final fractional coordinates, temperature parameters, bond distances, and bond angles for 1 can be found in the supplementary material (see paragraph at the end of paper concerning Supplementary Material).

Renal Blood Flow Assays. (a) Conscious Dogs. Intravenous Administration (Table II). Trained conscious female mongrel dogs in the postabsorptive state were used. Tap water (500 mL) was administered orally 1–1.5 h prior to study. Venous catheters were inserted into one jugular and one saphenous vein for blood sampling and infusion of sodium *p*-aminohippurate (PAH). The renal clearance of PAH was used as an estimate of the effective renal plasma flow. The urinary bladder was catheterized, and the animal was placed in a modified Pavlov sling. A priming dose of PAH (8 mg/kg) was given iv, and a constant infusion of PAH [0.3 (mg/kg)/min] in isotonic NaCl solution was begun. After an equilibration period of 30–45 min, the bladder was emptied, and two 30-min control clearance periods were obtained. A single iv dose of the test compound as the Na salt in aqueous solution was then given and four additional 30-min clearance periods were taken. Heparinized blood samples for measurement of plasma PAH concentration were taken at the midpoint of each urine collection period. Concentrations of PAH in plasma and urine were measured by standard automated methods (Technicon Autoanalyzer).

(b) Anesthetized Dogs. Intrarenal Arterial Administration (Table III). Dogs were anesthetized with vinbarbital, an electromagnetic flow probe was placed around the left renal artery, and a catheter was inserted into the aorta for blood pressure measurement. After a 30-min pretreatment period during which saline was infused into the renal artery, PGE_2 was infused at 10, 60, and 110 (ng/kg)/min each for 10 min in ascending dose order. The dogs were allowed to recover for 30 min, and **5b** as the Na salt in aqueous solution was injected into the renal artery at 10, 25, or 50 ng/kg. All dogs (14) received the graded PGE_2 infusions, and 4, 5, and 5 dogs received 10, 25, and 50 ng/kg of **5b**, respectively. Renal blood flow and BP were recorded during the 10-min infusion periods and for 180 min after injection of **5b**. Renal resistances were calculated, and the maximum decrease in RR during each period was used to calculate the percent change from pretreatment RR.

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Acknowledgment. We thank Mr. K. B. Streeter and his staff for elemental analyses and Dr. D. W. Cochran and Ms. J. S. Murphy for NMR spectra. We are indebted to Dr. B. H. Arison for interpretations of high-resolution NMR data and to H. Krenz for technical assistance with the X-ray diffraction experiments.

Registry No. (±)-8α,15α-**2a**, 84064-00-6; (±)-8α,15β-**2a**, 84064-01-7; (±)-8α,15α-**2b**, 84040-53-9; (±)-8α,15β-**2b**, 84040-54-0; (±)-**5a**, 84040-55-1; (+)-**5b**, 84040-56-2; (−)-**5b**, 84040-57-3; (±)-**5b**, 84064-02-8; (±)-**5b** Na salt, 84064-03-9; **6a**, 3884-92-2; **6b**, 72313-37-2; (±)-**7a**, 84040-58-4; (±)-**7b**, 84040-59-5; (±)-8α,15α-**8a**, 84040-60-8; (±)-8α,15β-**8a**, 84040-61-9; (±)-8α,15α-**8b**, 84040-62-0; (±)-8α,15β-**8b**, 84040-63-1; **9a**, 84040-64-2; **9b**, 84040-65-3; (±)-**10a**, 84040-66-4; (±)-**10b**, 84040-67-5; **11**, 72313-38-3; deethyl-**11**, 76865-45-7; (±)-**12**, 84040-68-6; (+)-**13**, 84040-69-7; (−)-**13**, 84064-04-0; CH_3CN, 75-05-8; 1-bromopentane, 110-53-2; (±)-1-chloro-3-octanol, 84040-70-0; (±)-3-acetoxy-1-chlorooctane, 84040-71-1; (±)-3-acetoxy-1-iodooctane, 84040-72-2; *p*-toluic acid, 99-94-5; 2-(2-bromoethyl)-1,3-dioxolane, 18742-02-4; 3-chloropropanal, 19434-65-2; cyclohexanone, 108-94-1; 2-(1-hydroxycyclohexyl)acetonitrile, 14368-55-9; 1-(2-aminoethyl)cyclohexanol, 39884-50-9.

Supplementary Material Available: Tables of fractional coordinates, temperature parameters, bond distances, and bond angles for (+)-**5b** (3 pages). Ordering information is given on any current masthead page.

Synthesis and Evaluation of 1- and 2-Substituted Fentanyl Analogues for Opioid Activity

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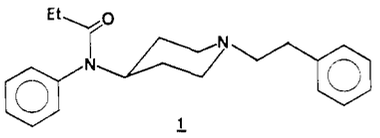
We synthesized fentanyl analogues that possess key groups common to the opioid peptides to investigate whether or not these two classes of compounds interact with common subsites on opioid receptors. The design of the analogues was based on the possibility of structural analogy between the two aromatic rings of fentanyl and the Tyr[1] and Phe[4] residues of the opioid peptides. The synthesized compounds showed very weak or no opioid activity as tested in the electrically stimulated longitudinal muscle of the guinea pig ileum or mouse vas deferens preparations. These results, together with those of reported studies, suggest that fentanyl and the opioid peptides interact with different subsites on either μ or δ receptors. Studies using the irreversible μ opioid receptor antagonist, β-funaltrexamine, indicate that fentanyl interacts preferentially with μ opioid receptors.

Fentanyl[1] (1) is the prototype of a series of analgesics



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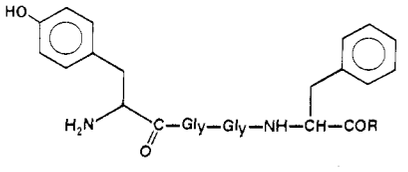

Opioid Peptides

known as the 4-anilidopiperidines. Compounds in the series are characterized by their potent analgesic properties. Structure–activity relationship studies of both fentanyl[1] and enkephalins[2] have suggested the possibility of analogies between them.[3] In the opioid peptides, the Tyr[1] and Phe[4] residues are important for opioid activity and probably interact with two complementary subsites on opioid receptors.[4,5] Since fentanyl (1) also contains two

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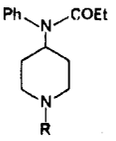
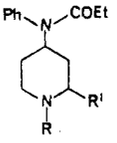
Table I. 1-Substituted Fentanyl Analogues



compd	R	mp, °C	R_f	$[\alpha]^{25}_D$, deg	yield,[a] %	formula	anal.
2·HOAc	Tyr-	172	0.55[b]	+12.49[c]	58	$C_{23}H_{29}N_3O_3 \cdot C_2H_4O_2 \cdot 2H_2O$	C, H, N
3·HOAc	Tyr-Gly-	157	0.45[b]	+17.5[d]	40	$C_{25}H_{32}N_4O_4 \cdot C_2H_4O_2 \cdot H_2O$	C, H, N
4·HOAc	Tyr-Gly-Gly-	93–105	0.13[e]	+7.69[f]	14	$C_{27}H_{35}N_5O_5$	C, H; N[g]

[a] Yield based on norfentanyl (5) (Scheme I). [b] Silica gel; EtOAc/MeOH/NH_4OH, 5:2:0.15. [c] c 0.91, MeOH. [d] c 1.0, MeOH. [e] Silica gel; EtOAc/MeOH/NH_4OH, 4:1:0.1. [f] c 0.13, MeOH, for the base form. [g] H: calcd, 6.92; found, 7.49. N: calcd, 13.74; found, 12.77.

Table II. 2-(Aminocarbonyl)fentanyl Analogues



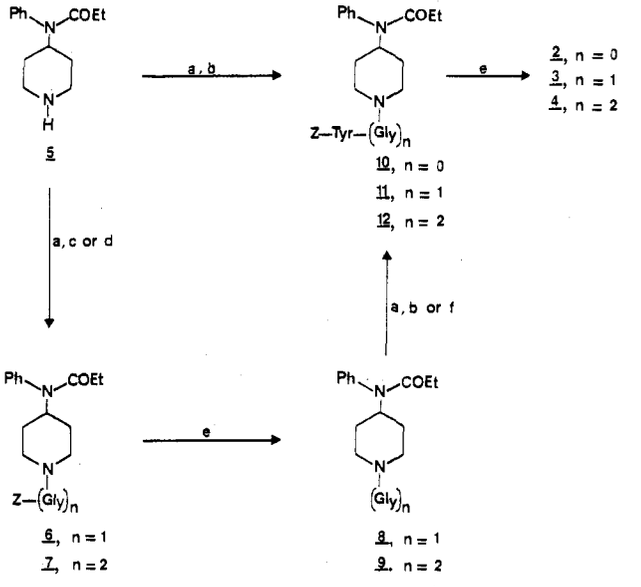
compd	R	R^1	stereochem	mp, °C	crystn solvent	yield,[a] %	formula
13	CH_2CH_2Ph	$CONH_2$	cis	161–162	2-propanol/*n*-hexane	71	$C_{23}H_{29}N_3O_2$
14	CH_3	$CONHCH_2Ph$	cis	188–189	acetone	41	$C_{23}H_{29}N_3O_2$
15·HCl	CH_3	$CONHCH_2Ph$	trans	237–238.5	acetone	42	$C_{23}H_{29}N_3O_2 \cdot HCl$
16	CH_2	$CONHCH_2CH_2Ph$	cis	70	Et_2O	55	$C_{24}H_{31}N_3O_2$
17·HCl	CH_3	$CONH(CH_2)_3Ph$	cis	184–185	EtOH/EtOAc	31	$C_{25}H_{33}N_3O_2 \cdot HCl$
18·HCl[c]	CH_3	$CONH(CH_2)_4Ph$	cis	97–99		30	$C_{26}H_{35}N_3O_2 \cdot HCl$
19	CH_3	CO-Gly-$NHCH_2CH_2Ph$	cis	103.5–105	Et_2O	45	$C_{26}H_{34}N_4O_3$

[a] Yield in the last step of the synthesis. [b] All compounds were within ±0.4% of theory for C, H, N analyses. [c] Purified by chromatography on a silica gel column (EtOAc/MeOH, 5:1); R_f 0.39.

aromatic residues that might conceivably bind with these subsites, we have explored this possibility by synthesizing compounds that possess structural features common to both fentanyl and enkephalin. In this paper we report on the synthesis of such compounds and their biological evaluation on the guinea pig ileum and mouse vas deferens preparations. Also, we report on the use of the irreversible μ opioid receptor antagonist, β-funaltrexamine,[6-8] to investigate the interaction of fentanyl with opioid receptor subtypes in these preparations.

Chemistry and Rationale. Two series of fentanyl–enkephalin hybrid analogues were synthesized. In the first series, the phenethyl group of fentanyl was replaced by Tyr(Gly)$_n$ (n = 0–2) residues of enkephalins (**2–4**, Table I). If the anilino phenyl ring of fentanyl mimics the phenyl ring of the Phe[4] residue, then it is conceivable that these derivatives may exhibit opioid activity. We accomplished the synthesis of **2–4** by coupling norfentanyl (**5**) to the protected amino acid or peptide using dicyclohexylcarbodiimide (DCC) and 1-hydroxybenzotriazole (HOBt) as illustrated in Scheme I. Intermediate **12** could not be separated from the precipitated dicyclohexylurea because of its poor solubility and, therefore, was prepared by coupling intermediate **9** with the *p*-nitrophenyl ester of *N*-Z-Tyr-OH. The benzyloxycarbonyl (Z) group was removed by hydrogenolysis over Pd/C to give the desired target compounds **2–4**.

Scheme I[a]



[a] a, DCC, HOBt; b, *N*-Z-Tyr-OH; c, *N*-Z-Gly-OH; d, *N*-Z-Gly-Gly-OH; e, H_2, Pd/C, HOAc or HCl; f, *N*-Z-Tyr-ONp (for **12**).

In the second series of compounds, the possibility was considered that the anilino phenyl ring of fentanyl may mimic the phenolic ring of the Tyr[1] residue. We therefore explored the effect of introducing an aminocarbonyl group at the 2-position of fentanyl (**13**, Table II), which may mimic the Try[1]–Gly[2] amide bond. Additionally, aminocarbonylfentanyl derivatives **14–19** were synthesized in

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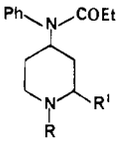
order to explore whether or not the aromatic moiety of the N-substituent would bind to the same receptor subsite that normally interacts with the phenyl ring of the Phe[4] residue of enkephalins. Compounds **13–19** were synthesized from the 2-aminocarbonyl intermediates **20–22**.[9] Thus, N-de-



	R	R'	
20	CH_3	$CONH_2$	cis
21	CH_3	$CONH_2$	trans
22	CH_2Ph	$CONH_2$	cis
23	H	$CONH_2$	cis
24	CH_3	COOH	cis
25	CH_3	COOH	trans

benzylation of **22** by hydrogenolysis over Pd/C afforded **23**, which was reacted with $PhCH_2CHO/NaBH_3CN$ to give target compound **13**. Attempts to hydrolyze the isomeric amides **20** and **21** to the corresponding amino acids **24** and **25** under acidic conditions (2–6 N HCl or 40% H_2SO_4 at 60 °C) resulted in concomitant hydrolysis of the 4-anilido function. Reacylation of the mixtures obtained with EtCOCl was not satisfactory due to the poor solubility of these intermediates in organic solvents and the trans to cis epimerization when the hydrolysis products of **21** were subjected to basic conditions. However, selective hydrolysis of both **20** and **21** to the corresponding amino acids **24** and **25** was accomplished with N_2O_4 in glacial acetic acid.[10] Other nitrosating agents ($NaNO_2$, *n*-BuONO, or $NOPF_6$) failed to afford the desired products under mild conditions. We accomplished the synthesis of target compounds **14–19** by coupling the appropriate amine [$Ph(CH_2)_{1-4}NH_2$] or amide [$PhCH_2CH_2NHCOCH_2NH_2$ (**26**)] with **24** and **25** using isobutyl chloroformate.[11]

Pharmacology. The opioid activity of fentanyl and its derivatives was evaluated in the electrically stimulated myenteric plexus longitudinal muscle of guinea pig ileum[12] (GPI) and mouse vas deferens[13] (MVD) preparations. The agonist activity of the synthesized compounds was tested for reversibility with 3×10^{-6} M naloxone. The compounds also were tested for antagonism to morphine and to [D-Ala[2],Met[5]]enkephalinamide in the GPI and MVD, respectively.

Fentanyl (**1**) was more potent than morphine in the GPI and MVD.[14] In the GPI, **1** had an IC_{50} of $3.45 \pm 0.45 \times 10^{-9}$ M ($n = 9$) compared to $3.31 \pm 0.94 \times 10^{-8}$ M ($n = 8$) for morphine. The IC_{50} of fentanyl in the MVD was $9.45 \pm 4.05 \times 10^{-9}$ M ($n = 11$), while that for morphine was $1.94 \pm 0.34 \times 10^{-7}$ M ($n = 3$). Compound **13**, which differs from fentanyl (**1**) only in having an aminocarbonyl group at the 2-position, was 110 and 450 times less potent than fentanyl in the GPI and MVD, respectively. None of the other fentanyl derivatives at a concentration of 1×10^{-6} M showed any significant opioid agonist or antagonist properties in the GPI or MVD preparations.

We investigated the agonist activity of fentanyl using GPI and MVD preparations that had been pretreated with β-funaltrexamine (β-FNA).[6-8] Since β-FNA is a highly selective, irreversible antagonist for morphine receptors (μ), these treated preparations are essentially devoid of this receptor subtype. We assessed the irreversible antagonism of β-FNA against the agonist response of fentanyl by determining the IC_{50} for fentanyl before (control) and after incubation of the tissue with β-FNA (treated). This is expressed as an IC_{50} ratio [IC_{50} (treated)/IC_{50} (control)]. In five different ileal preparations, the mean IC_{50} ratio for fentanyl was 26.94 ± 3.42. This value was not significantly different from that of morphine (~30) under similar conditions.[7] In the MVD, the IC_{50} ratios for fentanyl in three different preparations were 48.39, 15.86, and 64.77 (mean 43.0 ± 14.37). These values are also similar to the IC_{50} ratios obtained with morphine in the MVD preparation.[8]

Discussion

With the exception of **13**, which behaved as a feeble agonist, the target compounds (**2–4** and **14–19**) exhibited no opioid activity in the GPI or in the MVD preparations. Since none of the compounds displayed narcotic antagonist activity, the inactivity is probably due to loss of receptor affinity.

The fact that the $Tyr(Gly)_n$ derivatives **2–4** were inactive does not support the presence of a structural analogy between the norfentanyl residue and Phe[4] of the opioid peptides. Furthermore, the inactivity of **14–19** suggests that the anilino phenyl ring and the N(1)–C(2)–C(3) moiety of fentanyl are not functionally equivalent to the tyramine moiety of the Tyr[1] residue. This is consistent with the report[15] that phenolic substitution in the anilino phenyl ring or in the phenethyl group of fentanyl diminishes affinity for opioid receptors.

The apparent absence of a functional structural analogy between fentanyl and enkephalin implies that they bind either to different receptors or to different subsites at the same receptors. In their binding studies, Childer et al.[16] have shown that fentanyl has a higher affinity for morphine-selective receptors (μ) than for enkephalin-selective receptors (δ). The question of which opioid receptor subtype interacts with fentanyl was investigated in this study with β-funaltrexamine[6] (β-FNA), which is known to be a selective, irreversible antagonist for morphine receptors (μ) without affecting other receptor subtypes (δ, κ).[7,8] The data showed that β-FNA antagonizes fentanyl irreversibly in the GPI and MVD preparations and that this is comparable to that produced against morphine. This suggests that fentanyl interacts preferentially with the μ receptors that are present in these preparations. The selectivity of fentanyl toward μ receptors has also been supported by tolerance studies in the GPI and MVD preparations.[14]

Although fentanyl and morphine apparently bind to the same receptors, the two compounds show different structure–activity profiles. For example, the *N*-arylethyl group appears to be essential for potent agonist activity in the fentanyl series,[1] while in the morphine series this is not the case.[17] Another difference is the fact that phenolic substitution in fentanyl inhibits binding,[15] in contrast to

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the phenolic OH of morphine, which is known to contribute significantly to activity.[4] These data and the results of the present study suggest the interaction of fentanyl with μ opioid receptor subsites that are not common with those that bind morphine or the opioid peptides. This is consistent with the general principles of the multiple modality concept.[17-19]

Experimental Section

Melting points were determined in open capillary tubes with a Thomas-Hoover melting point apparatus and are uncorrected. Elemental analyses were performed by M-H-W Laboratories, Phoenix, AZ, and, unless otherwise specified, agree with theoretical values within ±0.4%. Optical rotation were obtained with a Perkin-Elmer 114 polarimeter. Infrared (Perkin-Elmer 281), ^{1}H NMR (300 NCI, XL-100, Varian HFT-80 and T-60D), and mass spectra (AEI MS-30, Finnigan) are consistent with the assigned structures. TLC analysis was carried out on precoated silica gel (GF) plates (0.25 mm), Analtech, Inc., Newark, DE. *N*-(Benzyloxycarbonyl)tyrosine *p*-nitrophenyl ester (*N*-Z-Tyr-ONp), *N*-Z-Gly-ONp, and *N*-Z-Gly-Gly-OH were obtained from Sigma Chemical Co., St. Louis, MO.

***N*-Phenyl-*N*-(4-piperidyl)propanamide (5).** This was prepared as reported[20] and was purified by crystallization from acetone/petroleum ether (30–60 °C): mp 89–90 °C (lit.[21] mp 83–85 °C).

General Procedure for Coupling 5 to the *N*-Benzyloxycarbonyl-Protected Amino Acid or Peptide (6, 7, and 10). To a stirred, ice-cold solution of either *N*-Z-Tyr-OH (0.52 g, 1.65 mmol) in MeCN (25 mL), *N*-Z-Gly-OH (1.29 g, 6.17 mmol), or *N*-Z-Gly-Gly-OH (1.99 g, 7.47 mmol) in a mixture of MeCN (25 mL) and DMF (10 mL) were added equivalent amounts of HOBt, DCC, and a MeCN (10 mL) solution of **5** (1 equiv). The mixture was stirred for 48 h at 25 °C in the preparation of **6** and **7** or at 0–5 °C in the case of **10**. After filtration and removal of solvent, the residue was taken in EtOAc and washed successively with 5% $NaHCO_3$, H_2O, 10% citric acid, and H_2O. The organic layer was dried (Na_2SO_4), and EtOAc was evaporated in vacuo. These procedures afforded compounds **6** [yield 0.72 g (83%); mp 73–75 °C (used without further purification)], **7** [yield 2.2 g (85%); mp 113–115 °C (acetone/*n*-hexane). Anal. ($C_{24}H_{29}N_3O_4$) C, H, N], and **10** [yield 2.74 g (76%); mp 124–127 °C (acetone/*n*-hexane). Anal. ($C_{26}H_{32}N_4O_5$) C, H, N].

***N*-(1-Tyrosyl-4-piperidyl)-*N*-phenylpropanamide Acetate (2·HOAc).** A solution of **10** (0.25 g, 0.48 mmol) in MeOH (20 mL) and glacial acetic acid (0.1 mL) was hydrogenated over 10% Pd/C (25 mg, 10% w/w) at 25 °C and atmospheric pressure for 4 h. The catalyst was filtered and the solvent was removed in vacuo. The residue was evaporated with *n*-hexane (3 × 25 mL), then washed with EtOAc (3 × 20 mL), and dried in vacuo to give 0.18 g (84%) of **2**·HOAc (Table I).

***N*-(1-Glycyl-4-piperidyl)-*N*-phenylpropanamide (8).** Compound **8**·HOAc was prepared from **6** as described for **2**·HOAc: yield 88%; mp 83–87 °C. Anal. ($C_{16}H_{23}N_3O_2 \cdot C_2H_4O_2$) C, H, N. The base **8** was prepared by dissolving **8**·HOAc in H_2O, followed by basification (Na_2CO_3) and extraction with EtOAc, mp 115–117 °C.

***N*-[1-(Glycylglycyl)-4-piperidyl]-*N*-phenylpropanamide Hydrochloride (9·HCl).** Compound **9**·HCl was prepared from **7** as described for **2**·HOAc with an equivalent amount of methanolic HCl instead of acetic acid: yield 80%; mp 102 °C dec. Anal. ($C_{18}H_{26}N_4O_3 \cdot HCl$) C, H, N.

***N*-[1-(Tyrosylglycyl)-4-piperidyl]-*N*-phenylpropanamide Acetate (3·HOAc).** To a stirred, ice-cold solution of *N*-Z-Tyr-OH (0.33 g, 1.04 mmol) in MeCN (20 mL) were added HOBt (0.166 g, 1.23 mmol), DCC (0.254 g, 1.23 mmol), and a solution of **8** (0.356 g, 1.23 mmol) in MeCN (10 mL). The mixture was stirred at 0–5 °C for 48 h. After filtration and evaporation of the solvent, the residue was washed with cold EtOAc (3 × 20 mL), dried (Na_2SO_4), and crystallized from a large volume of absolute EtOH to afford 0.42 g (58%) of **11**: mp 180–186 °C; $[\alpha]^{25}_D$ −13.6° (*c* 1.0, DMF); mass spectrum, *m/e* 451 ($M^+ - OCOCH_2Ph$). Anal. ($C_{33}H_{38}N_4O_6$) C, N; H: calcd, 6.52; found, 7.40. Deprotection of this product as described for **2**·HOAc afforded 92% of **3**·HOAc (Table I).

***N*-[1-(Tyrosylglycylglycyl)-4-piperidyl]-*N*-phenylpropanamide Acetate (4·HOAc).** To a mixture of *N*-methylmorpholine (0.28 mL, 2.6 mmol) and **9**·HCl (1 g, 2.6 mmol) in dry MeCN (15 mL) was added with stirring a suspension of *N*-Z-Tyr-ONp (1.05 g, 2.6 mmol) in dry MeCN (10 mL) at 0 °C. The mixture was stirred for 10 h at 0–5 °C and allowed to stand for 24 h at 15 °C. The solvent was removed in vacuo, and the residue was dissolved in EtOAc (75 mL) and washed successively with 5% $NaHCO_3$, H_2O (50 mL), 10% citric acid (2 × 50 mL), and H_2O (50 mL). The organic layer was dried (Na_2SO_4) and evaporated in vacuo, and the solid residue was washed repeatedly with ether to remove the remaining *p*-nitrophenol. Crystallization from MeOH/ether afforded 0.87 g (52%) of **12**: mp 145 °C dec. Anal. ($C_{35}H_{41}N_5O_7$) C, H, N. Deprotection of this product as described for **2**·HOAc afforded crude **4**·HOAc, which was chromatographed on a column (1 × 24 cm) of silica gel (EtOAc/MeOH/NH_4OH; 4:1:0.1) to give 42 mg (44%) of **4**: R_f 0.13; mp 114 °C; mass spectrum, *m/e* 510 ($M^+ + 1$). The acetate salt of **4** (Table I) was prepared by adding an equivalent amount of acetic acid to an ice-cooled solution of the product in MeOH, followed by evaporating MeOH and drying in vacuo.

***cis*-(±)-1-(2-Phenylethyl)-4-(*N*-phenylpropanamido)-piperidine-2-carboxamide (13).** To a solution of **22**[9] (0.59 g, 1.6 mmol) in glacial acetic acid (5 mL) was added 10% Pd/C (92 mg, 15.5%, w/w) and the mixture was hydrogenated at 25 °C and atmospheric pressure for 6 h. The mixture was filtered, and the solvent was removed in vacuo. The residue was treated with *n*-hexane several times, followed by evaporation in vacuo, and the solid residue that was obtained was crystallized from $CHCl_3$ to give 0.49 mg (91%) of **23**·HOAc: mp 138–140 °C; R_f 0.27 (*n*-hexane/EtOAc/MeOH/NH_4OH, 3:10:2:0.1); ^{1}H NMR ($CDCl_3$) δ 4.95–4.5 (m, 1 H, C_4 H); mass spectrum, *m/e* 276 ($M^+ + 1$). Anal. ($C_{15}H_{21}N_3O_2 \cdot C_2H_4O_2$) C, H, N.

A cooled (0 °C) solution of **23**·HOAc (100 mg, 0.298 mmol) and phenacetaldehyde (210 mg, 1.74 mmol) in MeOH (10 mL) was added in portions with stirring to $NaBH_3CN$[21] (18.7 mg, 0.3 mmol). After the addition, the mixture was stirred over molecular sieves (Linde 3Å) at room temperature for 8 h. The MeOH was removed in vacuo, and the residue was mixed with H_2O (10 mL), acidified with 10% HCl, and extracted with ether (2 × 15 mL). The aqueous layer was rendered basic with Na_2CO_3 and extracted with EtOAc (2 × 15 mL). The extract was dried and evaporated in vacuo, and the solid residue was crystallized from 2-propanol/*n*-hexane to give 81.3 mg (71%) of **13** (Table II): HBr salt, mp 149–153 °C.

***cis*-(±)-1-Methyl-4-(*N*-phenylpropanamido)piperidine-2-carboxylic Acid Hydrochloride (24·HCl).** To a solution of **20**[9] (1.94 g, 6.71 mmol) in a mixture of glacial acetic acid (25 mL) and CCl_4 (5 mL) was added anhydrous potassium acetate (1.2 g, 14.63 mmol). To this cooled (−20 °C) mixture was added dropwise, under nitrogen, with stirring and occasional shaking a 1.3% (w/v) solution of dinitrogen tetraoxide[7] (4.23 mmol) in glacial acetic acid over a period of 3 h. The mixture was kept at −20 °C for 12 h, and the solvents were removed in vacuo at room temperature. The residue was ice cooled, mixed with H_2O (25 mL) and then with 5% HCl (10 mL), and the mixture was extracted with EtOAc (2 × 25 mL). The aqueous layer was rendered basic (pH 12) with cold 10% NaOH and extracted with EtOAc (2 × 25 mL). The aqueous layer was acidified (pH 2) with 5% HCl, and H_2O was removed in vacuo at ambient temperature. The residue was extracted with 2-propanol (2 × 20 mL) and filtered, and the filtrate was evaporated. The resulting solid residue was washed with cold EtOAc (3 × 10 mL) and dried in vacuo to give 0.87 g (40%) of **24**·HCl, which was used without further purification: mp 172–180 °C dec; R_f 0.32 (EtOAc/MeOH/NH_4OH, 5:3:0.5); IR (KBr) 3420 (OH), 1735 (C=O, carboxy), 1650–1630 (C=O, anilide) cm^{-1}; ^{1}H NMR (D_2O) δ 3.75 (q, $J_{AX} + J_{BX}$ = 15.85 Hz, C_2 H); mass spectrum, *m/e* 290 (M^+).

***trans*-(±)-1-Methyl-4-(*N*-phenylpropanamido)-piperidine-2-carboxylic Acid Hydrochloride (25·HCl).** The

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title compound was prepared from **21**[9] by a procedure similar to that used for its cis isomer (**24**·HCl), except in this case the N_2O_4/HOAc solution was added to the reaction mixture in small portions over a period of 48 h at ≤−50 °C with occasional mixing. After complete addition, the mixture was kept at −50 to −30 °C for an additional 24 h and then worked up as described for **24**·HCl to afford 34% of crude **25**·HCl, which was used without purification: mp 235–237 °C dec; R_f 0.28 (EtOAc/MeOH/NH_4OH, 5:3:0.5); mass spectrum, m/e 291 ($M^+ + 1$).

α-Amino-*N*-(2-phenylethyl)acetamide (26). To a stirred, ice-cooled solution of *N*-Z-Gly-ONp (0.73 g, 2.2 mmol) in dry MeCN (10 mL) was added a solution of phenethylamine (0.72 g, 2.2 mmol) in MeCN (5 mL) and the mixture was stirred at 25 °C for 2 h. The solvent was removed in vacuo and the solid residue was washed repeatedly with 10% $NaHCO_3$ and then with H_2O. The EtOAc solution was evaporated to give 0.53 g (76%, based on *N*-Z-Gly-ONp) of *N*-Z-Gly-$NHCH_2CH_2Ph$ as a solid, mp 104–107 °C. This product (0.4 g, 1.28 mmol) was dissolved in MeOH (15 mL), 10% ethereal HCl (0.2 mL) was added and the mixture was hydrogenated over 10% Pd/C (40 mg, 10% w/w) at room temperature and atmospheric pressure for 6 h. The mixture was filtered and MeOH was evaporated. The residue was washed with cold ether and dried in vacuo to give 0.24 g (89%) of **26**·HCl as a solid, mp 85–86 °C. This was dissolved in H_2O and the solution was basified with $NaHCO_3$ and extracted with EtOAc. Removal of EtOAc gave **26** as an oil which solidified at −15 °C.

1-Methyl-*N*-substituted-4-(*N*-phenylpropanamido)-piperidine-2-carboxamides (14–19). To a stirred solution of **24**·HCl or **25**·HCl (1 equiv) in dry MeCN at −20 °C was added triethylamine (2 equiv), followed by isobutyl chloroformate[11] (1 equiv). After stirring for 15 min at −20 °C, a solution of the appropriate amine [$(Ph(CH_2)_{1-4}NH_2$] or **26** (1 equiv) in dry MeCN was added, and the mixture was stirred for 15 min at −10 °C and 24 h at 25–27 °C. The solvent was removed in vacuo, and the residue was mixed with H_2O, acidified (pH 3) with 10% HCl, and extracted with EtOAc. In the case of **17** and **18**, the organic layer was dried (Na_2SO_4) and EtOAc was removed to give the crude product, which was purified as its HCl salt as indicated in Table II. In the case of **14–16** and **19**, the aqueous layer was cooled (ice bath), basified (pH 12) with Na_2CO_3, and extracted with EtOAc. Drying and then removing the EtOAc gave the crude product, which was purified by crystallization of the base (**14**, **16**, and **19**) or the HCl salt (**15**) as shown in Table II. The HBr salts of **14** (mp 170–173 °C), **16** (mp 105–108 °C), and **19** (mp 98–101 °C) were prepared with an ethereal solution of HBr.

Guinea Pig Ileum Myenteric Plexus and Mouse Vas Deferens Preparations. This was performed according to modifications[7,8] of the published procedures of Kosterlitz et al.[12,13] The IC_{50} of fentanyl was determined in the GPI or MVD from the log dose–response curves. The preparations were then incubated with 2×10^{-7} β-FNA for 60 min. The agonist effect of β-FNA was washed until the tissue recovered its normal response. The IC_{50} ratio of fentanyl was then evaluated on the β-FNA-treated preparation. The IC_{50} ratio, which represents the IC_{50} of fentanyl after treatment with β-FNA divided by the control IC_{50}, was determined.

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β-Adrenergic Blocking Agents. 23. 1-[(Substituted-amido)phenoxy]-3-[[(substituted-amido)alkyl]amino]propan-2-ols

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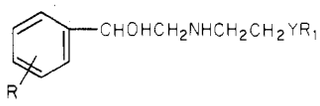
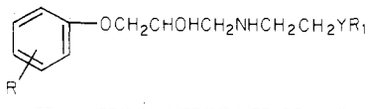
The synthesis of a series of 1-phenoxy-3-[(amidoalkyl)amino]propan-2-ols, in which the phenoxy ring is variously substituted with ortho and para amidic moieties, is described. Several of the compounds have β-blocking potency comparable to that of propranolol and cardioselectivity similar to that of practolol, when given intravenously to anesthetized cats. In contrast to previous findings with cardioselective β blockers, both ortho and para substitution give variable degrees of cardioselectivity. Potency, however, is favored by ortho substitution.

In two previous papers[1,2] we have shown that an amidic moiety in the side chain of an arylethanolamine, **1**, or an



1, Y = NHCO, NHCONH, $NHSO_2$



2, Y = NHCO, NHCONH, $NHSO_2$

(aryloxy)propanolamine, **2**, confers a high degree of cardioselectivity and β-adrenergic blocking potency. Furthermore, in earlier studies on (aryloxy)propanolamines, which were variously substituted in the aryloxy ring, we found that a para amidic substituent gave optimum cardioselectivity.[3] Other workers have reported similar findings[4-7] and cardioselectivity has also been achieved by replacing the isopropyl or *tert*-butyl substituent, conventionally used in β blockers with an (aryloxy)alkyl group in which the aryl ring has a para-amidic substituent.[8] We therefore considered it of interest to combine the above features by synthesizing a series of 1-[(substituted-amido)phenoxy]-3-[[(substituted-amido)alkyl]amino]-propanol-2-ols.

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