that the nature of the substituent in position one of the imidazole-2-thiol moiety plays an important role in determining inhibitory activity. Thus **3a–c** are 10^2–10^3-fold more potent inhibitors of DBH than previously described simple 1-alkylimidazole-2-thiols.[11] Kinetic characterization of the DBH inhibitory activity of **3a** and **3b**, compounds whose extraordinary potency may derive from a "multisubstrate" mode of interaction with DBH, will be described elsewhere.[4]

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Registry No. **1a**, 123-11-5; **1b**, 654-11-5; **1c**, 32085-88-4; **2a**, 54879-77-5; **2b**, 101471-18-5; **2c**, 101471-19-6; **3a**, 95333-64-5; **3b**, 95333-60-1; **3c**, 95333-81-6; **4**·HBr, 101471-20-9; **5**, 64248-63-1; DBH, 9013-38-1; $H_2NCH_2CH(OCH_3)_2$, 22483-09-6; 2,4-difluoroaniline, 367-25-9; 3,5-difluorobromobenzene, 461-96-1.

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Received December 13, 1985

Articles

Synthesis and Opioid Activity of Dermorphin Tetrapeptides Bearing D-Methionine *S*-Oxide at Position 2†

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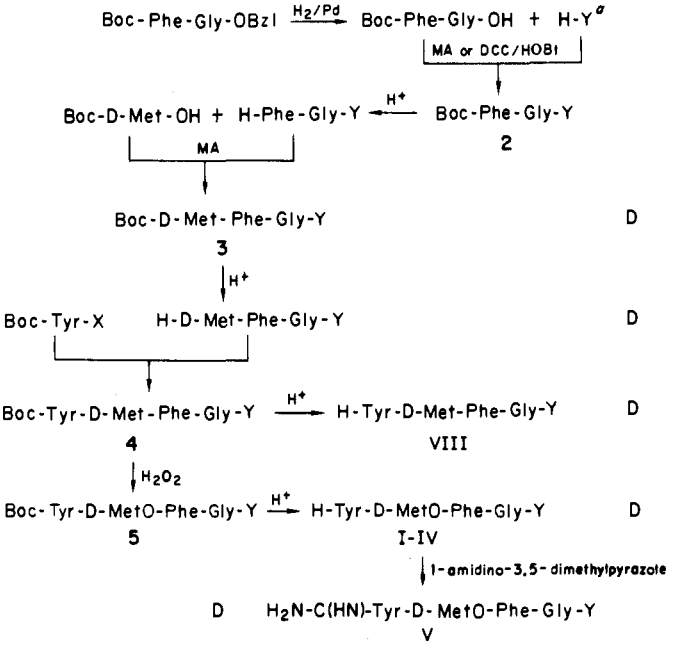
Received March 21, 1985

Eight new dermorphin tetrapeptides, X-Tyr-D-MetO-Phe-aa-Y (X = H, $H_2N{=}C(NH)$; aa = Gly, 2-aminoethanol, sarcosine; Y = NH_2, NH-alkyl), were prepared and tested for opioid activity. They show dose-related naloxone-reversible opioid effects in vitro and in vivo. H-Tyr-D-MetO-Phe-Gly-NH_2 (I) (guinea pig ileum IC_{50} = 13.6 nM; tail-flick ED_{50} = 1.97 pmol/mouse, icv, and 0.65 μmol/kg, sc), though less effective in the pheriphery, has central activities higher than those of dermorphin H-Tyr-D-Ala-Phe-Gly-Tyr-Pro-Ser-NH_2. Following intracerebroventricular or subcutaneous administrations in mice, I is about respectively 1500 and 17 times as potent an analgesic as morphine.

Dermorphin (H-Tyr-D-Ala-Phe-Gly-Tyr-Pro-Ser-NH_2) and its Hyp[6] analogue are opioid heptapeptides, originally isolated from frog skin[1] and characterized by the presence of a D-amino acid residue along their sequence.[2] Synthetic dermorphins have been prepared by different methods.[3] Opioid activities have been reported in the isolated longitudinal muscle strip of guinea pig ileum and mouse vas deferens,[3b,4] in cell-line preparations,[5] and in vivo in rat,[4a] mouse,[3b,4a] and human.[6] Our previous investigation concerned the synthesis and study of structure–activity relationships[4a,7] of 80 "small dermorphins"; we observed that tetrapeptides X-Tyr-D-Ala-Phe-aa-Y are potent analgesics after intracerebroventricular (icv) injection but relatively weak after subcutaneous (sc) administration.[7e,8] It has been reported that [D-Arg^2]- or [D-MetO^2]enkephalin[9,10] and the corresponding dermorphin peptides[11] retain high potency in vitro and/or in sc analgesia, the D-MetO^2 residue playing an important role.[12]

Accordingly, we report the synthesis and opioid activity

Scheme I. Synthesis of [D-Met^2]- and [D-MetO^2]dermorphin Tetrapeptide Analogues



[a] Y: see Table I (compounds I–V and VIII).

† Abbreviations according to IUPAC–IUB Commission on Biochemical Nomenclature, *Eur. J. Biochem.* **1984**, *138*, 9–37, are used throughout. Other abbreviations used are as follows: AcOH, acetic acid; Boc, *tert*-butyloxycarbonyl; DMF, dimethylformammide; Et_2O, diethyl ether; EtOAc, ethyl acetate; EtOH, ethyl alcohol; HPLC, high-performance liquid chromatography; IC, inhibitory concentration; MA, mixed anhydride; MeOH, methyl alcohol; mp, melting point; NMM, *N*-methylmorpholine; OMe, methyl ester; OSu, *N*-hydroxysuccinimidyl ester; PE, petrol ether; TEA, triethylamine; TFA, trifluoroacetic acid; THF, tetrahydrofuran; TLC, thin-layer chromatography.

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0022-2623/86/1829-0889$01.50/0

Table I. Physicochemical Properties of New Dermorphin-(1–4)-tetrapeptide Analogues (X-Tyr-D-MetO-Phe-aa-Y)

no.	X	aa	Y	mp, °C	$[\alpha]^{24}_D$	R_f[a]	formula	anal.
I	H	Gly	NH_2	146–148	+28.6[b]	0.54	$C_{25}H_{33}N_5O_6S\cdot C_2H_4O_2$	C, H, N, S
II	H	Gly	$NH\text{-}CH_2C_6H_5$	131–133	−6.9[c]	0.62	$C_{32}H_{39}N_5O_6S\cdot C_2H_4O_2$	C, H, N
III	H	Gly	D-$NHCH(CH_3)C_6H_5$	141–143	+54.2[b]	0.64	$C_{33}H_{41}N_5O_6S\cdot C_2H_4O_2$	C, H, N
IV	H	Gly	NH-Ad	161–163	+20.7[b]	0.67	$C_{35}H_{47}N_5O_6S\cdot C_2H_4O_2$	C, H, N
V	$H_2N\text{-}C(NH)$	Gly	D-$NHCH(CH_3)C_6H_5$	149–151	+13.3[d]	0.62	$C_{34}H_{43}N_7O_6S\cdot C_2H_4O_2$	C, H, N, S
VI	H	Sar	NH-Ad	159–161	+5.9[e]	0.68	$C_{36}H_{49}N_5O_6S\cdot C_2H_4O_2$	C, H, N
VII	H	$HNCH_2CH_2$	OH	93–95	+12.8[b]	0.57	$C_{25}H_{34}N_4O_6S$	C, H, N
VIII[f]	H	Gly	NH_2	175–177	+14.5[e]	0.56	$C_{25}H_{33}N_5O_5S$	C, H, N

[a] Thin-layer chromatography on silica gel: solvent system (A). [b] c 1.0 (methanol). [c] c 1.0 (dimethylformamide). [d] c 0.5 (methanol). [e] c 0.5 (dimethylformamide). [f] This compound contains D-Met[2].

Table II. Opioid Activity of Dermorphin Tetrapeptides in Vitro (Guinea Pig Ileum)

no.	compd	GPI:[a] IC_{50}, nM	rel potencies[b] c	d	e
I	H-Tyr-D-MetO-Phe-Gly-NH_2	13.6 ± 2.1	4.8	1	
II	H—NH-$CH_2C_6H_5$	21.0 ± 3.6	3	0.6	
III	H—D-NH-$CH(CH_3)C_6H_5$	72.1 ± 8.1	0.9	0.2	
IV	H—NH-Ad	22.0 ± 1.2	2.9	0.6	
V	$H_2N\text{-}C(NH)$—D-$NHCH(CH_3)C_6H_5$	35.6 ± 4.7	1.8	0.4	
VI	H—Sar-NH-Ad	25.5 ± 0.58	2.3	0.5	
VII	H—Gly-ol	16.7 ± 2.8	3.9	0.8	
VIII	H—D-Met—NH_2	97.6 ± 7.1	0.6	0.1	
I′	H-Tyr-D—Ala-Phe-Gly-NH_2	45.2 ± 3.2	1.4		1
II′	H—NH-$CH_2C_6H_5$	2.97 ± 0.5	22		15.2
III′	H—D-$NHCH(CH_3)C_6H_5$	0.59 ± 0.1	110		76.2
IV′	H—NH-Ad	2.05 ± 0.2	32		22
V′	$H_2N\text{-}C(NH)$—D-$NHCH(CH_3)C_6H_5$	0.28 ± 0.05	232		161
VI′	H—Sar-NH-Ad	2.68 ± 0.07	24		17
	dermorphin	1.41 ± 0.14	46		
	morphine	65.0 ± 4.6	1		

[a] The values are the means of five experiments ± SEM. [b] Relative potencies are on a molar basis: (c) morphine = 1, (d) I = 1, (e) I′ = 1.

of dermorphin tetrapeptides H-Tyr-D-MetO-Phe-aa, in which D-Ala[2] is substituted by D-methionine *S*-oxide, aa is Gly, Gly-ol, or Sar, and the C-terminus may bear different amide moieties. The analogue $H_2N{=}C(NH)$-Tyr-

Scheme II. Synthesis of [D-MetO[2],Gly-ol[4]]dermorphin Tetrapeptide Analogue

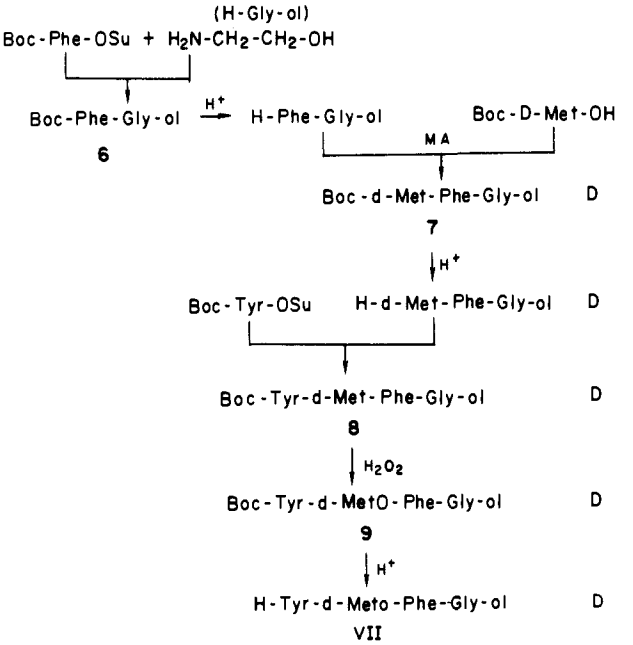

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Table III. Opioid Activity of Dermorphin Tetrapeptides in Vivo (Tail-Flick Test)

no.	compd	analgesia[a] icv: ED_{50}, pmol/mouse	analgesia[a] sc: ED_{50}, μmol/kg	rel potencies[b] icv	rel potencies[b] sc
I	H-Tyr-D-MetO-Phe-Gly-NH_2	1.97 (1.34-3.36)	0.65 (0.54-0.80)	1538	17
II	H—NH-$CH_2C_6H_5$	18.1 (9.43-35.1)	3.51 (2.29-4.48)	167	3.1
III	H—D-NHCH(CH_3)C_6H_5	83.6 (44.3-116)	5.81 (3.34-14.1)	36	1.8
IV	H—NH-Ad	30.4 (23.8-66.1)	3.93 (2.03-6.92)	100	2.8
V	H_2N-C(NH)—D-NHCH(CH_3)C_6H_5	32.0 (21.7-55.3)	4.98 (2.10-9.12)	95	2.1
VI	H—Sar-NH-Ad	60.6 (38.2-122)	4.82 (2.68-12.4)	50	2.3
VII	H—Gly-ol	8.80 (6.34-15.3)	2.79 (1.54-4.26)	344	3.9
VIII	H—D-Met—NH_2	87.5 (61.9-184)	12.1 (5.37-22.8)	35	0.9
I′	H-Tyr-D—Ala-Phe-Gly-NH_2	68.1 (46.1-120)	68.2 (28.1-99.7)	45	0.2
II′	H—NH-$CH_2C_6H_5$	33.1 (22.4-71.0)		92	
III′	H—D-NHCH(CH_3)C_6H_5	7.61 (5.46-10.6)	13.6 (8.9-20.1)	398	0.8
IV′	H—NH-Ad	5.12 (3.40-12.6)	18.8 (13.4-25.6)	591	0.6
V′	H_2N-C(NH)—D-NHCH(CH_3)C_6H_5	2.97 (2.22-4.36)	11.5 (7.66-17.3)	1020	0.9
VI′	H—Sar-NH-Ad	25.8 (17.6-44.2)	28.2 (18.4-43.2)	117	0.4
	dermorphin	11.4 (6.60-27.1)	2.75 (1.73-6.42)	266	4
	morphine	3030 (2207-4162)	10.9 (7.02-18.8)	1	1

[a] Analgesia of at least four doses of each compound was investigated. Each dose was tested for at least 10 animals. The ED_{50} was estimated at the time of peak activity. [b] Relative potencies are on molar basis (morphine = 1).

D-MetO-Phe-Gly-NH-Ad, whose terminal amino group is replaced by the guanidino function and whose C-terminus is amidated by 1-adamantanamine, was also obtained.

Results

Chemistry. The new tetrapeptides were obtained by conventional methods in solution, using active esters or mixed anhydrides, through stepwise addition of each amino acid starting from C-terminal glycine derivatives (Schemes I–II).

The [Sar^4] analogue VI was obtained, in turn, via 3 + 1 coupling (Scheme III), as described for related [Sar^4]-dermorphin peptides.[7c,13] Each Boc-peptide-sulfide (**4**, **8**, **12**) was oxidized with hydrogen peroxide in acetic acid to yield the (*R,S*)-sulfoxides (*5*, *9*, *13*), with no apparent sulfone contamination. Oxidation of methionine peptides by sodium metaperiodate[14] led to the same diastereoisomeric mixture of sulfoxides. The guanidino derivative V (Scheme I) was obtained by treating the pertinent peptide amide acetate IV with 1-amidino-3,5-dimethylpyrazole acetate.[7a]

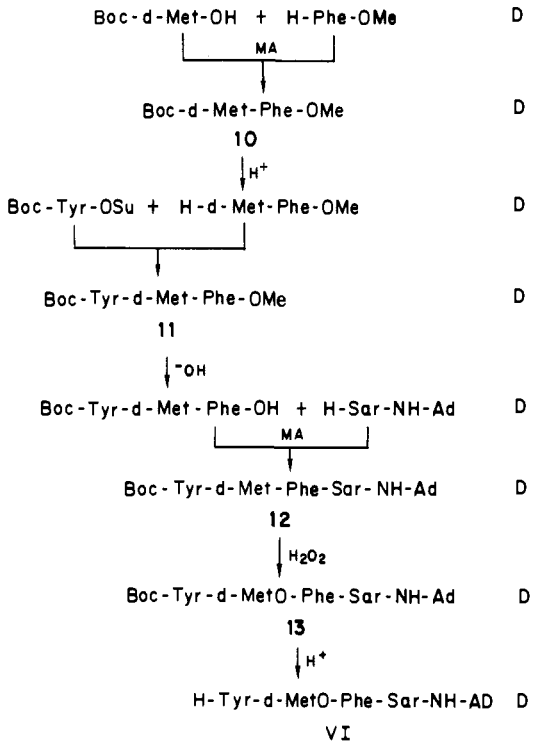
Treatment of compounds **4**, **5**, **9**, and **13** with trifluoroacetic acid in methylene chloride containing anisole (0.1%) gave the desired analogues (Schemes I–III).

Final purification was accomplished by partition chromatography on Sephadex G-25 or when necessary by HPLC. The homogeneity of I–VIII was checked by TLC, HPLC, and amino acid and elementary analyses (see Table I and Experimental Section).

Biological Activity. The new compounds were tested in vitro on electrically stimulated guinea pig ileum longitudinal muscle myenteric plexus preparation[15] (GPI) and in vivo by mouse tail-flick[16] assay after icv and sc administrations: the results are given in Tables II and III and compared with dermorphin and morphine. In order to permit easy comparison between the activity of [D-MetO^2] analogues and that of the corresponding D-Ala^2 tetrapeptides,[7a,c] peripheral and central activities of these parent peptides (compounds I′–VI′) are also shown in Tables II and III.

The in vitro tests indicated that substitution of D-MetO for D-Ala in the original dermorphin tetrapeptides I′–VI′ causes an increase of activity only in one case (compound I); a reduction of potency is evident in analogues II–VI. Replacement of the D-Ala^2 residue by D-Met is also de-

Scheme III. Synthesis of [D-MetO^2,Sar^4]dermorphin Tetrapeptide Analogue



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trimental: compound VIII is 7 and 2 times less potent than sulfoxide I or the reference D-Ala^2 tetrapeptide I′, respectively.

Within [D-MetO^2] analogues (I–VII), tetrapeptide VII, bearing Gly^4-ol as terminus, displays comparable activity to I. The peptide secondary amides (II–IV and VI) have reduced potency with respect to the primary amide I, showing a reversed trend in comparison with the corresponding [D-Ala^2] analogues (II′–IV′ and VI′). Finally, the guanidino amide V is more effective than III, but it is 130 times less potent than the D-Ala^2 derivative V′.

In the tail-flick test (Table III), icv administration of tetrapeptides I–VIII showed an antinociceptive action greater than morphine, confirming our previous data concerning compounds I′–VI′ and other dermorphin peptides.[7a-e] However, the hydrophobic compounds II–VI displayed an analgesic activity lower than both amide I and the glycinol derivative VII. Compound I is not only 35 times more active than the dermorphin-(1–4)-tetrapeptide I′ but it is the most potent dermorphin analogue so far synthesized by us, being 6 and 1500 times more potent than dermorphin and morphine, respectively.

After sc application, the antinociceptive action of compounds I–VIII proved again to parallel GPI and icv data. [D-MetO^2]dermorphin tetrapeptide I is 17 times as potent as morphine and 100 times more potent than the parent tetrapeptide I′. The tetrapeptide alcohol VII can also elicite an analgesia comparable to that of dermorphin; analogues II–VI containing bulky lipophilic amide moieties have lower activities than amide I, but they are still 2–3 times more potent than morphine or the [D-Ala^2] analogues.

Discussion

In comparison to the results obtained with compounds I′–V′ and other [D-Ala^2] dermorphin tetrapeptides,[7d] the dependence of activity on the stereochemical and lipophilic properties of the C-terminal substituents is reversed in the [D-MetO^2] analogues II–VI; the present trend agrees with that found by other investigators on [D-Arg^2] dermorphin tetrapeptides.[12b] Whereas the [D-Ala^2] analogues II′–VI′ may allow molecular flexibility and favor peptide–receptor interaction, the larger size of the D-MetO side chain in II–VI, when matched with bulky substituents at the C-terminus, may lead to structures less suitable for receptor binding. The reason for the remarkably enhanced activity of sulfoxide I with respect to D-Met^2 (VII) and [D-Ala^2] (I′) derivatives is not known at the present time. It may, however, be reasonable to consider that MetO^2 in the D configuration not only is critical for the existence of "active" conformers[17] but also provides an extra binding site for the receptors:[10b] this hypothesis is supported by the observed inactivity of [L-Met^2]- and [L-MetO^2]-dermorphin tetrapeptides.[4b,11] The data reported in Tables II and III indicate that, in comparison to morphine, the enhancement of antinociceptive action of all dermorphin-related peptides was not comparable to the potency on GPI preparation. Still, as the effect on mouse vas deferens of [D-Ala^2] analogoues[4b,7a] and I–VIII remains in the range of morphine (data not shown), it may be concluded that the potencies of dermorphin peptides on isolated peripheral muscle preparations do not completely reflect the interaction of these peptides with central receptors. Further, since K receptors do not seem to be particularly involved in the action of peptides similar to I and I′,[18] dermorphin peptides may interact, unlike morphine, with central μ_1 receptors more than with μ_2 receptors occurring in GPI and the brain.[19]

Finally, substitution of the more hydrophilic D-MetO^2 for D-Ala^2 residue may influence the pharmacokinetic properties, particularly the distribution and ability to permeate into the central nervous system. In fact, compounds I–VI showed considerable analgesic effects following sc injection, whereas the corresponding [D-Ala^2] analogues I′–VI′ displayed comparatively low activity after systemic administration, in agreement with the postulated role of D-MetO^2 in opioid peptide analgesia.[10,12a]

Experimental Section

Melting points were determined on a Tottoli apparatus in open capillaries and are uncorrected. Optical rotations were determined with a Perkin-Elmer 141 polarimeter with a 10-cm water-jacketed cell. HPLC analysis was performed on a JASCO liquid chromatograph equipped with a Varichrom UV variable-wavelength detector and an M660 solvent programmer. A Waters C-18 μ-Bondapak column (30 cm × 4 mm) was used in the HPLC system. All organic solvents were UV spectroscopic grade and were filtered and degassed prior to use. Analytical determination for each deprotected peptide was obtained by running a linear, 30-min gradient from 10% acetonitrile–90% 0.01 M ammonium formate, pH 4.0, to 60% acetonitrile–30% 0.01 M ammonium formate at a flow rate of 2 mL/min. The desired tetrapeptides eluted in the range of 20–30% acetonitrile. All analogues showed less than 1% impurities by analytical HPLC with monitoring at 210 and 280 nm. The amino acid composition was determined with a Carlo Erba 3A29 amino acid analyzer, after acid hydrolysis in constant-boiling HCl containing phenol (1%). In the amino acid analysis of Sar-peptides, sarcosine gave a single peak with a retention time between aspartic acid and serine. TLC was performed on precoated plates of silica gel F254 (from E. Merck) with use of the following solvent systems: (A) 1-butanol/AcOH/H_2O (6:1:5), (B) EtOAc/pyridine/AcOH/H_2O (60:20:6:11), (C) $CHCl_3$/MeOH/benzene (85:10:5), (D) $CHCl_3$/MeOH (1:1), (E) $CHCl_3$/AcOH/benzene (85:10:5), (F) EtOAc/MeOH (1:1), (G) $CHCl_3$/MeOH/30% ammonia (12:8:3). Ninhydrin 1% (Merck), fluorescamine (Hoffman-La Roche), and/or chlorine reagent were used as spray reagents. Samples were considered pure when they showed single spots with more than one solvent systems. Elemental analyses indicated by the symbols of the elements refer to data within ±0.4% of the theoretical values. Analyses were carried out after the products were dried for 12 h at 50 °C (0.2 torr). Open column chromatography was run on silica gel 60 (70–230 mesh, Merck), unless stated otherwise.

Coupling Procedures. Method A. To a stirred solution (0.5–0.8 M) of Boc-protected amino acid or Boc-protected peptide (1 mmol) in DMF, 1 equiv of *N*-methylmorpholine (NMM) was added; the mixture was cooled to −10 °C, treated with isobutyl chloroformate (IBCF) (1 equiv), and allowed to react for 2–3 min. A precooled solution of amino component hydrochloride or trifluoroacetate (1.1 mmol) in DMF (0.4–0.6 M) was added to the mixture, followed by NMM (1.1 equiv). The reaction mixture was stirred for 1 h at −10 °C and 2–3 h at 0–10 °C and then diluted with EtOAc (100 mL). The solution or suspension was washed consecutively with brine, 0.5 N $KHSO_4$, brine, 5% $NaHCO_3$, and brine. The organic phase was dried ($MgSO_4$), filtered, and evaporated to dryness. The residue was crystallized from appropriate solvents or purified by column chromatography.

Method B. To a solution of the carboxy component (2 mmol) in DMF (10 mL) were added the amino acid component (2 mmol),

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NMM (2 mmol if the amino component was in the protonated form), 1-hydroxybenzotriazole (HOBt) (2 equiv), and DCC (2.1 mmol) in the above order at 0 °C. The reaction mixture was stirred for 2 h at 0 °C and 24 h at room temperature; *N,N'*-dicyclohexylurea (DCU) was filtered off, and the solution was diluted with EtOAc (100 mL) and worked up as described in method A.

Deprotection. Method C. Boc protecting groups were removed by treating the peptide with aqueous 90% TFA (1:10 w/v) containing anisole (1 mL) for 30–40 min. The solvent was evaporated in vacuo at 0 °C, and the residue was triturated with Et_2O or petrol ether (PE); the resulting solid peptide was collected and dried.

Method D. Hydrogenations were carried out in the indicated solvent at atmospheric pressure and room temperature in the presence of 10% palladized charcoal (catalyst to peptide ratio, 1:9, w/w). The reaction mixture was filtered through a Celite bed and evaporated to dryness. The residue was treated as described above in method C.

Boc-Phe-Gly-OH (1). According to general deprotection procedure D, Boc-Phe-Gly-OBzl[3b] (8.24 g, 20 mmol) in MeOH (200 mL) was hydrogenated for 4 h. The resulting crude **1** was recrystallized from MeOH–Et_2O (5.81 g, 90%): mp 167–169 °C; $[\alpha]^{22}_D$ −4.9° (*c* 1.0, MeOH). Anal. ($C_{16}H_{22}N_2O_5$) C, H, N.

Preparation of Boc-Phe-Gly-amides (2). Boc-Phe-Gly-NH_2 (2a). According to general coupling procedure B, **1** was treated with the ammonium salt of HOBt.[20] Crude **2a** was recrystallized from EtOAc (85%): mp 68–70 °C; $[\alpha]^{22}_D$ +9.3° (*c* 1.0, MeOH). Anal. ($C_{16}H_{23}N_3O_4$) C, H, N.

Boc-Phe-Gly-NH-$CH_2C_6H_5$ (2b), from **1** and benzylamine (procedure B). Crude **2b** was recrystallized from EtOH–Et_2O (81%): mp 155–157 °C; $[\alpha]^{22}_D$ +16.4 (*c* 1.0, MeOH). Anal. ($C_{23}H_{29}N_3O_4$) C, H, N.

Boc-Phe-Gly-D-NH-$CH(CH_3)C_6H_5$ (2c), from **1** and (*R*)-(+)-α-methylbenzylamine (procedure B). Crude **2c** was purified by flash chromatography[21] on silica gel with use of the solvent system D. It was recrystallized from EtOAc–Et_2O (68%): mp 143–145 °C; $[\alpha]^{22}_D$ +45.5° (*c* 1.0, MeOH). Anal. ($C_{24}H_{31}N_3O_4$) C, H, N.

Boc-Phe-Gly-NH-Ad (2d), from **1** and 1-adamantanamine (procedure A). It was reprecipitated from EtOAc–PE (74%): mp 170–172 °C; $[\alpha]^{22}_D$ +7.7 (*c* 1.0, MeOH). Anal. ($C_{26}H_{37}N_3O_4$) C, H, N.

Preparation of Boc-D-Met-Phe-Gly-amides (3). Boc-D-Met-Phe-Gly-NH_2 (3a). Compound **2a** (3.2 g, 10 mmol) was converted to its trifluoroacetate salt (deprotection procedure C) and reacted with the mixed anhydride prepared from Boc-D-Met-OH (2.49 g, 10 mmol) according to coupling method A. Crude **3a** was recrystallized from AcOEt–Et_2O (3.8 g, 84%): mp 91–93 °C; $[\alpha]^{22}_D$ +18.7 (*c* 1.0, MeOH). Anal. ($C_{21}H_{32}N_4O_5S$) C, H, N, S. The following compounds were obtained by the same procedure.

Boc-D-Met-Phe-Gly-NH-$CH_2C_6H_5$ (3b) (82%): mp 123–125 °C; $[\alpha]^{22}_D$ −8.0° (*c* 1.0, DMF). Anal. ($C_{28}H_{38}N_4O_5S$) C, H, N.

Boc-D-Met-Phe-Gly-D-NH-CH (CH_3)C_6H_5 (3c) (79%): mp 178–180 °C; $[\alpha]^{22}_D$ +14.1 (*c* 1.0, DMF). Anal. ($C_{29}H_{40}N_4O_5S$) C, H, N.

Boc-D-Met-Phe-Gly-NH-Ad (3d) (81%): mp 175–177 °C; $[\alpha]^{22}_D$ −7.6° (*c* 1.0, DMF). Anal. ($C_{31}H_{46}N_4O_5S$) C, H, N.

Preparation of Boc-Tyr-D-Met-Phe-Gly-amides (4). Boc-Tyr-D-Met-Phe-Gly-NH_2 (4a). According to the deprotection procedure C, **3a** (4.5 g, 10 mmol) was treated with TFA. The resulting H-D-Met-Phe-Gly-NH_2 trifluoroacetate in DMF (30 mL) containing TEA (1.54 mL, 11 mmol) was reacted with Boc-Tyr-OSu (3.7 g, 10 mmol). The reaction mixture was stirred for 8 h at room temperature, diluted with EtOAc (200 mL), and worked up as in method A. The crude product was reprecipitated from EtOH–Et_2O (5 g, 81%): mp 125–127 °C; $[\alpha]^{22}_D$ +21.7° (*c* 1.0, MeOH). Amino acid analysis: Tyr 0.98; Met 0.97; Phe 1.02; Gly 1.00. Anal. ($C_{30}H_{41}N_5O_7S$) C, H, N.

Boc-Tyr-D-Met-Phe-Gly-NH-$CH_2C_6H_5$ (4b), from H-D-Met-Phe-Gly-NH-CH_2-C_6H_5 and Boc-Tyr-OSu. Compound **4b** was recrystallized from EtOAc (79%): mp 157–159 °C; $[\alpha]^{22}_D$ −11.2° (*c* 1.0, DMF). Anal. ($C_{37}H_{47}N_5O_7S$) C, H, N.

Boc-Tyr-D-Met-Phe-Gly-D-NH-$CH(CH_3)C_6H_5$ (4c), from H-D-Met-Phe-Gly-D-NH-$CH(CH_3)C_6H_5$ trifluoroacetate and Boc-Tyr-OSu. The title compound was reprecipitated from EtOH–Et_2O (76%): mp 113–115 °C; $[\alpha]^{22}_D$ +45.1° (*c* 1.0, MeOH). Anal. ($C_{38}H_{49}N_5O_7S$) C, H, N, S.

Boc-Tyr-D-Met-Phe-Gly-NH-Ad (4d), from H-D-Met-Phe-Gly-NH-Ad trifluoroacetate and Boc-Tyr-OSu. The crude **4d** was purified by flash chromatography[21] on silica gel with use of the solvent system F. Compound **4d** was recrystallized from EtOH–H_2O (72%): mp 159–161 °C; $[\alpha]^{24}_D$ −13.4° (*c* 1.0, DMF). Amino acid analysis: Tyr 0.99; Met 0.96; Phe 1.03; Gly 1.00.

Preparation of Boc-Tyr-D-MetO-Phe-Gly-amides (5). Each Boc-peptide-sulfide (**4a–d**) (1 mmol) was dissolved in acetic acid (15 mL) and cooled to 0 °C, and 11.2 M H_2O_2 (0.1 mL, 1.1 mmol) was added. After 1 h at 20 °C, the mixture was slowly poured into vigorously stirred Et_2O (150 mL) and the product isolated by filtration. The resulting (*R,S*)-sulfoxide was reprecipitated from MeOH–Et_2O (85–91%). The following compounds were obtained.

Boc-Tyr-D-MetO-Phe-Gly-NH_2 (5a): mp 131–133 °C; $[\alpha]^{22}_D$ +21.8° (*c* 1.0, MeOH); R_f 0.51 (D), 0.33 (C). Anal. ($C_{30}H_{41}N_5O_8S$) C, H, N, S.

Boc-Tyr-D-MetO-Phe-Gly-NH-$CH_2C_6H_5$ (5b): mp 154–156 °C; $[\alpha]^{22}_D$ −11.3° (*c* 0.99, DMF); R_f 0.52 (D), 0.35 (C). Anal. ($C_{37}H_{47}N_5O_8S$) C, H, N, S.

Boc-Tyr-D-MetO-Phe-Gly-D-NH-$CH(CH_3)C_6H_5$ (5c): mp 127–129 °C; $[\alpha]^{22}_D$ +45.3 (*c* 1.0, MeOH); R_f 0.53 (D), 0.35 (C). Anal. ($C_{38}H_{49}N_5O_8S$) C, H, N.

Boc-Tyr-D-MetO-Phe-Gly-NH-Ad (5d): mp 145–147 °C; $[\alpha]^{22}_D$ −13.4° (*c* 1.0, DMF); R_f 0.57 (D), 0.40 (C). Anal. ($C_{40}H_{55}N_5O_8S$) C, H, N, S.

Boc-Phe-Gly-ol (6). To an ice-cold solution of ethanolamine (0.66 mL, 11 mmol) in DMF (20 mL), Boc-Phe-OSu (3.62 g, 10 mmol) was added. The reaction mixture was stirred at 0–5 °C for 10 h, diluted with EtOAc (150 mL), and worked up as coupling procedure A. The resulting **6** was reprecipitated from EtOAc–Et_2O (2.6 g, 84%): mp 93–95 °C; $[\alpha]^{22}_D$ +4.4 (*c* 1.0, MeOH). Anal. ($C_{16}H_{24}N_2O_4$) C, H, N.

Boc-D-Met-Phe-Gly-ol (7). Compound **6** (2.46 g, 8 mmol) was converted to its trifluoroacetate salt (deprotection procedure C) and reacted with the mixed anhydride prepared from Boc-D-Met-OH (1.76 g, 7 mmol) according to the coupling method A. Crude **7** was recrystallized from EtOAc–Et_2O (2.6 g, 83%): mp 84–86 °C; $[\alpha]^{22}_D$ +8.2 (*c* 1.0, MeOH). Anal. ($C_{21}H_{33}N_3O_5S$) C, H, N.

Boc-Tyr-D-Met-Phe-Gly-ol (8) was prepared as **4a** from Boc-Tyr-OSu and H-D-Met-Phe-Gly-ol trifluoroacetate (obtained from **7** following procedure C). Compound **8** was recrystallized from AcOEt–Et_2O (79%): mp 132–134 °C; $[\alpha]^{22}_D$ +9.1 (*c* 1.0, MeOH). Anal. ($C_{30}H_{42}N_4O_7S$) C, H, N.

Boc-Tyr-D-MetO-Phe-Gly-ol (9) was prepared as **5** from **8**; it was reprecipitated from $CHCl_3$–Et_2O (89%): mp 121–123 °C; $[\alpha]^{24}_D$ +8.6 (*c* 1.0, MeOH). Anal. ($C_{30}H_{42}N_4O_8S$) C, H, N, S.

Boc-D-Met-Phe-OMe (10) was obtained as oil from Boc-D-Met-OH and H-Phe-OMe according to the coupling procedure A (88%): $[\alpha]^{24}_D$ +16.3 (*c* 0.98, MeOH). Anal. ($C_{20}H_{30}N_2O_5S$) C, H, N.

Boc-Tyr-D-Met-Phe-OMe (11) was prepared as **4a** from Boc-Tyr-OSu and H-D-Met-Phe-OMe trifluoroacetate (obtained from **10** following procedure C). It was recrystallized from AcOEt–Et_2O (81%): mp 112–114 °C; $[\alpha]^{22}_D$ +18.9° (*c* 1.0, MeOH). Amino acid analysis: Tyr 0.99, Met 1.01, Phe 1.00. Anal. ($C_{29}H_{39}N_3O_7S$) C, H, N.

Boc-Tyr-D-Met-Phe-Sar-NH-Ad (12). To a solution of **11** (5.73 g, 10 mmol) in MeOH (50 mL) was added 1 N NaOH (11 mL), and the mixture was stirred for 2 h while the progress of the hydrolysis was checked by TLC, with solvent system B. After the evaporation of the methanol, the mixture was diluted with water (50 mL) and washed with EtOAc (2 × 30 mL). The precooled aqueous phase was then acidified with solid citric acid and extracted with EtOAc (3 × 50 mL). The organic extract was washed with brine, dried, and evaporated to dryness. The resulting Boc-Tyr-D-Met-Phe-OH (3.8 g, 7 mmol) was condensed (procedure A) with H-Sar-NH-Ad[7c] (2.06 g, 8 mmol). Crude **12**

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was purified with use of a column (2 × 45 cm) and solvent system C. The resulting solid was recrystallized from EtOH–Et_2O (4.1 g, 77%): mp 123–125 °C; $[\alpha]^{22}_D$ +9.9 (c 1.0, DMF). Amino acid analysis: Tyr 0.99; Met 0.97; Phe 1.00; Sar 1.01. Anal. ($C_{41}H_{57}N_5O_7S$) C, H, N.

Boc-Tyr-D-MetO-Phe-Sar-NH-Ad (13) was prepared from 12 as described for 5: mp 135–137 °C; $[\alpha]^{24}_D$ +10.1 (c 1.0, DMF). Anal. ($C_{41}H_{57}N_5O_8S$) C, H, N, S.

Preparations of Free Tetrapeptides I–IV and VI–VIII. Each Boc-protected tetrapeptide was deprotected according to procedure C. The resulting free compound (1 mmol) was dissolved in 0.5 N acetic acid (3 mL) and passed through a 2 × 50 cm Sephadex G-25 column, with solvent system A. The tetrapeptide trifluoroacetates were converted into the corresponding acetates through anion-exchange resin DE52 Whatman (acetate form) with use of 0.2 N acetic acid as eluting solvent. The fractions containing the peptide were collected and lysophilized to constant weight (85–90%). The analogues VII and VIII were obtained as free bases. Characterization of the final products are summarized in Table I.

Synthesis of Guanidino-tetrapeptide Acetate·H_2N-C-(NH)-Tyr-D-MetO-Phe-Gly-D-NH-CH(CH_3)C_6H_5 (V). The title compound was prepared by amidination of IV (1 mmol) with 1-amidino-3,5-dimethylpyrazole acetate (1.2 mmol) as in ref 7a. The crude V was reprecipitated from EtOH–AcOEt and purified by column chromatography on silica gel (2 × 60 cm) in the solvent system G. The fractions containing the pure compound were evaporated to dryness, and the residue was crystallized from AcOH–Et_2O (51%). Chracterization of V is summarized in Table I.

Pharmacological Assays. All tetrapeptides and reference compounds were assayed on electrically stimulated guinea pig ileum (GPI) with use of the conditions of Kosterlitz and Watt.[15] Dose–response curves were drawn on at least three ponts and the IC_{50}, i.e., the concentration of compound necessary to inhibit the amplitude of electrically induced twitch by 50%, was determined. Naloxone (1.4 nmol/L, i.e., the pA_2 value against dermorphin) was a potent antagonist of peptides tested at IC_{50} concentration. The analgesic potency of tetrapeptides was estimated in Swiss–Webster mice weighing 23–25 g. The tail-flick test was essentially that described by Janssen,[16] using water at 55 °C as nociceptive stimulus. Tests were made prior to and at various times after icv and sc administration of each compound in saline (4 μL). The average reaction time in control animals was 1 s. Complete analgesia was assumed to be present when no reaction appeared 10 s after application of noxious stimulus. Percent analgesia was calculated according to the formula $(T - T_0/10 - T_0) \times 100$ (T = reaction time (seconds) after administration of compound; T_0 = "normal" reaction time before injection of compound; 10 = cut off time). The specificity of the effects was tested by pretreating the animals with naloxone hydrochloride (0.5–1 mg/kg sc). In all cases, the antagonist prevented any analgesic effect.

Acknowledgment. We express our appreciation to L. Minozzi for skillful technical assistance and to Prof. F. D'Angeli and Prof. C. Bianchi for their comments and suggestions and for reading the manuscript. This research was supported by the Italian C.N.R. grant and in part by the Ministry of Public Education: Research project "Chimica, Struttura e Funzione di Peptidi".

Registry No. 1, 25616-33-5; **2a**, 42726-70-5; **2b**, 100571-96-8; **2c**, 100571-97-9; **2d**, 89661-89-2; **3a**, 100571-98-0; **3b**, 100571-99-1; **3c**, 100572-00-7; **3d**, 100572-01-8; **4a**, 100572-02-9; **4b**, 100572-03-0; **4c**, 100572-04-1; **4d**, 100572-05-2; **5a** (*R*-sulfoxide), 100572-06-3; **5a** (*S*-sulfoxide), 100679-98-9; **5b** (*R*-sulfoxide), 100572-07-4; **5b** (*S*-sulfoxide), 100679-99-0; **5c** (*R*-sulfoxide), 100572-08-5; **5c** (*S*-sulfoxide), 100680-00-0; **5d** (*R*-sulfoxide), 100572-09-6; **5d** (*S*-sulfoxide), 100680-01-1; **6**, 87976-65-6; **7**, 100572-10-9; **8**, 100572-11-0; **9** (*R*-sulfoxide), 100572-12-1; **9** (*S*-sulfoxide), 100680-02-2; **10**, 99909-57-6; **11**, 100572-13-2; **12**, 100572-14-3; **13** (*R*-sulfoxide), 100572-15-4; **13** (*S*-sulfoxide), 100680-03-3; I, 100572-16-5; I·$C_2H_4O_2$, 100572-17-6; II, 100572-18-7; II·$C_2H_4O_2$, 100572-19-8; III, 100572-20-1; III·$C_2H_4O_2$, 100680-04-4; IV, 100572-21-2; IV·$C_2H_4O_2$, 100572-22-3; V, 100572-23-4; V·$C_2H_4O_2$, 100680-05-5; VI, 100572-24-5; VI·$C_2H_4O_2$, 100572-25-6; VII, 100572-26-7; VIII, 87619-62-3; I′, 78700-75-1; II′, 83579-03-7; III′, 83603-32-1; IV′, 83579-08-2; V′, 100572-27-8; VI′, 94849-58-8; Boc-Phe-Gly-OBzl, 42280-29-5; Boc-D-Met-OH, 5241-66-7; Boc-Tyr-OSu, 20866-56-2; H-D-Met-Phe-Gly-NH-CH_2-C_6H_5, 100572-28-9; H-D-Met-Phe-Gly-D-NH-CH(CH_3)C_6Hs·trifluoroacetate, 100572-30-3; H-D-Met-Phe-Gly-NH-Ad·trifluoroacetate, 100572-32-5; Boc-Phe-OSu, 3674-06-4; H-D-Met-Phe-Gly-ol·trifluoroacetate, 100572-34-7; H-Phe-OMe, 2577-90-4; H-D-Met-Phe-Gly-NH_2·trifluoroacetate, 100572-38-1; H-D-Met-Phe-OMe·trifluoroacetate, 100572-36-9; Boc-Tyr-D-Met-Phe-OH, 100572-39-2; H-Sar-NH-Ad, 100572-40-5; benzylamine, 100-46-9; (*R*)-(+)-α-methylbenzylamine, 3886-69-9; 1-adamantanamine, 768-94-5; ethanolamine, 141-43-5.

7-Aroyl-2,3-dihydrobenzo[*b*]furan-3-carboxylic Acids and 7-Benzoyl-2,3-dihydrobenzo[*b*]thiophene-3-carboxylic Acids as Analgesic Agents

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Received August 13, 1985

The synthesis of a series of 7-aroyl-2,3-dihydrobenzo[*b*]furan-3-carboxylic acids and 7-benzoyl-2,3-dihydrobenzo-[*b*]thiophene-3-carboxylic acids is described. The isomeric 4-benzoyl-1,3-dihydrobenzo[*c*]furan-1-carboxylic acid was also prepared. Compounds were evaluated for analgesic activity in the mouse phenyl-*p*-quinone-induced writhing test. Selected compounds were tested for their ability to produce gastric damage in fasted mice and for inhibition of prostaglandin synthetase activity in vitro. Zomepirac was used as a reference. Structure–activity relationships are discussed. One of the compounds, 7-benzoyl-5-chloro-2,3-dihydrobenzo[*b*]furan-3-carboxylic acid (**2c**), combined potent analgesic activity with low gastric irritancy.

Gastrointestinal symptoms including mucosal damage, bleeding, and ulceration are the most common side effects of peripherally acting analgesic agents that inhibit prostaglandin synthetase.[1] One of the aims of our research program has been to identify compounds that combine the analgesic potency of zomepirac[2] (1) with a high level of gastric tolerance. In this paper we describe the synthesis and pharmacology of a series of 5-substituted 7-aroyl-

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