60% MeOH in 1% TFA. If necessary, further purification to homogeneity was performed by semipreparative reversed-phase HPLC [20–50% MeOH (linear gradient) in 0.1% TFA]. Semipreparative HPLC under the same conditions was also used to separate the two diastereoisomers of the Atc[3] and oMePhe[3] analogues. Final products were obtained as lyophilisates. Homogeneity of the peptides was established by TLC and by HPLC under conditions identical with those described above. All peptides were at least 98% pure, as judged from the HPLC elution profiles. Analytical data are presented in Table III. In the case of H-Tyr-D-Orn-D,L-oMePhe-Glu-NH_2 the configurational assignment of the separated diastereoisomers was made on the basis of amino acid analyses of peptide hydrolysates that had been incubated with L-amino acid oxidase.

Binding Assays and Bioassays. Opioid receptor binding assays using rat brain membrane preparations were performed as reported in detail elsewhere.[24] [^{3}H]DAGO, [^{3}H]DSLET, and [^{3}H]U69,593 at respective concentrations of 0.72, 0.78, and 0.80 nM were used as radioligands and incubations were performed at 0 °C for 2 h. IC_{50} values were determined from log dose–displacement curves and K_i values were calculated from the obtained IC_{50} values by means of the equation of Cheng and Prusoff,[25] using values of 1.3, 2.6, and 2.9 nM for the dissociation constants of [^{3}H]DAGO, [^{3}H]DSLET, and [^{3}H]U69,593, respectively.[26-28]

The GPI[29] and MVD[30] bioassays were carried out as reported in detail elsewhere.[24,31] A log dose–response curve was determined with [Leu^5]enkephalin as standard for each ileum and vas

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preparation, and IC_{50} values of the compounds being tested were normalized according to a published procedure.[32] K_e values for naloxone as antagonist were determined from the ratio of IC_{50} values obtained in the presence and absence of a fixed naloxone concentration.[33]

Molecular Dynamics Studies. Dyamics simulations were carried out by using the software package SYBYL (Tripos Associates, St. Louis, MO) on a VAXstation 3500. Molecules were viewed on an Evans & Sutherland PS330 computer graphics display terminal and a Hewlett-Packard HP7475 plotter was used for the preparation of the figures. The simulations were carried out with a step size of 1 fs and data were recorded for analysis every 50 fs. Starting conformations were low-energy conformers obtained in a molecular mechanics study (Wilkes, B. C.; Schiller, P. W., manuscript in preparation). After an equilibration period of 2 ps the simulations were carried out for 100 ps at 600 K. The elevated temperature of 600 K was chosen in order to obtain better insight into the accessible conformational space, since conformational transitions are expected to be more frequent at 600 K than at 300 K. Each dynamics trajectory was analyzed for torsion angles (Figures 2 and 3), and conformations were sampled every 5 ps to generate Figure 4.

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Registry No. 1, 135943-98-5; 2, 136030-31-4; 3, 135943-99-6; (L,D,L,L)-4, 136031-52-2; (L,D,D,L)-4, 135944-04-6; 5, 135972-37-1; (L,D,L,L)-6, 135972-38-2; (L,D,D,L)-6, 136031-53-3; 8, 135944-00-2; 9, 136030-32-5; 10, 135944-01-3; 11, 135944-02-4; 12, 135944-03-5; (±)-AcNHCH(CN)COOEt, 90877-70-6; 2-$MeC_6H_4CH_2Cl$, 552-45-4; H-Phe-OH, 63-91-2; H-DL-oMePhe-OH, 22888-51-3; H-Aic-OH, 27473-62-7; H-DL-Atc-OH, 6331-63-1; H-Tic-OH, 74163-81-8; H-Hfe-OH, 943-73-7; H-D-Hfe-OH, 82795-51-5; H-Nal-OH, 55516-54-6; H-N^{α}MePhe-OH, 2566-30-5; Fmoc-C^{α}MePhe-OH, 135944-05-7; Fmoc-DL-oMePhe-OH, 135944-06-8; Fmoc-Aic-OH, 135944-07-9; Fmoc-DL-Atc-OH, 135944-08-0; Fmoc-Hfe-OH, 132684-59-4; Fmoc-D-Hfe-OH, 135944-09-1; Fmoc-Nal-OH, 96402-49-2; Fmoc-N^{α}MePhe-OH, 77128-73-5; Fmoc-Tic-OH, 136030-33-6; Fmoc-Phe-OH, 35661-40-6; Fmoc-D-Phe-OH, 86123-10-6; Fmoc-Glu(OBu-t)-OH, 71989-18-9; Fmoc-D-Orn(Boc)-OH, 118476-89-4; Boc-Tyr(Boc)-OH, 20866-48-2; H-C^{α}MePhe-OH, 23239-35-2; 2-indanone, 615-13-4; 2-spirohydantoinindan, 27473-61-6; (±)-2-spirohydantointetralin, 6270-37-7.

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Synthesis and Evaluation of Some Nitrobenzenesulfonamides Containing Nitroisopropyl and (Ureidooxy)methyl Groups as Novel Hypoxic Cell Selective Cytotoxic Agents

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Basic nitrobenzenesulfonamides containing nitroisopropyl and (ureidooxy)methyl groups were prepared and evaluated as novel hypoxic cell selective cytotoxic agents. In vitro, *N*-(2-aminoethyl)-*N*-methyl-3-nitro-4-(1-methyl-1-nitroethyl)benzenesulfonamide hydrochloride (11) proved to be preferentially toxic to hypoxic EMT6 mammary carcinoma cells. At 1 mM concentration in vitro, 11 reduced the surviving fraction of these hypoxic cells to 3×10^{-3} with no effect on aerobic cells. In radiation experiments, 11 appeared to function as a hypoxic cell radiosensitizer as well as a selective cytotoxic agent. However, administration of 11 at 200 mg/kg ip or 100 mg/kg iv to BALB/c mice implanted with solid EMT6 tumors produced no evidence of significant in vivo cytotoxic or radiosensitizing activity. *N*-Methyl-*N*-[2-(methylamino)ethyl]-3-nitro-4-[(ureidooxy)methyl]benzenesulfonamide hydrochloride (20) showed slight differential toxicity toward EMT6 cells at 3 mM concentration and radiosensitizing activity comparable to misonidazole at 1 mM concentration.

Introduction

Convincing evidence is now available that solid tumors possess significant numbers of hypoxic cells[1] which adversely affect the efficacy of radiotherapy[2] and chemotherapy[3] regimens. However, from a medicinal chemistry perspective, this apparent obstacle to successful therapy can conceivably be exploited to provide novel approaches for the design of hypoxia selective agents. For example, reduction of nitro groups by the reducing environment of hypoxic cells is one strategy which has been utilized for the activation of latent cytotoxic compounds.[4-6]

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