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Synthesis and Pharmacological Characterization of Novel, Potent and Low Clearance GLP-2 Analogues

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Introduction

GLP-2, **1**, is a 33 amino acid peptide released from intestinal L-cells following food ingestion and acts at G protein coupled GLP-2 receptors in the small intestine and colon to promote intestinal growth and increase nutrient absorption. Native hGLP-2 has a high systemic clearance (CL) due in part to proteolytic cleavage of its N-terminus by dipeptidyl peptidase IV (DPP4), limiting its potential clinical use. A DPP4 resistant analogue, teduglutide, [Gly^2]hGLP-2 (**2**), displays similar intestinotrophic properties with an improved pharmacokinetic profile [1]. **2** is in clinical trials in patients with short bowel syndrome [2] and Crohn's disease [3]. Two other analogues with C-terminal hexalysine extensions, ZP1846 and ZP1848 are also in clinical trials for the treatment of chemotherapy-induced diarrhea and for the treatment of Crohn's disease, respectively [4].

In search of GLP-2 agonists pharmacologically superior to compounds currently in clinical development, we synthesized and biologically evaluated (*in vitro* receptor potency and selectivity, *in vivo* rat pharmacokinetics), a series of analogues based on [Gly^2]hGLP-2 (1-30) peptide amides where the Met^{10} residue was replaced by the more stable isosteric norleucine. Based on our internal data and literature [5], positions 11 and 16 were selected for modifications. The most promising modifications were then incorporated in full length 1-33 peptides. Here we report on the discovery of potent, low-clearance and clinically relevant GLP-2 analogues.

Results and Discussion

Based on our preliminary C-terminal truncation study (results not shown here) the 1-30 peptide amide was selected for initial SAR studies. To prevent side reactions associated with aspartimide formation [5] due to the presence of the Asp^3-Gly^4 motif, peptides were synthesized by Fmoc SPPS up to position 5 and coupling the protected 1-4 fragment prepared separately on trityl resin. The introduction of single hydrophobic residues in positions 11 or 16 resulted in analogues nearly as potent *in vitro* as the natural hormone, **1**. Compounds with D-aromatic amino acids in position 11 (**3-5**) or aromatic/aliphatic L-amino acids in position 16 (**6-9**) were the most potent in the series. When combined, these modifications resulted in compounds equipotent in vitro with **1** (i.e. **14**). Some analogues modified in position 11 (e.g. **3**, **4**) showed decreased selectivity vs. hGLP-1 receptor. The selectivity was considerably improved when the L-amino acid residues in this position were replaced with their D-enantiomers (**11**, **12**, respectively). The introduction of aromatic D-amino acid residues in position 11 yielded compounds with greatly improved pharmacokinetic profiles in rat as illustrated by their low systemic clearance (CL) values after iv administration (e.g. the D-3-Cpa^{11}, compound **4**). Combination of hydrophobic modifications in positions 11 and 16 led to compounds **13-15** with

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34
hGLP-2, 1	H	A	D	G	S	F	S	D	E	M	N	T	I	L	D	N	L	A	A	R	D	F	I	N	W	L	I	Q	T	K	I	T	D	OH
teduglutide, 2	H	G	D	G	S	F	S	D	E	M	N	T	I	L	D	N	L	A	A	R	D	F	I	N	W	L	I	Q	T	K	I	T	D	OH
Compounds 3-15	H	G	D	G	S	F	S	D	E	Nle	Xaa	T	I	L	D	Yaa	L	A	A	R	D	F	I	N	W	L	I	Q	T	K	NH_2			
Compounds 16-19	H	G	D	G	S	F	S	D	E	Nle	Xaa	T	I	L	D	Yaa	L	A	A	R	D	F	I	N	W	L	I	Q	T	K	I	T	D	R

Fig. 1. Sequences of GLP-2 analogues synthesized in this study.

Table 1. Pharmacological profile of GLP-2 analogues

Analogue	Structure[a]		In vitro profile[b]			Rat PK
	Xaa^{11}	Yaa^{16}	hGLP-2 EC_{50} (nM)	hGLP-1 EC_{50} (nM)	Selectivity	CL (ml/kg/min)
1	Asn	Asn	0.07	>1000[c]	>14000	25
2	Asn	Asn	0.09	520	5700	9.9
3	D-Phe	Asn	0.09	120[d]	1300	3.3
4	D-Cpa	Asn	0.09	60	660	0.51
5	D-Thi	Asn	0.10	80[d]	800	1.1
6	Asn	Leu	0.10	>1000[c]	>10000	0.84
7	Asn	Cha	0.10	>1000[c]	>10000	0.41
8	Asn	Tyr	0.11	>1000[c]	>9000	1.2
9	Asn	Phe	0.14	>1000[c]	>7100	NT[e]
10	Phe	Asn	0.15	16	100	NT[e]
11	Cpa	Asn	0.16	8.9	55	NT[e]
12	D-3-Cpa	Asn	0.11	45	400	0.32
13	D-Phe	Phe	0.09	>1000[c]	>11000	0.30
14	D-Phe	Tyr	0.07	90[d]	120	0.48
15	D-Phe	Leu	0.08	>1000[c]	>11000	0.30
16	D-Phe	Leu	0.03	>1000[c]	>33000	0.27
17	D-Phe	Leu	0.03	>1000[c]	>33000	0.22
18	D-Phe	Phe	0.06	>1000[c]	>16000	0.24
19	D-Phe	Phe	0.06	>1000[c]	>16000	0.15

*[a] **1** has Ala and **2-19** have Gly in pos. 2. **1, 2** have Met and **3-19** have Nle in pos. 10. R is OH for **1, 2, 17** and **19** and NH_2 for all other compounds; [b] cell based functional assays of receptor activation; [c] No agonism up to the highest concentration tested, 1000 nM; [d] Partial agonist; [e] Not tested*

further reduced CL values in rat. The full length peptides **16-19** were equipotent or more potent *in vitro* than the parent hormone (analogues **16**, **17** were 2-fold more potent than **1**). CL values were additionally decreased in peptides **16-19** as compared to shortened analogues **13-15**. The C-terminal acid peptides **17** and **19** had pharmacological profiles similar to their corresponding primary amide compounds **16** and **18**.

A series of potent and selective GLP-2 analogues modified in position 11 and/or 16 with pharmacokinetic characteristics superior to that of native hormone and/or teduglutide have been discovered. A member of this series, compound **16** (FE 203799), is a potent, selective and low CL analogue that has been selected for clinical development as a potential treatment of gastrointestinal diseases and disorders. More comprehensive accounts on the pharmacological profile of FE 203799 and related compounds will be presented elsewhere.

References

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