benzylamine hydrochloride (55.55 mM), 5-(aminomethyl)-3-aryldihydrofuran-2(3*H*)-one hydrochloride (5.55 mM) with benzylamine hydrochloride (55.55 mM), and a control containing only the buffer. To these solutions, was added MAO-B (20 μL, 3 mg/mL). The mixtures were incubated at 25 °C, and the MAO activity was assayed as described above.

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Analgesic Dipeptide Derivatives. 7. 3,7-Diamino-2-hydroxyheptanoic Acid (DAHHA) Containing Dipeptide Analogues of the Analgesic Compound H-Lys-Trp(Nps)-OMe

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A series of diastereomeric dipeptides, analogues of the analgesic compound H-Lys-Trp(Nps)-OMe (**2**), containing 3,7-diamino-2-hydroxyheptanoic acid (DAHHA) and 2-[(*o*-nitrophenyl)sulfenyl]tryptophan [Trp(Nps)] has been synthesized. These compounds were tested as enkephalin-degrading aminopeptidases (APs), AP-M and AP-B inhibitors, and analgesics. The inhibitory potencies and the antinociceptive effects depended on the stereochemistry of the compounds. (2*S*,3*R*)-DAHHA-L-Trp(Nps)-OMe (**26d**) was a highly potent and selective enkephalin-degrading APs inhibitor, with an IC_{50} value in the 10^{-8} M range. Although this derivative was about 10^3-fold more potent than **2** against these enzymes, their antinociceptive effects were completely similar. These results indicate that the inhibitory capacity of this series of Trp(Nps)-containing dipeptides against enkephalin-degrading enzymes is not an important factor for their antinociceptive effects.

In previous papers,[1,2] it was reported that the synthetic dipeptide H-Lys-Trp(Nps)-OH [Nps = (*o*-nitrophenyl)-sulfenyl] (**1**) and its methyl ester **2** exhibited a naloxone-reversible analgesia in mice, comparable with that of the enkephalin analogue D-Ala^2-Met-enkephalinamide (DAME), regarding both maximum effect and the time-course of analgesia. Studies to establish the structural requirements for the antinociceptive effect of **1** and **2** showed the need for a basic amino acid,[1,3] the importance of the Nps moiety,[1,2,4] since no analgesia was found with the unsubstituted dipeptide H-Lys-Trp-OH (**3**), and the dependence on the absolute configuration of each amino acid of the activity.[5] Studies on the mechanism of action of **1** appear to indicate that these Trp(Nps)-containing dipeptides do not act directly on opioid receptors, but their antinociceptive effects could be possible explained by a mixture of a moderate enkephalin-degrading aminopeptidase inhibition and Met-enkephalin-releasing properties.[1] We considered that a structural modification able to increase the aminopeptidase (AP) inhibitory potency of these dipeptide derivatives could help to clarify the participation of this inhibition in the observed analgesic effect and, therefore, in the mode of action. With this aim, the lysine residue of **2** has been replaced by the hydroxy-substituted homologue, 3,7-diamino-2-hydroxyheptanoic acid (DAHHA) whose α-hydroxy group could mimic the tetrahedral intermediate formed during the substrate hydrolysis by APs. This concept has been applied to explain the potent inhibition of APs by the natural compound [(2*S*,3*R*)-3-amino-2-hydroxy-4-phenylbutanoyl]-L-leucine [(2*S*,3*R*)-AHPBA-Leu, bestatin (**4**)],[6-9] which protects the endogenous enkephalins, released from K^+ depolarized brain slices, from degradation by this type of enzymes.[10,11] This paper deals with the synthesis, inhibitory properties against enkephalin-degrading APs, and the antinociceptive activity of a series of stereoisomeric dipeptide derivatives **5**, in which the absolute configuration of each asymmetric

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(1) García-López, M. T.; Herranz, R.; González-Muñiz, R.; Naranjo, J. R.; De Ceballos, M. L.; Del Río, J. Antinociceptive Effects in Rodents of the Dipeptide Lys-Trp(Nps) and Related Compounds. *Peptides* **1986**, *7*, 39–43.
(2) García-López, M. T.; González-Muñiz, R.; Molinero, M. T.; Naranjo, J. R.; Del Río, J. Analgesic Dipeptide Derivatives. 3. Synthesis and Structure-Activity Relationships of *o*-Nitrophenyl-Modified Analogues of the Analgesic Compound H-Lys-Trp(Nps)-OMe. *J. Med. Chem.* **1987**, *30*, 1658–1663.
(3) García-López, M. T.; González-Muñiz, R.; Herranz, R.; Molinero, M. T.; Del Río, J. Analgesic Dipeptide Derivatives. II. Synthetic Dipeptides Containing a *C*-Terminal 2-(2-Nitrophenylsulfenyl)tryptophan Residue. *Int. J. Peptide Protein Res.* **1987**, *29*, 613–620.
(4) García-López, M. T.; González-Muñiz, R.; Molinero, M. T.; Del Río, J. Analgesic Dipeptide Derivatives V. Sulfenyl Modified Analogs of the Analgesic Compound Lysyl-2-(*o*-nitrophenylsulfenyl)tryptophan Methyl Ester. *Anal. Quim. C* **1988**, *84*, 312–316.
(5) García-López, M. T.; González-Muñiz, R.; Molinero, M. T.; Del Río, J. Analgesic Dipeptide Derivatives. 4. Linear and Cyclic Analogues of the Analgesic Compounds Arginyl-2-[(*o*-nitrophenyl)sulfenyl]tryptophan and Lysyl-2-[(*o*-nitrophenyl)-sulfenyl]tryptophan. *J. Med. Chem.* **1988**, *31*, 295–300.
(6) Umezawa, H.; Aoyagi, T.; Suda, H.; Hamada, M.; Takeuchi, T. Bestatin, an Inhibitor of Aminopeptidase B, Produced by Actinomycetes. *J. Antibiot.* **1976**, *29*, 97–99.
(7) Ocain, T. D.; Rich, D. H. Synthesis of Sulfur-Containing Analogues of Bestatin. Inhibition of Aminopeptidases by α-thiolbestatin Analogues. *J. Med. Chem.* **1988**, *31*, 2193–2199.
(8) Gordon, E. M.; Godfrey, J. D.; Delaney, N. G.; Asaad, M. M.; Von Lange, D.; Cushman, D. W. Design of Novel Inhibitors of Aminopeptidases. Synthesis of Peptide-Derived Diamino Thiols and Sulfur Replacement Analogues of Bestatin. *J. Med. Chem.* **1988**, *31*, 2199–2211.
(9) Harbeson, S. L.; Rich, D. H. Inhibition of Arginine Aminopeptidase by Bestatin and Arphamenine Analogues. Evidence for a New Mode of Binding to Aminopeptidases, *Biochemistry* **1988**, *27*, 7301–7310.
(10) Schwartz, J. C. Metabolism of Enkephalins and the Inactivating Neuropeptidase Concept. *TINS* **1983**, 45–48.

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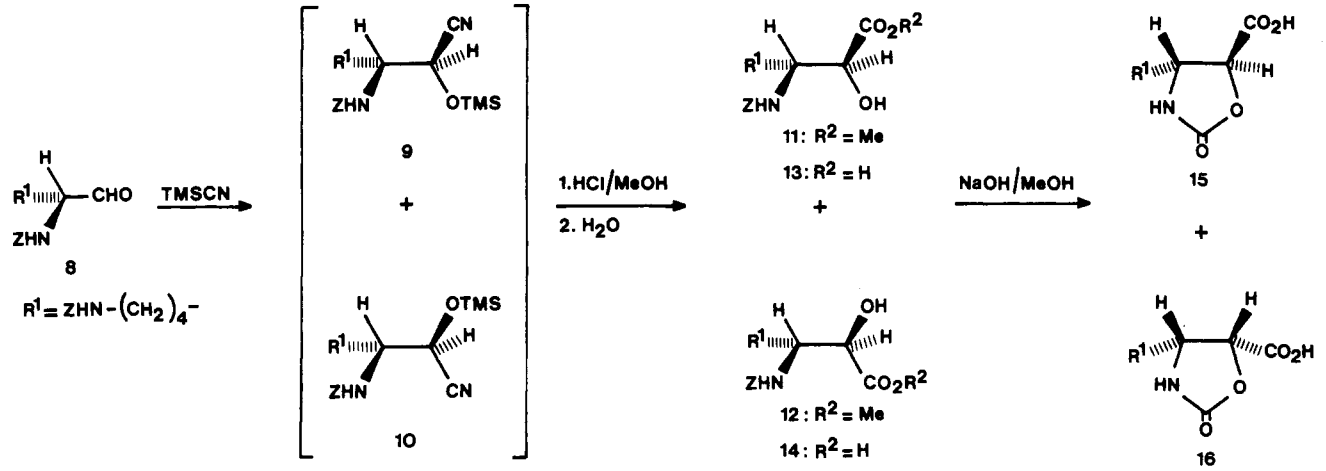
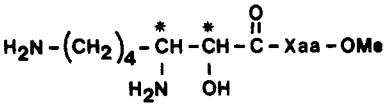
Scheme I



center has been varied systematically. The inhibition of AP-B by these derivatives is also reported. All biological data are compared with those of the parent compound **2**, the unsubstituted tryptophan analogues **6** and **3**, and bestatin. For further comparative purposes the bestatin



analogues **7**, in which the AHPBA residue has been replaced by DAHHA, have also been synthesized and included in the biological assays.

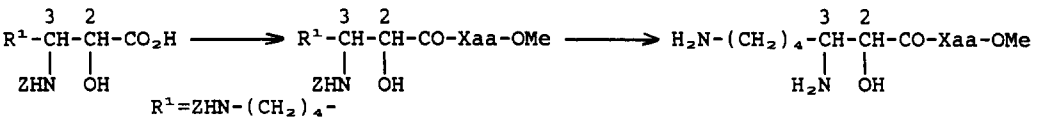
Results

Chemistry. As indicated in the Scheme I, the N-diprotected DAHHA **13** and **14** were prepared via our method for the stereoselective synthesis of (2*S*,3*R*)- and (2*R*,3*S*)-3-amino-2-hydroxy acids, recently reported.[12] Thus, reaction of bis(*N*-Z)-D-lysinal (**8**), freshly prepared[13] by the method of Fehrentz and Castro,[14] with trimethylsilyl cyanide (TMSCN) in dry dichloromethane yielded a (4:1) mixture of the corresponding *threo*- and *erythro*-*O*-(trimethylsilyl)cyanohydrins **9** and **10**, which was directly transformed into a 4:1 mixture of the 2-hydroxy esters **11** and **12**, by treatment with dry methanolic hydrogen chloride, followed by in situ hydrolysis of the imidate hydrochloride intermediates. The methyl esters **11** and **12** could not be separated by chromatography, using different elution systems. The ratio of diastereoisomers **11** and **12** was determined by the measurement of the integrals of the two singlets, corresponding to the methyl ester groups, in the ^{1}H NMR spectrum of the mixture. This mixture of **11** + **12** was saponified to yield the 4:1 mixture of the acids **13** + **14**, which could not be separated. The C-2 configuration of these compounds was established on the basis of the ^{1}H NMR spectrum of the corresponding 2-oxazolidinones.[15] Thus, the major threo isomer methyl ester **11** gave the 2-oxazolidinone **15**, with a H_4,H_5 trans disposition, as indicated by its J value of 4.5 Hz, while the minor erythro isomer **16** had a $J_{4,5}$ value of 9 Hz, consistent with a cis disposition. Similarly, a 4:1 mixture of 2*R*,3*S*, **20**, and 2*S*,3*S*, **21**, enantiomers of **13** and **14**, respectively, were obtained from bis(*N*-Z)-L-lysinal (**17**).

The dipeptide derivatives **22a–25c**, indicated in Scheme II, were prepared by coupling of the corresponding (4:1) mixture of *threo*- and *erythro*-β-amino-α-hydroxy acids **13** + **14** or **20** + **21** with the methyl esters of L-Trp, D-Trp, and L-Leu, respectively, via the DCC method,[16] in the presence of 1-hydroxybenzotriazole. In this way, the 4:1 threo:erythro mixtures **22** + **23** and **24** + **25** were obtained, which could only be separated by chromatography when the C-3 configuration of the β-amino-α-hydroxy acid residue and that of the coupled amino acid were different as in **22a** and **23a**, **22c** and **23c**, or **24b** and **25b**. Removal of the *N*-Z protecting groups by hydrogenolysis, in the presence of 10% Pd/C, yielded the corresponding deprotected dipeptides **26a–29c**. Treatment of the Trp-containing dipeptides **26a**, **26b** + **27b** (4:1), **28a** + **29a** (4:1), and **28b** with *o*-nitrobenzenesulfonyl chloride (Nps-Cl), in dry 1 N HCl in dioxane,[3,17,18] afforded the respective Trp(Nps)-containing dipeptides **26d**, **26e** + **27e** (4:1), **28d** + **29d** (4:1), and **28e**. The UV spectra of the Trp(Nps)-containing dipeptides (Table V) showed two absorption maxima at 355 nm (ε, 3.065 × 10^3) and 280 nm (ε, 1.070 × 10^4), in agreement with those reported in the literature for dipeptides containing this substituted amino acid.[3,18] As in the case of all the Trp(Nps)-containing dipeptides

(11) Chaillet, P.; Marçais-Collado, H.; Costentin, J.; Yi, C.; De la Baume, S.; Schwartz, J. C. Inhibition of Enkephalin Metabolism by, and Antinociceptive Activity of, Bestatin, an Aminopeptidase Inhibitor. *Eur. J. Pharmacol.* **1983**, *86*, 329–336.
(12) Herranz, R.; Castro-Pichel, J.; Vinuesa, S.; García-López, M. T. An Improved One-Pot Method for the Stereoselective Synthesis of the (2*S*,3*R*)-3-Amino-2-hydroxy Acids: Key Intermediates for Bestatin and Amastatin. *J. Org. Chem.* **1990**, *55*, 2232–2234.
(13) Coy, D. H.; Hocart, S. J.; Sasaki, Y. Solid Phase Reductive Alkylation Techniques in Analogue Peptide Bond and Side Chain Modification. *Tetrahedron* **1988**, *44*, 835–841.
(14) Fehrentz, J. A.; Castro, B. An Efficient Synthesis of Optically Active α-(*t*-Butoxycarbonylamino)-aldehydes from α-Amino Acids. *Synthesis* **1983**, 676–678.
(15) Suda, H.; Takita, T.; Aoyagi, T.; Umezawa, H. The Structure of Bestatin. *J. Antibiot.* **1976**, *29*, 100–101.
(16) Barany, G.; Merrifield, R. B. Solid-Phase Peptide Synthesis in *The Peptides. Analysis, Synthesis, Biology*; Gross, E., Meienhofer, J., Eds.; Academic Press, Inc.: New York, 1980; Vol. 2, pp 1–284.
(17) Fontana, A.; Marchiori, F.; Moroder, L.; Scoffone, E. New Removal of Sulfenyl Groups in Peptide Synthesis. *Tetrahedron Lett.* **1966**, 2985–2987.
(18) Scoffone, E.; Fontana, A.; Rocchi, R. Sulfenyl Halides as Modifying Reagents for Polypeptides and Proteines. I. Modification of Tryptophan Residues. *Biochemistry* **1968**, *7*, 971–979.

Scheme II. Dipeptide Synthesis



No.	C-2	C-3	→	No.	C-2	C-3	Xaa	→	No.	C-2	C-3	Xaa
				22a	S	R	L-Trp		**26a**	S	R	L-Trp
				23a	R	R	L-Trp		**27a**	R	R	L-Trp
13+14	R,S	R		**22b+23b**	R,S	R	D-Trp		**26b+27b**	R,S	R	D-Trp
				22c	S	R	L-Leu		**26c**	S	R	L-Leu
				23c	R	R	L-Leu					
									26d	S	R	L-Trp(Nps)
									26e+27e	R,S	R	D-Trp(Nps)
				24a+25a	R,S	S	L-Trp		**28a+29a**	R,S	S	L-Trp
20+21	R,S	S		**24b**	R	S	D-Trp		**28b**	R	S	D-Trp
				25b	S	S	D-Trp		**29b**	S	S	D-Trp
				24c+25c	R,S	S	L-Leu		**28c+29c**	R,S	S	L-Leu
									28d+29d	R,S	S	L-Trp(Nps)
									28e	R	S	D-Trp(Nps)

previously reported,[2-5] a significant shielding of the Nps H-6 ($\delta \approx 6.6$) was observed (Table V).

Biological Results and Discussion

AP-M Inhibition. Taking into account that enkephalins are mainly degraded by two membrane-bound APs,[19] one of which was characterized and identified[20-22] as AP-M (EC 3.4.11.2), the dipeptides **26a–28e** were first tested as inhibitors of the activity of this enzyme, using L-leucine β-naphthylamide (Leu-NA) as substrate.[6] As shown in Table I, the IC_{50} values of all the compounds reflect the large importance of the stereochemistry on the inhibitory potency. Thus, a very large difference appears in the IC_{50} values of the two enantiomers **26a** and **28b** or **26d** and **28e**. The best inhibitors were compound **26a** and the Nps-substituted analogue **26d**, which were more than 100- and 35-fold more potent than the corresponding Lys-containing models **3** and **2**, respectively. Compounds **26a** and **26d** were also more efficiently recognized by AP-M than bestatin (factor of 2–3). Although compound **26d** was only slightly more potent than **26a**, in general, the introduction of the Nps residue produced, at least, a 10-fold increase in the inhibitory potency. Finally, replacement of the AHPBA residue in bestatin by DAHHA moiety to give **26c** lead to a loss of activity, by a factor of 10.

Table I. Inhibitory Potency of the Dipeptides **26e–28e** on AP-M, Purified Membrane-Bound Rat Brain APs, and AP-B

no.	AP-M[a] IC_{50} (μM)	enkephalin-degrading APs IC_{50} (μM)	AP-B[a] IC_{50} (μM)
26a	10.2	0.21	>1000
27a	502	45.53	>1000
26b + 27b	826	-	>1000
26c	201	2.01	481
26d	7	0.024	101
26e + 27e	91.2	0.33	486
28a + 29a	>1000	-	500
28b	>1000	-	>1000
29b	>1000	-	>1000
28c + 29c	>1000	-	500
28d + 29d	852	32.65	583
28e	225	10.9	982
2	261	18	400
3	>1000	-	>1000
4	19.4	0.30	6

[a] Values are the mean of 4–5 experiments with 3–5 different concentrations of inhibitor. SE were less than 10% of the mean.

Purified Membrane-Bound Rat Brain APs Inhibition. Compounds **26a**, **27a**, **26c**, **26d**, **26e + 27e**, **28d + 29d**, and **28e**, with values of IC_{50} in the 10^{-4}–10^{-6} M range against AP-M were tested as inhibitors of purified membrane-bound rat brain APs using ^{3}H-Leu-enkephalin as substrate.[23] As indicated in Table I, all them were more potent (factors of 10–300) against enkephalin-degrading APs than against AP-M. As in the case of AP-M, compound **26d**, containing (2*S*,3*R*)-DAHHA and L-Trp(Nps) components, was the best inhibitor with a value of IC_{50} in the 10^{-8} M range. This compound, having the same absolute configuration in the amino acid residues as bestatin, was about 10-fold better than this natural dipeptide and 10^3-fold better than the Lys-containing analogue **2**. Com-

(19) Hersch, L. B. Characterization of Membrane-Bound Aminopeptidases from Brain: Identification of the Enkephalin-Degrading Aminopeptidase. *J. Neurochem.* **1985**, 1427–1435.
(20) Giros, B.; Gros, C.; Solhonne, B.; Schwartz, J. C. Characterization of Aminopeptidases Responsible for Inactivating Endogenous (Met[5]) Enkephalin in Brain Slices Using Peptidase Inhibitors and Anti-Aminopeptidase M antibodies. *Mol. Pharmacol.* **1986**, *29*, 281–287.
(21) Solhonne, B.; Gros, C.; Pollard, H.; Schwartz, J. C. Major Localization of Aminopeptidase M in Rat Brain Microvessels. *Neuroscience* **1987**, *22*, 225–232.
(22) Fournie-Zaluski, M. C. Design and Evaluation of Inhibitors of Enkephalin Degrading Enzymes. *Neurochem. Int.* **1988**, *12*, 375–382.
(23) Malfroy, B.; Swerts, J. P.; Guyon, A.; Roques, B. P.; Schwartz, J. C. High Affinity Enkephalin-Degrading Peptidases in Brain is Increased after Morfine. *Nature* **1978**, *276*, 523–526.

Table II. Analgesic Response to the Dipeptides 26a–28e

no.	dose, μg/mouse icv	% change in reaction time (min)[a] 5	30	60
saline	–	14 ± 8	−2 ± 10	−15 ± 7
26a	5	60 ± 16*	17 ± 9	14 ± 10
26b + 27b	5	10 ± 9	−1 ± 6	−5 ± 11
26c	5	62 ± 20*	21 ± 15	23 ± 15
26d	1	124 ± 20*	63 ± 14*	47 ± 15*
	5	146 ± 8*	80 ± 14*	74 ± 13*
	10	243 ± 10*	[b]	[b]
26e + 27e	1	19 ± 15	13 ± 11	−2 ± 11
	5	80 ± 10*	56 ± 8*	37 ± 12
28a + 29a	5	26 ± 13	19 ± 9	−4 ± 12
28b	5	22 ± 8	−1 ± 10	2 ± 15
28c + 29c	5	−1 ± 15	10 ± 14	15 ± 11
28d + 29d	1	25 ± 7	15 ± 6	−8 ± 10
	5	75 ± 11*	64 ± 12*	43 ± 13*
28e	1	19 ± 5	21 ± 18	−1 ± 16
	5	59 ± 20*	75 ± 14*	55 ± 22*
2	0.5	80 ± 20*	40 ± 9*	20 ± 7
	1[c]	130 ± 20*	60 ± 10*	35 ± 6
4	25	26 ± 9	3 ± 7	−3 ± 7
	100	11 ± 8	41 ± 12*	59 ± 16*

[a] Results are the means ± SE obtained with groups of 10–12 mice. (*) Significant change ($p < 0.05$ or better, Student's t test). [b] Signs of strong neurotoxicity. [c] Signs of weak neurotoxicity, consisting of motor incordination, respiratory disturbances, and barrel rotations; at 5/μg this compound was strongly neurotoxic.

pounds **26a** and the diastereomeric mixture **26e + 27e** were as efficient as bestatin in inhibiting enkephalin-degrading APs.

None of these DAHHA-containing dipeptides inhibited the enkephalin-degrading neutral endopeptidase (NEP, EC 3.4.24.11).

AP-B Inhibition. Since bestatin is a potent AP-B (EC 3.4.11.6) inhibitor, inhibition which is related with the immunomodifier activity of **4**,[24] the dipeptides **26a–28e** were also evaluated against this enzyme, associated with the surface of murine L cells, using L-lysine β-naphthylamide (Lys-NA) as substrate.[25,26] As shown in Table I, only the Leu- or Trp(Nps)-containing dipeptides **26c** and **28c + 29c** or **26d**, **26e + 27e**, **28d + 29d**, and **28e** exhibited a poor inhibition, with IC_{50} values in the 10^{-4} M range. Contrary to that expected, due to the preference of AP-B for substrates with a basic *N*-terminal amino acid (Lys or Arg) and to the higher affinity of L-lysinethiol as compared to L-leucinethiol,[27] replacement of the AHPBA residue of bestatin by the DAHHA moiety produced a loss of activity, by a factor of 70. While this work was in progress Harbeson et al.[9] reported an IC_{50} of 4.8 μM for the (1:1) mixture **28c + 29c** in AP-B isolated from rat-liver tissue; however, they did not give a reference either of its synthesis or of its structural characterization.

Antinociceptive Activity. The antinociceptive effect in mice of the dipeptide derivatives **26a–28e** in the tail-flick test, given by the icv route are listed in Table II. All the compounds containing Trp(Nps) exhibited antinociceptive activity but, similarly to the diastereomeric Trp(Nps)-containing dipeptides previously reported,[5] the effect depended on the absolute configuration of each asymmetric center. In all cases, the analgesia was blocked by previous administration of naloxone, 5 mg/kg sc, given 15 min before the injection. The highest activity was shown by compound **26d**, which produced analgesic effects at 1 μg/mouse, similar to those of the model compound **2** at the same dose, 5 and 30 min after injection, but in contrast to this model, there were no signs of neurotoxicity up to a dose of 10 μg/mouse. As with compound **2**, the peak antinociceptive effect of the (2*S*,3*R*)-DAHHA-containing dipeptide **26d** was observed 5 min after administration. However compound **26d** had a longer response, since analgesia was also observed 60 min after injection. A sustained analgesic effect was obtained from 5 to 60 min following the icv administration (5 μg/mouse) of **26e + 27e** or **28e**.

The lower analgesic activity of **26a** with respect to the corresponding Nps-substituted derivative **26d** reflects, one more time, the importance of this substituent for the activity of this series of dipeptides.

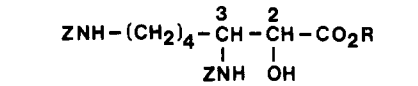
It is interesting to note that substitution of the AHPBA residue by DAHHA in bestatin resulted in more activity, since a 20-fold higher dose of the natural dipeptide was required to produce a similar analgesia to that produced by **26c**, 5 min after injection. However the response of **26c** had a very short duration, since it was extinguished at 30 min, while a very sustained analgesia was obtained for 30–60 min, following the icv administration of bestatin at 100 μg/mouse. When the results of antinociception (Table II) and APs inhibition (Table I) are compared, it can be seen that although the new compounds **26a**, **26e**, **26d**, **26e + 27e**, and **28e** and bestatin show higher AP-M and enkephalin-degrading APs inhibitory potency than the model compound **2**, they do not show higher antinociceptive effect. This lack of relation between enkephalin-degrading enzymes inhibition and antinociceptive effect has been previously reported for bestatin and other peptidase inhibitors.[10,28]

Conclusions

In summary, with the end of increasing the inhibitory potency of H-Lys-Trp(Nps)-OMe (**2**) against enkephalin-degrading aminopeptidases, we have synthesized a series of diastereomeric dipeptides in which the Lys moiety has been replaced by the β-amino-α-hydroxy homologue, DAHHA. Among these compounds, those containing (2*S*,3*R*)-DAHHA and L-Trp(Nps) (**26d**) and a 4:1 mixture of (2*S*,3*R*)- and (2*R*,3*R*)-DAHHA and D-Trp(Nps) (**26e + 27e**) were, respectively, 1000- and 100-fold more potent as enkephalin-degrading APs inhibitors than the model compound **2**. Additionally, compound **26d** was more potent (factor of 10) than bestatin against these APs but, in contrast, it was a very poor AP-B inhibitor ($IC_{50} = 10^{-4}$ M). Therefore this DAHHA-containing dipeptide was a highly efficient and selective enkephalin-degrading APs inhibitor. In spite of the large difference in the inhibitory potencies on these APs between **2** and **26d**, these compounds showed a similar antinociceptive activity. Therefore the main result of this study is that in this series of Trp(Nps)-containing dipeptides an increase in the inhibitory effect on enkephalin-degrading APs is not bound to an increase in

(24) Umezawa, H., Ed. *Small Molecular Immunomodifiers of Microbial Origin. Fundamental and Clinical Studies of Bestatin.* Pergamon Press: Oxford, 1981.
(25) Aoyagi, T.; Suda, H.; Nagai, M.; Ogawa, K.; Suzuki, J.; Takeuchi, T.; Umezawa, H. Aminopeptidase Activities on the Surface of Mammalian Cells. *Biochim. Biophys. Acta* **1976**, *452*, 131–143.
(26) Umezawa, H.; Ishizuka, M.; Aoyagi, T.; Takeuchi, T. Enhancement of Delayed-type Hypersensitivity by Bestatin, an Inhibitor of Aminopeptidase B and Leucine Aminopeptidase. *J. Antibiot.* **1976**, *29*, 857–859.
(27) Ocain, T. D.; Rich, D. H. *L*-Lysinethiol: A subnanomolar Inhibitor of Aminopeptidase B. *Biochem. Biophys. Res. Commun.* **1987**, *145*, 1038–1042.
(28) González-Muñiz, M. R.; García-López, M. T.; Gómez-Monterrey, I. M.; Harto-Martínez, J. R. Inhibitors of Enzymes Involved in the Degradation Process of Eukephalins. A New Approach in the Treatment of Pain. *Farm. Clin.* **1989**, *6*, 9–30.

Table III. Analytical and Spectroscopic Data of Bis(*N*-Z)DAHHA and Their Methyl Esters



no.	C-2	C-3	R	yield, %	formula[a]	^{1}H NMR (Me_2SO-d_6) δ 2-H	3-H	3-NH	CH_3	$J_{2,3}$ (Hz)
11 + 12	*S*	*R*	CH_3	50[b]	$C_{24}H_{30}N_2O_7$	4.07	3.76	6.90	3.56	2.4
(4:1)	*R*	*R*	CH_3			3.98	3.68	7.16	3.58	2.7
18 + 19	*R*	*S*	CH_3	56[b]	$C_{24}H_{30}N_2O_7$	4.07	3.76	6.90	3.56	2.4
(4:1)	*S*	*S*	CH_3			3.98	3.68	7.16	3.58	2.7
13 + 14	*S*	*R*	H	90	$C_{23}H_{28}N_2O_7$	3.98	3.80	6.82	–	3.6
(4:1)	*R*	*R*	H			4.08	3.80	6.87	–	3.9
20 + 21	*R*	*S*	H	93	$C_{23}H_{28}N_2O_7$	3.98	3.80	6.82	–	3.6
(4:1)	*S*	*S*	H			4.08	3.80	6.87	–	3.9

[a] Anal. C, H, N. [b] Overall yield from bis(*N*-Z)Lys.

the analgesic activity. This fact indicates that the AP inhibition by these compounds is not an important factor for its mode of action as analgesics.

Experimental Section

Chemical Methods. Melting points were measured with a Kofler hot-stage apparatus and are uncorrected. Elemental analyses were obtained using a Heraeus CHN-O-RAPID instrument. ^{1}H NMR spectra were recorded with a Varian XL-300 spectrometer (300 MHz), using Me_4Si as internal standard and $CDCl_3$ or Me_2SO-d_6 as sample solvents. UV absorption spectra were taken with a Perkin-Elmer 550 SE spectrophotometer, using MeOH as sample solvent. Analytical TLC was performed on aluminum sheets coated with a 0.2-mm layer of silica gel 60 F_{254} (Merck). Silica gel 60 (230–400 mesh) (Merck) was used for column chromatography. Compounds were detected with UV light (254 nm).

Synthesis of 4:1 Mixtures of (2*S*,3*R*)- and (2*R*,3*R*)-Bis(*N*-Z)DAHHA (11 + 12) and (2*R*,3*S*)- and (2*S*,3*S*)-Bis(*N*-Z)DAHHA Methyl Esters (18 + 19). General Procedure. TMSCN (0.71 g; 7.2 mmol) was added to a solution of N^{α},N^{ϵ}-bis(benzyloxycarbonyl)-D- or -L-lysinal[29] (8 or 17) (7 mmol), freshly prepared in 90% yield from the corresponding N^{α},N^{ϵ}-bis(benzyloxycarbonyl)-D- or -L-lysine by the method of Fehrentz and Castro,[14] in dry dichloromethane (50 mL), and the solution was stirred at room temperature for 5 days. Then the reaction mixture was evaporated, and the crude mixture of *threo*- and *erythro*-*O*-(trimethylsilyl)cyanohydrins was dissolved in a dry and cooled at 0 °C, 3:1 Et_2O/MeOH mixture, previously saturated with HCl (70 mL). This solution was stirred below 5 °C for 24 h, then, keeping the temperature below 10 °C, ice water (15 mL) was added, and the stirring was kept for 24–48 h [until the disappearance of the imidate intermediate was detected by TLC (5:1 $CHCl_3$–MeOH)]. The reaction mixture was concentrated (10 mL) and extracted with dichloromethane (3 × 50 mL). The organic extracts were washed with water (30 mL) and brine (30 mL), dried over Na_2SO_4, and evaporated. The residue was purified by flash chromatography with hexane–ethyl acetate mixtures as eluants. The yield and analytical and spectroscopic data for the β-amino-α-hydroxy acid methyl esters thus obtained, 11 + 12 and 18 + 19, are summarized in Table III.

Synthesis of 4:1 Mixtures of (2*S*,3*R*)- and (2*R*,3*R*)-Bis(*N*-Z)DAHHA (13 + 14) and (2*R*,3*S*)- and (2*S*,3*S*)-Bis(*N*-Z)DAHHA (20 + 21). General Procedure. NaOH (2.4 mmol) was added to a solution of the 4:1 mixture of methyl esters 11 + 12 or 18 + 19 (2 mmol) in a 1:1 dioxane–water mixture (50 mL), and the solution was stirred at room temperature for 1 h. Then, the reaction mixture was concentrated (≈20 mL), diluted with water (40 mL), and extracted with dichloromethane (3 × 40 mL). The aqueous phase was acidified to pH 3–4 with Dowex 50W-X4 resin. The resin was filtered and washed with dichloromethane (50 mL). The aqueous phase was extracted with dichloromethane (3 × 50 mL), and the organic extracts were dried over Na_2SO_4 and evaporated to give quantitatively the corresponding mixture of bis(benzyloxycarbonyl)DAHHA, 13 + 14 or 20 + 21, whose analytical and spectroscopic data are summarized in Table III.

4:1 Mixture of (4*R*,5*S*)- and (4*R*,5*R*)-4-[4-[(Benzyloxycarbonyl)amino]butanoyl]-2-oxo-5-oxazolidinecarboxylic Acids (15 + 16). NaOH (6 N, 1 mL, 2 mmol) was added to a solution of the mixture of methyl esters 11 + 12 (1 mmol) in MeOH (30 mL), and after stirring at room temperature for 2 h, the reaction mixture was evaporated. The residue was taken up in water (20 mL), washed with dichloromethane (2 × 20 mL), and acidified to pH 3–4 with Dowex 50W-X4 resin. The resin was filtered off and washed with ethyl acetate (30 mL). The aqueous phase was extracted with ethyl acetate (2 × 30 mL), and the combined organic extracts were dried over Na_2SO_4 and evaporated to give the 4:1 mixture of 15 + 16 as a foam in a 80% yield. It was not possible to separate 15 and 16 by chromatography; ^{1}H NMR (Me_2SO-d_6) δ 1.35 (m, 6 H, $(CH_2)_3CH$), 2.98 (m, 2 H, ZNH-CH_2), 3.68 (m, 0.8 H, 4-H, 14), 4.01 (m, 0.2 H, 4-H, 15), 4.59 (d, 0.8 H, $J_{4,5}$ = 4.5 Hz, 5-H, 14), 5.01 (d, 0.2 H, $J_{4,5}$ = 9 Hz, 5-H, 15), 5.01 (s, 2 H, C_6H_5-CH_2), 7.32 (m, 5 H, C_6H_5), 7.96 (s, 1 H, *N*H-Z). Anal. ($C_{16}H_{20}O_6N_2$) C, H, N.

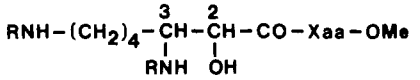
Synthesis of the Protected DAHHA-Containing Dipeptides 22a–25c. General Procedure. To a solution of the 4:1 mixture of *threo*- and *erythro*-bis(*N*-Z)DAHHA, 13 + 14 or 20 + 21 (1.3 mmol), and L-Trp, D-Trp, or L-Leu methyl ester hydrochlorides (1.6 mmol) in dry THF (13 mL) were added, at 0 °C, 1-hydroxybenzotriazole (1.6 mmol) and triethylamine (1.3 mmol). After 30 min of stirring at 0 °C, DCC (1.3 mmol) dissolved in dry dichloromethane (20 mL) was added, and the stirring was continued at room temperature for 24 h. Solvents were removed under reduced pressure, and the residue was purified by flash chromatography, using hexane–ethyl acetate mixtures as eluants. In this way the corresponding 4:1 mixtures of *threo*- and *erythro*-bis(*N*-Z)DAHHA-containing dipeptides were obtained, of them only those in which the C-3 configuration of the DAHHA residue was different from the configuration of the C-terminal amino acid could be separated by a new flash chromatography using hexane–ethyl acetate mixtures as eluants. The analytical and more significative spectroscopic data of the dipeptides **22a**, **23a**, **22b** + **23b**, **22c**, **24a** + **25a**, **24b**, **25b**, and **24c** + **25c**, thus obtained, are summarized in Table III.

Synthesis of the Protected DAHHA-Containing Dipeptides 26a–27c and 28a–29c. General Procedure. A solution of the protected bis(*N*-Z)DAHHA-containing dipeptides **22a**, **23a**, **22b** + **23b**, **22c**, **24a** + **25a**, **24b**, **25b**, or **24c** + **25c** (0.83 mmol) in a 0.4 N HCl solution in MeOH (50 mL) was hydrogenized in the presence of 10% Pd/C (47 mg), at 2 atm of H_2 pressure and at room temperature for 1 h. The catalyst was filtered off and washed with MeOH (10 mL), and the solvents were removed under reduced pressure. The residue was purified by flash chromatography, using $CHCl_3$–MeOH mixtures as eluants. The deprotected dipeptides **26a–26c** and **28a–29c** are summarized in Table IV.

Synthesis of the DAHHA-Trp(Nps)-OMe Dipeptides 26d, 26e + 27e, 28d + 29d, and 28e. General Procedure. To a solution of the deprotected DAHHA-containing dipeptides **26a**, **26b** + **27b**, **28a** + **29a**, or **28b** (0.22 mmol) in a 0.3 N HCl solution in MeOH (3 mL) was added Nps-Cl (0.27 mmol), and the mixture was stirred at room temperature for 30 min. Removal of the

(29) Ito, A.; Takahashi, R.; Baba, Y. A New Method to Synthesize α-Aminoaldehydes. *Chem. Pharm. Bull.* **1975**, *23*, 3081–3087.

Table IV. Analytical and Spectroscopic Data of the DAHHA-Containing Dipeptides Synthesized



no.	R	Xaa	yield, %	formula[a]	^{1}H NMR (Me_2SO-d_6) δ DAHHA 2-H	DAHHA 3-H	DAHHA $J_{2,3}$ (Hz)	Xaa α-H	Xaa NH
22a	Z	L-Trp	72	$C_{35}H_{40}N_4O_8$[c]	3.88	3.74	3.2	4.58	7.82
23a	Z	L-Trp	18	$C_{35}H_{40}N_4O_8$[c]	3.92	3.76	2.7	4.59	7.82
22b + 23b	Z	D-Trp	85	$C_{35}H_{40}N_4O_8$	3.89	3.73	3.4	4.57	7.94
	Z	D-Trp			3.96	3.73	2.8	4.57	7.76
22c	Z	L-Leu	81	$C_{30}H_{41}N_3O_8$	3.92	3.75	3.2	4.36	7.90
23c	Z	L-Leu	15	$C_{30}H_{41}N_3O_8$	4.00	3.76	2.8	4.36	7.81
24a + 25a	Z	L-Trp	91	$C_{35}H_{40}N_4O_8$	3.89	3.73	3.4	4.57	7.94
	Z	L-Trp			3.96	3.73	2.8	4.57	7.76
24b	Z	D-Trp	73	$C_{35}H_{40}N_4O_8$	3.88	3.74	3.2	4.58	7.82
25b	Z	D-Trp	16	$C_{35}H_{40}N_4O_8$[c]	3.92	3.76	2.7	4.59	7.82
24c + 25c	Z	L-Leu	90	$C_{30}H_{41}N_3O_8$	3.91	3.75	3.6	4.31	8.10
	Z	L-Leu			4.01	3.76	3.1	4.31	7.92
26a	H	L-Trp	62	$C_{19}H_{28}N_4O_4 \cdot 2HCl$ $MeOH \cdot 2H_2O$[b]	4.10	3.16	4.6	4.60	8.27
27a	H	L-Trp	57	$C_{19}H_{28}N_4O_4 \cdot 2HCl$ $MeOH \cdot 2H_2O$[b]	4.25	3.33	2.7	4.61	8.13
26b + 27b	H	D-Trp	65	$C_{19}H_{28}N_4O_4 \cdot 2HCl$	4.10	3.14	4.2	4.62	8.03
	H	D-Trp		$MeOH \cdot 2H_2O$[b]	4.26	3.14	2.2	4.62	8.21
26c	H	L-Leu	79	$C_{14}H_{29}N_3O_4 \cdot 2HCl$ $MeOH \cdot 2H_2O$[b]	4.09	3.21	3.1	4.61	8.37
28a + 29a	H	L-Trp	67	$C_{19}H_{28}N_4O_4 \cdot 2HCl$	4.10	3.14	4.2	4.62	8.03
	H	L-Trp		$^1/_2MeOH \cdot 2H_2O$[b]	4.26	3.14	2.2	4.62	8.21
28b	H	D-Trp	59	$C_{19}H_{28}N_4O_4 \cdot 2HCl$ $^1/_2MeOH \cdot 2H_2O$[b]	4.10	3.16	4.6	4.60	8.27
29b	H	D-Trp	61	$C_{19}H_{28}N_4O_4 \cdot 2HCl$ $^1/_2MeOH \cdot 2H_2O$[b]	4.25	3.33	2.7	4.61	8.13
28c + 29c	H	L-Leu	72	$C_{14}H_{29}N_3O_4 \cdot 2HCl$	4.12	3.20	2.7	4.60	8.36
	H	L-Leu		$MeOH \cdot 2H_2O$[b]	4.28	3.20	2.7	4.61	8.32

[a] Anal. C, H, N. [b] MeOH was also detected by ^{1}H NMR. [c] Unsatisfactory C, H, N results.

Table V. Analytical and Spectroscopic Data of DAHHA-Trp(Nps)-OMe Dipeptides

no.	yield, %	formula[a]	λ_{max} (EtOH)	^{1}H NMR (Me_2SO-d_6) δ 2-H	3-H	$J_{2,3}$ (Hz)	α-H	3′-H	4′-H	5′-H	6′-H[c]
26d	62	$C_{25}H_{31}N_5O_6S \cdot 2HCl$ $MeOH \cdot 2H_2O$[b]	355 281	3.89	3.16	3.6	4.59	8.29	7.43	7.57	6.68
26e + 27e	58	$C_{25}H_{31}N_5O_6S \cdot 2HCl$[d]	355	4.05	3.20	3.6	4.65	8.27	7.40	7.56	6.65
		$^1/_2MeOH \cdot 2H_2O$[b]	280	4.10	3.20	2.1	4.65	8.27	7.41	7.56	6.65
28d + 29d	57	$C_{25}H_{31}N_5O_6S \cdot 2HCl$	355	4.05	3.20	3.6	4.65	8.27	7.40	7.56	6.65
		$MeOH \cdot 2H_2O$[b]	280	4.10	3.20	2.1	4.65	8.27	7.41	7.56	6.65
28e	60	$C_{25}H_{31}N_5O_6S \cdot 2HCl$ $2MeOH \cdot 2H_2O$[b]	355 280	3.89	3.16	3.6	4.59	8.29	7.43	7.57	6.68

[a] Anal. C, H, N, S. [b] MeOH was also detected by ^{1}H NMR. [c] Calculated value = 7.58 ppm. [d] Unsatisfactory C, H, N results.

solvent left a residue, which was purified by flash chromatography, using $CHCl_3$–MeOH mixtures as eluants. The analytical and more significative spectroscopic data of the Trp(Nps)-containing dipeptides **26d**, **26e + 27e**, **28d + 29d**, or **28e** are summarized in Table IV.

Biological Methods. Materials. The following commercial compounds were used: bestatin, Tyr, Tyr-Gly-Gly, Lys-NA, Leu-NA, and Fast Garnet GBC (Sigma UK), ^{3}H-Leu-enkephalin (the Radiochemical Centre, Amersham, UK). Microsomal (AP-M, EC 3.4.11.2) porcine kidney leucine-aminopeptidase was purchased from Sigma (UK). Mouse L cells were grown in Dulbecco's modified Eagle's medium and 10% fetal calf serum. L Cells' media and serum were supplied by Flow Labs (UK). Male ICR swiss albino mice weighing 20–25 g and male Wistar rats (250–300 g) were used. Animals had free access to water and food. Mice were housed in the behavioral room at least 2 days before testing, which was done at the same time of the day.

AP-M Assays. This activity was determined by the described method.[6] A solution of 2 mM L-Leu-NA (0.25 mL) in 0.1 M solution of Tris-HCl buffer (0.5 mL) at pH 7.0 was added to distilled water with or without an inhibitor in a series of test tubes in a 37 °C bath. After 3 min the enzyme solution (0.05 mL) was added and mixed well. Exactly 30 min later, the reaction was stopped by adding a 1 mg/mL solution of the stabilized diazonium salt Garnet GBC (1 mL) in acetic acid buffer at pH 4.2, containing 10% Tween 20. After standing for 15 min at room temperature, absorbancy was read at 525 nm. The reaction was also carried without enzyme solution, and the result was taken as blank.

Enkephalin-Degrading APs and NEP Assays. A membrane preparation from rat striatum was obtained according to a previously described method.[23] After a 10-min preincubation, incubations (15 min, 25 °C) were started by addition of ^{3}H-Leu-enkephalin (20 nM final concentration) and different concentrations of the tested inhibitors. The reaction was stopped by adding 1 M HCl (10 μL). ^{3}H-Metabolites (^{3}H-Tyr and ^{3}H-Tyr-Gly-Gly) were separated from intact ^{3}H-Leu-enkephalin by TLC on silica gel sheets, using the migration system 2-propanol–EtOAc–AcOH (2:2:1). The spots, detected by ninhydrin in EtOH solution, were removed, the peptide extracted with 1 mL of MeOH, and the radioactivity determined by liquid scintillation spectrometry. The IC_{50} values were determined by linear regression analysis from the inhibition curves constructed with at least six increasing concentrations of the inhibitors.

AP-B Assays. Cell surface-associated AP-B activities were determined following the Aoyagi method.[25] The incubation mixture consisted of 2 mM L-Lys-NA (0.25 mL), Hank's balanced salt solution (0.65 mL), and distilled water (0.1 mL) with or without the inhibitor. After 3 min of incubation (37 °C), the

mixture was added to monolayer cultures of mouse L cells (5×10^5 cells), and the incubation was stopped after 30 min by adding the stabilized diazonium salt Garnet GBC (1 mL, 1 mg/mL) in 1 M acetic acid buffer at pH 4.2, containing 10% Tween 20. The mixture was left at room temperature for 15 min and centrifuged, and its absorbance was measured at 525 nm.

Antinociceptive Activity. Antinociception was evaluated in mice by means of the tail-flick test,[30] immersing the tail into water at 52 °C, using a cutoff time of 10 s. The observer was blind to the compound injected. Results were expressed as percentage change in reaction time vs predrug score (1.9–2.5 s). A group of mice injected with saline was also tested in parallel. Saline administration had no effect on the tail-flick latency at any time postinjection.

Acknowledgment. The present work has been supported by Comisión Interministerial de Ciencia y Tecnología (Plan Nacional Project FAR 88-0298). We thank Francisco Caballero for help in typing this manuscript.

(30) Nott, M. W. Potentiation of Morphine Analgesia by Cocaine in Mice. *Eur. J. Pharmacol.* **1968**, *5*, 93–99.

Registry No. **8**, 138207-66-6; **11**, 138207-67-7; **12**, 138207-68-8; **13**, 138207-69-9; **14**, 138207-70-2; **15**, 138207-71-3; **16**, 138207-72-4; **17**, 119372-45-1; **18**, 138207-73-5; **19**, 138207-74-6; **20**, 138207-75-7; **21**, 138207-76-8; **22a**, 138207-77-9; **22b**, 138257-65-5; **22c**, 138207-78-0; **23a**, 138257-64-4; **23b**, 138257-66-6; **23c**, 138257-67-7; **24a**, 138257-68-8; **24b**, 138257-70-2; **24c**, 138257-72-4; **25a**, 138257-69-9; **25b**, 138257-71-3; **25c**, 138257-73-5; **26a**·2HCl, 138207-79-1; **26a** (free base), 138257-74-6; **26b**·2HCl, 138331-15-4; **26b** (free base), 138257-76-8; **26c**·2HCl, 138207-80-4; **26c** (free base), 138257-78-0; **26d**·2HCl, 138207-81-5; **26d** (free base), 138257-79-1; **26e**·2HCl, 138331-23-4; **26e** (free base), 138257-80-4; **27a**·2HCl, 138331-14-3; **27a** (free base), 138257-75-7; **27b**·2HCl, 138331-16-5; **27b** (free base), 138257-77-9; **27e**·2HCl, 138331-24-5; **27e** (free base), 138257-81-5; **28a**·2HCl, 138331-17-6; **28a** (free base), 138257-82-6; **28b**·2HCl, 138331-19-8; **28b** (free base), 138257-84-8; **28c**·2HCl, 138331-21-2; **28c** (free base), 138257-86-0; **28d**·2HCl, 138331-25-6; **28d** (free base), 138257-88-2; **28e**·2HCl, 138331-27-8; **28e** (free base), 138257-90-6; **29a**·2HCl, 138331-18-7; **29a** (free base), 138257-83-7; **29b**·2HCl, 138331-20-1; **29b** (free base), 138257-85-9; **29c**·2HCl, 138331-22-3; **29c** (free base), 138257-87-1; **29d**·2HCl, 138331-26-7; **29d** (free base), 138257-89-3; AP-M, 9054-63-1; AP-B, 9073-92-1; AP(enkephalin degrading), 75496-63-8; H-Trp-OMe·HCl, 7524-52-9; H-D-Trp-OMe·HCl, 41222-70-2; H-Leu-OMe·HCl, 7517-19-3.

Development of High-Affinity 5-HT_3 Receptor Antagonists. 1. Initial Structure–Activity Relationship of Novel Benzamides

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This report describes the development of novel benzamides which are orally active, highly potent, specific antagonists of 5-HT_3 receptors. Described in this first report are the structure–activity relationships that led to novel structures with improved potency and selectivity. From this series of compounds, (*S*)-**28** was identified and selected for further evaluation as a 5-HT_3 receptor antagonist. Compared with 5-HT_3 antagonists such as GR 38032F, BRL 43694, and metoclopramide, (*S*)-**28** was most active in (a) inhibiting binding to 5-HT_3 receptor binding sites in rat entorhinal cortex with an K_i value of 0.19 nM and (b) blocking cisplatin-induced emesis in the ferret with an ED_{50} value determined to be 9 μg/kg po.

Introduction

In the past decade there have been significant advances in our understanding of the biochemistry and physiology of the neurotransmitter serotonin (5-HT). Much of this progress was stimulated by the discovery of multiple 5-HT receptor subtypes and by the subsequent design of pharmacological agents selective for these sites. During recent years, there has been intense effort aimed at identification and functional characterization of 5-HT receptor subtypes and preparation of ligands with both potent binding affinity and receptor subtype specificity. One of these receptor subtypes is the 5-HT_3 receptor through which 5-HT acts to excite enteric neurons.[1] Ligand-binding and functional studies have shown that the 5-HT_3 receptor is found in both the peripheral[2] and central nervous system.[3] In enteric neurons and in autonomic neurons, activation of 5-HT_3 receptors produces depolarization and causes neurotransmitter release.[4] A somewhat selective 5-HT_3 agonist, 2-methylserotonin has been described[4a] and 5-HT_3 antagonists have been described which exhibit a variety of pharmacological effects. Several have displayed potent antagonism of chemotherapy or radiation-induced emesis in man.[5] In various animal models there has been some indication that these drugs may have utility in the treat-

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(1) Round, A.; Wallis, D. I. Further Studies on the Blockade of 5-HT Depolarization of Rabbit Vagal Afferent and Sympathetic Ganglion Cells by MDL-72222 and Other Antagonists. *Neuropharmacology* **1987**, *26*, 39.

(2) Richardson, B. P.; Engel, G.; Donatsch, P.; Stadler, P. A. Identification of Serotonin M-Receptor Subtypes and Their Specific Blockade by a New Class of Drugs. *Nature* **1985**, *316*, 126–131.

(3) Kilpatrick, G. J.; Jones, B. J.; Tyers, M. B. Identification and Distribution of 5-HT_3 Receptors in Rat Brain Using Radioligand Binding. *Nature* **1987**, *330*, 746–748. Barnes, N. M.; Costall, B.; Naylor, R. J.; Tattersall, F. D. Identification of 5-HT_3 Recognition Sites in the Ferret Area Postrema, *J. Pharm. Pharmacol.* **1988**, *40*, 586–588.

(4) Fox, A. T.; Morton, I. K. M. An Examination of the 5-HT_3 Receptor Mediating Contraction and Evoked [^{3}H]-Acetylcholine Release in the Guinea-Pig Ileum. *Br. J. Pharmacol.* **1990**, *101*, 553–558.

(5) Carmichael, J.; Cantwell, B. M. J.; Edwards, C. M.; Rapeport, W. G.; Harris, A. L. The Serotonin Type 3 Receptor Antagonist BRL 43694 and Nauseau and Vomiting Induced by Cisplatin. *Br. Med. J.* **1988**, *297*, 110–111.

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