Structure-Activity Relationships of N^2-Substituted Guanines as Inhibitors of HSV1 and HSV2 Thymidine Kinases

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A series of N^2-phenylguanines was synthesized and tested for inhibition of the thymidine kinases encoded by Herpes simplex viruses type 1 and type 2. Compounds with hydrophobic, electron-attracting groups in the meta position of the phenyl ring such as *m*-trifluoromethyl (*m*-CF_3PG, IC_{50} = 0.1 μM) were the most potent inhibitors of both enzymes. Many derivatives were significantly more potent against the type 2 thymidine kinase, and can effectively discriminate between the two enzymes. Among other N^2-substituted guanines, alkyl and benzyl derivatives were moderately potent inhibitors, and the type 2 enzyme was again more sensitive than the type 1 enzyme. None of the compounds inhibited the thymidine kinase isolated from the host HeLa cell line, suggesting that members of this class of compounds may be useful nonsubstrate, antiviral compounds for latent herpesvirus infections.

Several N^2-substituted guanines and their nucleosides display selective inhibitory activity toward the Herpes simplex virus type 1 (HSV1) thymidine kinase (TK) relative to the human enzyme in vitro and in vivo.[1,2] The nucleoside forms are nonsubstrate inhibitors of HSV1 TK in that they are resistant to phosphorylation by the viral enzyme.

N^2-Phenyl-2′-deoxyguanosine (PhdG) is the most potent derivative of this class of compounds with a K_i value of 0.3 μM, and its action is competitive with the enzyme substrates thymidine (TdR) and deoxycytidine.[1] We have suggested that PhdG, as a drug prototype, has potential as a selective antiherpes agent in cells of nondividing tissues and as a novel molecular probe of the structure and function of HSV1 thymidine kinase.[1] Recently, we have shown that PhdG inhibits the analogous enzyme derived from the type 2 variant of herpes simplex virus (HSV2) with a K_i of 0.7 μM,[2] a potency nearly as great as that against the HSV1 enzyme.

We are interested in the structural basis for the anti-TK activity of this class of compounds and in the use of these analogues to compare the TK encoded by HSV2 with that from HSV1. To further these investigations, we have chosen the base analogue N^2-phenylguanine (**1**) as a structural starting point. This compound is a moderately potent HSV1 TK inhibitor (IC_{50} = 8 μM), and preliminary studies suggested that substituents in the phenyl ring may profoundly alter potency of the resulting compounds.[1,2] Base analogues, furthermore, have several advantages over nucleosides for this purpose: they do possess activity against the kinases of interest even in the absence of a sugar moiety, they cannot be substrates for the enzymes, and they are more readily synthesized than nucleosides for structure-activity studies. Our approach has been to modify the structure of the N^2-substituent of guanine to develop structure-activity relationships that may allow us to map the inhibitor binding sites and to gain further insight into similarities or differences between the viral enzymes.

Results and Discussion

Chemistry. The guanine derivatives synthesized for this work were prepared by reaction between 2-bromohypoxanthine and the appropriate amine in refluxing 2-methoxyethanol, as described previously for **1**, **10**, **11**, **14**, and **17**.[1,3] Yields and physical properties of the new compounds are summarized in Table I. Compound **29**, N^2-*n*-hexyl-2′-deoxyguanosine, was synthesized by direct displacement of the halogen from 2-bromo-2′-deoxyinosine with *n*-hexylamine in ethanol at 120 °C.

TK Inhibitory Assays. The thymidine kinases from HSV1 and HSV2 and that from the host HeLa cell line were isolated and purified as described previously,[1,2] and were assayed by measuring the extent of phosphorylation of [^{3}H]thymidine with ATP as phosphate donor. The results of inhibitor testing presented in Tables II and III are tabulated as IC_{50} values, representing the concentrations of test compounds giving 50% inhibition of enzyme activity at a fixed concentration of thymidine (1 and 2 μM for the type 1 and 2 thymidine kinases, respectively). IC_{50} values for several of these compounds have been found to be close to and proportional to K_i values measured by standard kinetic experiments;[1,2] the activities reported in this paper are, therefore, considered to represent relative binding affinities of the analogues for the enzymes. None of the compounds tested at concentrations up to 1 mM inhibited the HeLa TK assayed in the presence of 3 μM thymidine.

HSV1 TK Inhibition. Structure-activity relationships for inhibition of the HSV1 enzyme can be summarized on the basis of results presented in Table II. Compared with the unsubstituted N^2-phenylguanine, **1**, substituents in the meta position are seen to have a more significant effect to increase inhibitory activity than those in the para position, and disubstituted derivatives having both meta and para groups are generally weak, although more potent than those with a para group alone. Hydrophobic groups in the meta position increased activity as observed for compounds **2**-**5**, but the hydrophilic CH_2OH group of **6** decreased activity. The most potent HSV1 TK inhibitor, **3**, has the hydrophobic, highly electron attracting CF_3 group in the meta position. Although para-substituted compounds were generally weaker than **1**, an exception to this trend is compound **7**, which was 8-fold more active than the prototype compound. The IC_{50} values for the disubstituted compounds do not follow an obvious pattern, nor are they simply additive contributions from the individual substituents. Among disubstituted compounds only the 3,4-dibromo analogue **13** was significantly more

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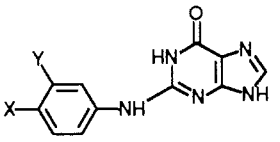
Table I. Physical Data for N^2-Phenylguanine Derivatives

no.	yield, %	mp, °C	crystn solvent	formula (anal.)	^{1}H NMR,[a] δ (ppm) 2-NH	8-H
2	80	231–234	MeOH–H_2O	$C_{11}H_8N_5OCl$ (C, H, N)	9.06	7.97
3	90	229–231	EtOH	$C_{12}H_8N_5OF_3$ (C, H, N[b])	9.52	8.32
5	30	239–241	ME[c]–H_2O, 3:1	*d*	8.56	7.82
6	36	>350	*e*	$C_{12}H_{11}N_5O_2$ (C, H, N)	8.72	7.81
7	46	>350	DMF–MeOH	$C_{11}H_8N_5OBr$ (C, H, N)	8.80	7.71
8	75	335–337	EtOH	$C_{13}H_{13}N_5O{\cdot}0.17H_2O$ (C, H, N)	8.52	7.77
9	43	265	DMF	$C_{12}H_8N_5OF_3{\cdot}0.33H_2O$ (C, H, N)	9.11	7.95
12	41	237–240	DMF–H_2O, 1:9	$C_{11}H_9N_5O_2{\cdot}H_2O$ (C, H, N)	8.29	7.71
13	36	>350	DMF	$C_{11}H_7N_5OBr_2{\cdot}0.25H_2O$ (C, H, N)	8.32	7.86
15	46	>350	DMF	$C_{11}H_7N_5OClF{\cdot}0.13H_2O$ (C, H, N)	8.87	7.79
16	59	>350	DMF–H_2O, 1:2	$C_{12}H_{10}N_5OCl{\cdot}0.5H_2O$ (C, H, N)	8.72	7.93
18	34	263–265	MeOH	$C_{15}H_{11}N_5O{\cdot}0.33MeOH$ (C, H, N)	8.88	8.26
19	27	>350	DMF	$C_{11}H_7N_5OF_2{\cdot}0.25DMF$ (C, H, N)	8.88	7.80
20	60	>350	DMF	$C_{11}H_7N_5OCl_2$ (C, H, N)	8.94	8.15
23	32	286–288	75% EtOH	$C_9H_{13}N_5O{\cdot}0.17H_2O$ (C, H, N)	6.19 (t)	7.66
24	55	287–289	EtOH	$C_{11}H_{17}N_5O{\cdot}0.17H_2O$ (C, H, N)	6.21 (t)	7.62
25	46	259–262	DMF–EtOH, 1:2	$C_{10}H_{15}N_5O_2{\cdot}0.25H_2O$ (C, H, N)	6.19 (t)	7.67
26	54	225–228	MeOH	$C_{12}H_{11}N_5O{\cdot}H_2O$ (C, N, H[f])	6.67 (br t)	7.68
27	23	212–215	EtOH	$C_{12}H_{10}N_5OCl{\cdot}0.5H_2O$ (C, H, N)	6.80 (t)	7.70
28	44	284–285	MeOH	$C_{12}H_{10}N_5OCl{\cdot}0.25H_2O$ (C, H, N)	6.74 (br t)	7.61

[a] All other resonances as expected. NMR spectra were determined in DMSO-d_6. [b] N: calcd 22.04; found 21.41. [c] ME: 2-methoxyethanol. [d] This compound did not give satisfactory elemental analyses after several attempts. [e] Purified by precipitation with acetic acid from a solution in 1 M NaOH. [f] H: calcd 5.05; found 4.39.

Table II. Effect of N^2-Phenylguanine Derivatives on the Activity of HSV1 and HSV2 Thymidine Kinases



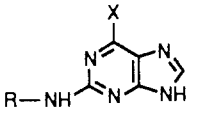
no.	X	Y	IC_{50},[a] μM HSV1 TK	HSV2 TK
1	H	H	8[b]	1.6
2	H	Cl	1.3[c]	0.35
3	H	CF_3	0.15	0.1
4	H	C_2H_5	2.5[b]	4
5	H	*n*-C_3H_7	3.3	3
6	H	CH_2OH	25	20
7	Br	H	1[c]	0.6
8	C_2H_5	H	20	25
9	CF_3	H	30	40
10	CH_3	H	50[b]	6[c]
11	*n*-C_4H_9	H	50[b]	50[c]
12	OH	H	140	20
13	Br	Br	3	1.3
14	CH_3	C_2H_5	7[b]	8
15	F	Cl	8	2.5
16	CH_3	Cl	12	2.5
17	$-CH_2CH_2CH_2-$		15[b]	10
18	$-CH{=}CH-CH{=}CH-$		40	10
19	F	F	50[c]	2.2[c]
20	Cl	Cl	100	30

[a] Concentration causing 50% inhibition of TdR phosphorylation; the TK assay included TdR at concentrations of 1 and 2 μM for the HSV1 and HSV2 enzymes, respectively, and was performed as described in the Experimental Section. [b] From ref 1. [c] From ref 2.

active than 1 and most were much weaker inhibitors.

HSV2 TK Inhibition. Similar trends were observed for inhibition of the HSV2 variant of TK by the same series of compounds as summarized in Table II. However, the type 2 enzyme appears to be intrinsically more sensitive to many of the inhibitors than the HSV1 enzyme. For example, there was a 5–8-fold increase in activity against HSV2 TK by compounds **1**, **2**, **10**, **12**, and **16** and more than a 20-fold increase for the 3,4-difluoro derivative, **19**. However, the most potent inhibitor, N^2-[*m*-(trifluoromethyl)phenyl]guanine (**3**), showed the same activity against both enzymes (Table II).

Table III. Effect of 2-Substituted Purines on the Activity of HSV1 and HSV2 Thymidine Kinases



no.	R	X	IC_{50},[a] μM HSV1 TK	HSV2 TK
1	C_6H_5	OH[b]	8	1.6
21	C_6H_5	Cl	55	8
22	C_6H_5	NH_2	>100	10
23	*n*-C_4H_9	OH	25	20
24	*n*-C_6H_{13}	OH	55	10
25	$(CH_2)_5OH$	OH	100	40
26	$CH_2C_6H_5$	OH	45[c]	2[c]
27	$CH_2C_6H_4$(3-Cl)	OH	10	14
28	$CH_2C_6H_4$(4-Cl)	OH	8.5	5.5

[a] Concentration causing 50% inhibition of TdR posphorylation; the TK assay included TdR at concentrations of 1 and 2 μM for the HSV1 and HSV2 enzymes, respectively, and was performed as described in the Experimental Section. [b] Keto tautomeric structure implied. [c] From ref 2.

These results cannot be interpreted in terms of identical binding sites for this class of inhibitors on the herpesvirus TKs, if it is assumed that the mechanism of inhibition of both enzymes is identical. Previous studies with PhdG indicate that its inhibition of HSV1 TK was classically competitive with the substrates TdR and deoxycytidine.[1] Similarly, compounds **2** and **7** were competitive with both substrates on HSV1 TK, and N^2-(3,4-difluorophenyl)-guanine (**19**) and compound **26** (see below) were found to be competitive with TdR as inhibitors of HSV2 TK.[2] Therefore, it is likely that N^2-phenylguanines and related inhibitors of herpesvirus TKs bind the enzymes at their active sites. The differential response of HSV1 and HSV2 TKs to inhibitors suggests structural differences between those sites. Previous studies have shown that the type 1 and type 2 enzymes differ in their ability to accept alternate substrates[4] as well as in their sensitivity to other inhibitors. For example, 5′-ethynylthymidine was about

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4-fold more potent as an inhibitor of the HSV1 compared to the HSV2 TK.[5] In contrast, among 5'-amino-5'-deoxythymidines and 5'-amino-2',5'-dideoxy-5-iodouridines, active TK inhibitors were 2–19-fold more potent against the HSV2 than the HSV1 enzyme.[6] The behavior of the latter series is similar to that reported for certain N^2-phenylguanines in this work. It may be possible to further discriminate between the viral TKs by enhancing the selectivity of substituted N^2-phenylguanines.

Other 2-Substituted Purines. Table III summarizes results of screening of the thymidine kinases against several other types of purine and guanine derivatives. Replacement of the 6-oxo group of **1** by the chloro and amino groups decreased activity against both enzymes by the resulting compounds, **21** and **22**, but the HSV2 enzyme was still more sensitive than the HSV1 enzyme. Apparently, the 6-oxo group is not absolutely required for activity, but the presence of this group, and the consequent "guanine" tautomeric structure, is optimal for inhibitor binding to both enzymes.

N^2-Substituents on guanine other than phenyl or naphthyl (cf. **18**) afforded active TK inhibitors (Table III). Thus, *n*-alkyl derivatives (**23–25**) and benzyl derivatives (**26–28**) were all moderately active inhibitors of HSV1 TK, and the activities of chloro-substituted N^2-benzylguanines approached that of **1**. Interestingly, in nearly all cases, the HSV2 enzyme was considerably more sensitive than the HSV1 enzyme. This is seen most strikingly with N^2-benzylguanine (**26**), which is over 20-fold more active as an inhibitor of the HSV2 TK, although the substituted derivatives **27** and **28** were about equiactive. It is likely that the benzyl compounds bind the active sites on the enzymes; for example, like certain prototype phenylguanines cited above, **26** inhibited the HSV2 TK competitively with thymidine.[2]

Effect of Deoxyribonucleosides on TKs. In our initial screen of guanine derivatives, we found that the 9-(2-deoxy-β-D-ribofuranosyl) derivative of **1**, PhdG, was 27-fold more potent than **1** as an inhibitor of HSV1 TK.[1] However, we later found that PhdG was only 2-fold more potent than **1** against the type 2 enzyme.[2] We were curious if other base/nucleoside pairs would display the same pattern of activities on the two TKs. N^2-*n*-Hexyl-2'-deoxyguanosine (**29**) was synthesized for this purpose. It showed potent inhibition of both type 1 and 2 enzymes, with K_i values of 2.6 and 2 μM, respectively, but the base form, **24**, had IC_{50} values of 55 and 10 μM, respectively, against the two enzymes (Table III). Thus, the enhancement of activity resulting from the deoxyribofuranosyl group on **24** was 21-fold for HSV1 TK, but, as with PhdG, only 5-fold for the HSV2 enzyme.

Conclusions

The results presented in this paper confirm that guanine derivatives substituted at the exocyclic nitrogen form a class of selective and potent inhibitors of the herpesvirus types 1 and 2 thymidine kinases. A trend of greater sensitivity of the HSV2 enzyme toward certain analogues was observed. Preliminary attempts to derive quantitative structure–activity relationships (QSAR) of the Hansch type[7] for the data presented in Table II, as we have reported for a related series of inhibitors of a DNA polymerase,[8] were consistent with the qualitative results described above. Given the limited number of compounds tested, we were unable to generate equations with good enough correlation coefficients to quantify our observations. The results do confirm that the contribution of meta substituents with positive π values was most important to binding. In addition, the electronic parameter σ_m contributed significantly to binding, an observation reflected in the highest potency against both enzymes by compounds **2** and **3**, each bearing a hydrophobic, electron-attracting meta substituent. It will be necessary to test more analogues to provide sufficient data for quantifying inhibition of the thymidine kinases by this series of compounds.

The pattern of potencies of various inhibitor bases and their deoxyribonucleosides toward the two kinases suggests structural differences between the enzymes. In no case, however, have we observed greater sensitivity of the HSV1 enzyme relative to the HSV2 enzyme. Further manipulations of the most potent type 1 TK inhibitors are being done to determine if analogues with this property may be found.

As modified *bases*, these inhibitors cannot be phosphorylated by the viral TKs, and, like the prototype nucleoside PhdG, they can interact with the active sites of the viral TKs to competitively inhibit the formation of thymidine 5'-monophosphate. Thus, N^2-phenylguanines and related compounds may have potential as selective new antiherpes agents under conditions where TK expression is required for virus replication. Indeed, PhdG has been shown to inhibit TdR phosphorylation by HSV1 TK in cultured cells at a concentration that had minimal cytotoxicity.[1] Tests to determine if selected HSV TK inhibitors can suppress activation of latent herpesviruses in cell cultures are in progress.

Experimental Section

All new compounds were fully characterized by ^1H NMR and elemental analyses (C, H, N); analyses were done by the Microanalysis Laboratory, University of Massachusetts, Amherst, MA, and agree to ±0.4% of calculated values, except where noted in Table I. NMR spectra were obtained at 60 MHz with a Perkin-Elmer R-12B/Nicolet TT7 Fourier-transform spectrometer; chemical shifts are reported in ppm (δ) from internal tetramethylsilane. Melting points were determined on a Mel-Temp apparatus and are uncorrected. Compound **21** was prepared as reported,[1] and the synthesis of compound **22** will be published elsewhere (Talanian, Dresler, and Wright, manuscript to be submitted).

N^2-Substituted Guanines. Compounds **1–20** and **23–28** were obtained by reaction of 2-bromohypoxanthine and the appropriate amine as previously described.[1,3] The yields and properties of new compounds are presented in Table I.

***N^2-n*-Hexyl-2'-deoxyguanosine (29).** 2-Bromo-2'-deoxyinosine (132 mg, 0.4 mmol) was suspended in ethanol (4 mL) and treated with *n*-hexylamine (1.21 g, 12 mmol). The suspension in a glass screw-cap tube was placed in a steel bomb and heated at 120 °C for 18 h. After chilling on ice for 4 h, the solvent was evaporated, and the residue was adsorbed to silica gel and placed on top of a silica gel column (2.25 g, 70–230 mesh) prepared in chloroform. After washing with chloroform (100 mL), the product was eluted with 10% methanol in chloroform containing 0.1% triethylamine (450 mL). After removal of solvent, the residue was crystallized from aqueous ethanol and then water to give 59 mg (58%) of **29**: mp 181–183 °C; UV (pH 12.5) λ_{max} 259.5 nm (ε 11 400); ^1H NMR (Me_2SO-d_6) δ 10.3 (s, 1 H, 1-H), 7.85 (s, 1 H, 8-H), 6.33 (br s, 1 H, 2-NH), 6.14 (pseudo t, 1 H, 1'-H, J_{av} = 6.9 Hz), all other deoxyribose and alkyl resonances as expected.

Enzymes and Enzyme Assays. The source of HSV1(17syn$^+$) and HSV2(G) strains and cell lines for cell culture have been described.[1,2] The herpesvirus thymidine kinases were isolated

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(7) Hansch, C. Structure–Activity Relationships; Cavallito, C. J., Ed.; Pergamon Press: London, 1973; Vol. 1, pp 69–116.
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from virus-infected HeLa cells that were deficient in the host thymidine kinase (TK^-), and the cellular enzyme was obtained from normal TK^+ HeLa cells. The standard TK assay, outlined in detail in ref 1, measured radioactivity retained on DEAE paper disks after incubation of [^{3}H]TdR with enzyme and ATP as phosphate donor for 30 min at 37 °C. The HSV1, HSV2, and cellular TKs were assayed in the presence of TdR at 1, 2, and 3 μM, respectively. Inhibitors were dissolved in dimethyl sulfoxide and diluted into assay mixtures; control assays contained an equal amount of dimethyl sulfoxide.

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Registry No. 1, 40769-49-1; 2, 123994-67-2; 3, 123994-68-3; 4, 123994-69-4; 5, 123994-70-7; 6, 123994-71-8; 7, 123994-72-9; 8, 123994-73-0; 9, 123994-74-1; 10, 57338-66-6; 11, 83173-14-2; 12, 123994-75-2; 13, 123994-76-3; 14, 104715-65-3; 15, 123994-77-4; 16, 123994-78-5; 17, 83173-13-1; 18, 123994-79-6; 19, 123994-80-9; 20, 123994-81-0; 21, 114300-74-2; 22, 81613-41-4; 23, 114300-69-5; 24, 123994-82-1; 25, 123994-83-2; 26, 5711-37-5; 27, 123994-84-3; 28, 123994-85-4; 29, 123994-86-5; m-$ClC_6H_4NH_2$, 108-42-9; m-$CF_3C_6H_4NH_2$, 98-16-8; m-$PrC_6H_4NH_2$, 2524-81-4; m-$HOCH_2C_6H_4NH_2$, 1877-77-6; p-$BrC_6H_4NH_2$, 106-40-1; p-$EtC_6H_4NH_2$, 589-16-2; p-$CF_3C_6H_4NH_2$, 454-14-1; p-$HOC_6H_4NH_2$, 123-80-8; $BuNH_2$, 109-73-9; $H_3C(CH_2)_5NH_2$, 111-26-2; $HO(CH_2)_5NH_2$, 2508-29-4; $PhCH_2NH_2$, 100-46-9; m-$ClC_6H_4CH_2NH_2$, 4152-90-3; p-$ClC_6H_4CH_2NH_2$, 104-86-9; thymidine kinase, 9002-06-6; 2-bromohypoxanthine, 87781-93-9; 2-bromo-2′-deoxyinosine, 123994-87-6; 3,4-dibromobenzenamine, 615-55-4; 3-chloro-4-fluorobenzenamine, 367-21-5; 3-chloro-4-methylbenzenamine, 95-74-9; 2-naphthalenamine, 91-59-8; 3,4-difluorobenzenamine, 3863-11-4; 3,4-dichlorobenzenamine, 95-76-1.

Synthesis and Structure–Activity Relationships of Dynorphin A-(1–8) Amide Analogues

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In order to study the structure–activity relationships of dynorphin A-(1–8) amide [Dyn(1–8)-NH_2], 20 analogues were synthesized by the solution method. Their biological activities were determined in the three bioassays [guinea pig ileum (GPI), mouse vas deferens (MVD), and rabbit vas deferens (RVD)] and in the mouse tail-pinch test after intravenous administration. Some analogues that showed interesting activity in the bioassays and/or in the analgesic tests were further characterized in μ-, δ-, and κ-representative binding assays. The obtained data indicate that modification of the enkephalin segment to give metabolically stable analogues with high affinity and selectivity for the κ receptor is strictly limited and that introduction of MeArg in position 7 protects the Arg^6–Arg^7 bond from enzymatic degradation without potency drop and change of opioid receptor selectivity. [$MeTyr^1$,$MeArg^7$,D-Leu^8]Dyn(1–8)-NHEt (18) [IC_{50} (nM) = 0.3 (GPI), 7.4 (MVD), and 2.6 (RVD); tail pinch ED_{50} (mg/kg) = 0.75] showed opioid activity similar to that of dynorphin A in the three bioassays and relatively high κ-receptor selectivity in the binding assays and produced a 2.5-fold more potent analgesic effect than morphine. [D-Cys^2-Cys^5,$MeArg^7$,D-Leu^8]Dyn(1–8)-NHEt (20) showed a 40–60-fold more potent opioid activity than 18 in the three bioassays and produced a 3.4-fold more potent analgesic effect than 18. In the binding assays, however, 20 showed higher affinity for μ and δ receptors than for the κ receptor.

Dynorphin A (Dyn) isolated either from porcine pituitary[1,2] or from porcine duodenum[3] is a 17 amino acid opioid peptide containing the sequence of [Leu]enkephalin at its N-terminal. In the guinea pig ileum longitudinal muscle preparation both Dyn and its fragment, Dyn(1–13), are about 700-fold more potent than [Leu]enkephalin and are relatively insensitive to naloxone inhibition.[1,2] It has been postulated that Dyn is the endogenous ligand for the κ opioid receptor.[4,5]

Recently, we demonstrated that Dyn(1–8) still shows opioid activity similar to that of Dyn in the presence of peptidase inhibitors and that a metabolically stable analogue of Dyn(1–8), [$MeTyr^1$,$MeArg^7$,D-Leu^8]Dyn(1–8)-NHEt (18), not only retains opioid receptor selectivity similar to that of Dyn but also produces a more potent analgesic effect than morphine even when administered subcutaneously into mice.[6,7] In the present study we have focussed our attention on the synthesis and study of structure–activity relationships of the Dyn(1–8)-NH_2 analogues. Although there have been several reports on modification and structure–activity relationships of Dyn fragments,[8-18,26] no previous systematic study to find

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