Acta Pharmacologica Sinica (2010) 31: 1026–1030
 1671-4083/10 $32.00
www.nature.com/aps

Perspective

Non-peptidic glucose-like peptide-1 receptor agonists: aftermath of a serendipitous discovery

Ming-wei WANG*, Qing LIU, Cai-hong ZHOU

The National Center for Drug Screening and the State Key Laboratory of New Drug Research, Shanghai Institute of Materia Medica, Chinese Academy of Sciences, Shanghai 201203, China

Glucagon-like peptide-1 (GLP-1) receptor is an ideal target in the development of incretin-based therapies for diabetes and obesity. Two approaches have been adopted: GLP-1 receptor agonists that mimic the effects of native GLP-1 and dipeptidyl peptidase-4 inhibitors that increase endogenous GLP-1 levels. During the past two decades, search for orally active, non-peptidic GLP-1 receptor agonists has been the focal point of research and development activities in many multinational pharmaceutical companies. Such efforts have not resulted in any success thus far. Serendipitous discovery of substituted cyclobutanes represented by Boc5 as a new class of GLP-1 receptor agonists led us to believe that a small molecule approach to class B G-protein coupled receptor agonism is no longer a fantasy but a reality. However, major obstacles still pose great challenges, and the reasons of which are discussed in this perspectives.

Keywords: type 2 diabetes mellitus; glucagon-like peptide-1; G-protein coupled receptor; agonist

Acta Pharmacologica Sinica (2010) 31: 1026–1030; doi: 10.1038/aps.2010.107; published online 2 Aug 2010

Type 2 diabetes mellitus (T2DM) is a progressive, complex metabolic disorder mainly characterized by insulin resistance and hyperglycemia[1]. The prevalence of diabetes, of which T2DM accounts for more than 90% of patients, is rising at an alarming rate in certain parts of the world[2]. Although the exact incidence in China is arguable, the age-adjusted prevalence of diabetes and pre–diabetes was reported to be 9.7% and 15.5%, respectively[3, 4]. T2DM has thus become a global heath concern due to its complications, such as cardiovascular conditions that result in increased morbidity and mortality. While the currently available anti-diabetic agents are effective in lowering glucose, some of them, including insulin, sulfonylureas and thiazolidinediones, are often limited by weight gain and/or hypoglycemia. However, recent emergence of incretin-based therapies, exemplified by glucagon-like peptide-1 (GLP-1) mimetics and dipeptidyl peptidase-4 (DPP-4) inhibitors, will drastically transform the landscape of diabetes care[5]. GLP-1 maintains glucose homeostasis through several mechanisms that include, but not limited to, stimulating insulin secretion, suppressing glucagon production, improving β-cell mass and function, inhibiting food intake and slowing gastric emptying[6]. Therefore, GLP-1 based incretin therapy is designed to target the fundamental defects of T2DM, capable of reducing both glycosylated hemoglobin (HbA1c) and body weight, and has potential benefits on blood pressure, lipids, and other surrogate markers, leading to decreased cardiovascular risk[7, 8].

The native GLP-1 has a very short half-life (<2 min) mainly because of the degradation by DPP-4 and neutral endopeptidase (NEP)[9]. Two approaches have been employed: GLP-1 receptor agonists that mimic the effects of native GLP-1 and DPP-4 inhibitors that increase endogenous GLP-1 levels[7]. The former is represented by two peptidic mimetics, Exenatide[10, 11] and Liraglutide[12], approved by the US Food and Drug Administration (FDA) for the treatment of T2DM. Other peptides that are under development include long-acting human GLP-1 analog CJC-1131[13], GLP-1 analog-Fc fusion protein LY2189265[14] and pegylated GLP-1 analog LY2428757[15], recombinant human serum albumin exendin–4 conjugated protein CJC-1134-PC[16], Taspoglutide[17], as well as AVE0010[18].

Unlike peptidic mimetics that require injections, DPP-4 inhibitors (such as Sitagliptin, Vildagliptin and Saxagliptin)[19–21] are small in molecular nature, orally active, and transported and stored at ease. Administration of DPP-4 inhibitor has led to improved plasma concentrations of endogenous GLP-1 and marked reduction of HbA1c, but its effect on body weight has been neutral[21]. Thus, oral formulation of

* To whom correspondence should be addressed.
E-mail wangmw@mail.shcnc.ac.cn
Received 2010-06-01 Accepted 2010-07-01

GLP-1 analogs remains a development option[22].

It is conceivable that an orally active, non-peptidic GLP-1 receptor agonist could resolve the issues mentioned above. Indeed, this has been the focal point of drug discovery efforts in many multinational pharmaceutical companies. Such efforts have not resulted in any success thus far.

The GLP-1 receptor belongs to the glucagon-secretin B family of the G protein-coupled receptors (GPCRs). A characteristic structural feature of this family is a long and structurally complex extracellular amino-terminal domain (N-domain) containing six conserved cysteine residues that form disulfide bonds important for stabilizing the folded protein. The N-domain is connected to a juxtamembrane domain (J-domain) of the seven membrane-spanning α-helices with intervening loops and a C-terminal tail[23]. From a biological perspective, class B GPCRs are highly attractive therapeutic targets, but the search for non-peptidic modulators (agonists in particular) has been met with great difficulties. Very few 'druggable' small molecule ligands have been identified for this class of GPCRs. With reference to GLP-1 receptors, only a very small number of non-peptidic agonists have been reported over the years as summarized below in Table 1.

Of the compounds listed, diallylmethylamine derivative is more like a DPP-4 inhibitor than a GLP-1 receptor agonist, based on the limited information available in the public domain[29]. The remaining eight molecules possess fairly diverse structural features. Despite what were described by the inventors for these compounds in terms of orthosteric, allosteric, ago-allosteric, inverse, or partial/full agonists, their functional varieties are manifested in the following manners: (1) inducing both biochemical and cellular responses without *in vivo* activities[24, 27]; (2) behaving like an antagonist in cell-based assays (*eg*, T0632)[27]; (3) promoting native ligand binding to the receptor *in vitro*[32]; (4) demonstrating a full range of GLP-1 properties including *in vivo* efficacy[25, 26]; and (5) enhancing GLP-1 activities via binding to the receptor[33]. Since no distinct commonality could be found in the structures of these five types of compounds, their binding to the GLP-1 receptor must be realized via different sites. Among them, Boc5 is the only one that demonstrated therapeutic benefits *in vivo*[25,26]. While peptidic GLP-1 mimetics displayed some side effects such as nausea and vomiting in the clinic[9], both normal and diabetic *db/db* mice are highly tolerable to Boc5[25] such that a similar reaction on the conditioned taste aversion required a dose well beyond the therapeutic window[26]. Long-term toxicity (≥3 month) has yet to be determined. It appears that Boc5 is neither metabolized by nor interacts with the cytochrome P450 *in vivo* exhibiting a half-life ranging from 12.1 to 35.4 h in mice and rats, respectively when injected intraperitoneally. However, its oral bioavailability is extremely poor due to metabolism by esterase in the gut (unpublished data).

The current binding model of GLP-1 receptor is a two-step mechanism where initially the C-terminal part of the peptide ligand interacts with the N-domain of the receptor, thereby conferring high affinity. In the second step, the N-terminal part of the ligand interacts with the core domain of the receptor (transmembrane helices and connecting loops), leading to activation and signal transduction[23]. Both the N-domain and J-domain of the receptor are needed for this interaction, and the former is also critical for ligand selectivity between glucagon and GLP-1. Molecular elucidation of such an interaction is nearly impossible due to the inherent complexity of GPCR structures. However, attempts were made using both the crystal structure of human GLP-1 receptor N-terminal extracellular domain[34] and computational simulation[35]. These studies have implicated that activation of GLP-1 receptor by an agonist is related to some intrinsic conformation changes. There may be two major states of the GLP-1 receptor structure: (1) the inactive state, in which the orthosteric agonist-binding site is partially blocked as the consequence of the relative motions between the N-domain and the transmembrane domain; and (2) the active state, in which the orthosteric agonist-binding site is fully accessible[35]. Binding of an agonist makes a GLP-1 receptor stay in the latter state, as in the case of Boc5[25], while interaction with an inverse agonist such as T0632 favors the former state[23, 27]. Compound 2 (quinoxaline derivative), which binds the GLP-1 receptor at an allosteric site, rigidifies its structure with an open binding site to improve the activity of a full agonist[35].

Obviously, the exact requirements needed for a small molecule to fully mimic GLP-1 have yet to be understood. Compounds that confer the conventional wisdom[36] such as 'Rule of 5' do not display any *in vivo* bioactivities, while molecules that demonstrate therapeutic benefits in animal models of T2DM and obesity such as Boc5 are considered not druggable because of poor oral bioavailability. Serendipitous discovery of substituted cyclobutanes represented by Boc5 as a new class of GLP-1 receptor agonists led us to believe that a small molecule approach to class B GPCR agonism is no longer a fantasy but a reality[37].

Nonetheless, major obstacles still pose great challenges in terms of developing an orally active non-peptidic GLP-1 receptor agonist. To what extent should such a molecule look like from a medicinal chemistry point of view? Can it possess a suitable molecular size that meets the requisite ADME/T profile while maintaining a full range of incretin-like efficacy? Can it be produced economically, so that it can sufficiently compete against its peptidic counterparts? These are the questions that must be addressed in order to sustain the vitality of a research program in this particular area.

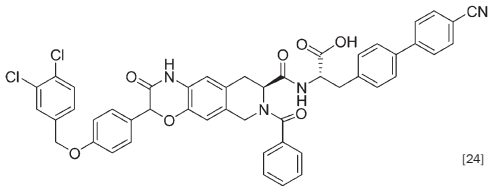
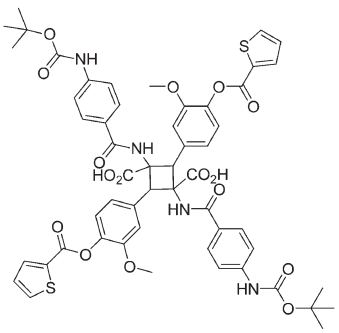
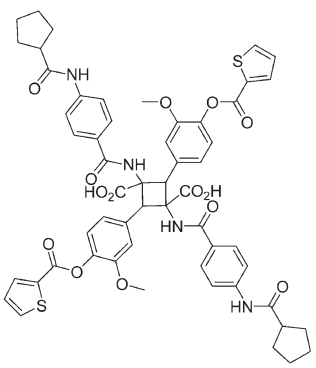
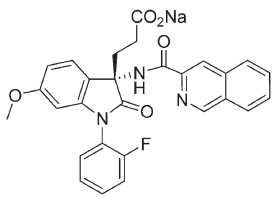
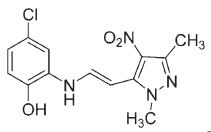
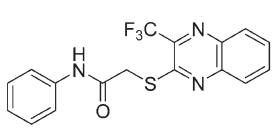
Acknowledgements

Project was supported in part by the Ministry of Science and Technology (2009ZX09302-001), Chinese Academy of Sciences (KSCX2-YW-R17 and KSCX1-02-2), Shanghai Municipality Science and Technology Development Fund (No 074319114, 08ZR1403600, 08DZ2291300, and 09DZ2291200).

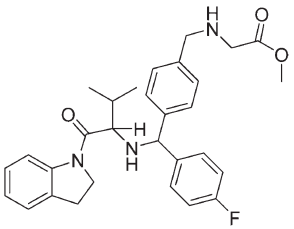
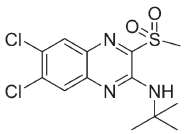
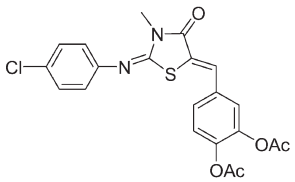
References

1 Rodbard HW, Blonde L, Braithwaite SS, Brett EM, Cobin RH, Handelsman Y, *et al*. American Association of Clinical Endocrinologists medical guidelines for clinical practice for the management of

Table 1. The structures of small molecule GLP-1 receptor agonists and their claimed biological activities *in vitro* and *in vivo*.

Structure	Receptor binding	Cellular effect	Animal study
 [24]	Exhibited 68% binding activity *vs* [^{125}I]GLP-1	EC_{50}: 145 nmol/L Cell activation: 99.6%	Not reported
 Boc5, full agonist[25]	K_i: 1.47 μmol/L	EC_{50}: 2.73 μmol/L Caused the release of insulin from normal rat islets[22]	Diabetic *db/db* mice: reduction in food intake, body weight, gastric emptying, blood glucose, HbA1c, insulin resistance, *etc*[25, 26]
 S4P, partial agonist[25]	K_i: 287 nmol/L	EC_{50}: 1.08 μmol/L At concentrations up to 10 μmol/L evoked maximally only 37% of the GLP-1 response	Diabetic *db/db* mice: HbA1c was robustly lowered[25]
 T0632, inverse agonist[27]	IC_{50}: 0.62 μmol/L (wild type receptor) (C347-QQRY mutant receptor)	Decreased the basal GLP-1R activity during a short incubation while increased the surface distribution of the C347-QQRY GLP-1R mutant after a long incubation; right-shifted the dose-response curve of GLP-1[28]	Not reported
 Compound A[27]	IC_{50}: 5.94 μmol/L (wild type receptor) IC_{50}: 5.10 μmol/L (mutant receptor)	Increased cAMP production	Not reported
 Compound B[27]	IC_{50}: 25 μmol/L (wild type receptor) IC_{50}: 20 μmol/L (mutant receptor)	Increased cAMP production	Not reported

(Continued)

Structure	Receptor binding	Cellular effect	Animal study
 [29]	Not reported	Not reported	Enhanced blood concentrations of GLP-1 30 min after dosing
 Ago-allosteric modulator[30–32]	Enhanced GLP-1 binding EC_{50}: 0.032 μmol/L)[32]	EC_{50}: 0.16 μmol/L E_{max} (%): 85% Caused the release of insulin from normal mouse islets and a perfused rat pancreas[32]	Not reported
 6Cu[33]	IC_{50}: 3.52 nmol/L Exhibited 36% binding *vs* [^{125}I] GLP-1	EC_{50}: 0.40 nmol/L (in the presence of GLP-1)	Dose-dependently inhibited acute food intake in normal mice (ED_{50}: 0.90 mg)

diabetes mellitus. Endocr Pract 2007; 13 Suppl 1: 1–68.
2 Wild S, Roglic G, Green A, Sicree R, King H. Global prevalence of diabetes: estimates for the year 2000 and projections for 2030. Diabetes Care 2004; 27: 1047–53.
3 Yang W, Lu J, Weng J, Jia W, Ji L, Xiao J, *et al*. Prevalence of diabetes among men and women in China. N Engl J Med 2010; 362: 1090–101.
4 Shi Z. Prevalence of diabetes among men and women in China. N Engl J Med 2010; 362: 2425; author reply 2426.
5 Unger J, Parkin CG. Type 2 diabetes: an expanded view of pathophysiology and therapy. Postgrad Med 2010; 122: 145–57.
6 Neumiller JJ. Differential chemistry (structure), mechanism of action, and pharmacology of GLP-1 receptor agonists and DPP-4 inhibitors. J Am Pharm Assoc (2003) 2009; 49 Suppl 1: S16–29.
7 Chia CW, Egan JM. Role and development of GLP-1 receptor agonists in the management of diabetes. Diabetes Metab Syndr Obes 2009; 2: 37.
8 Wysham CH. New perspectives in type 2 diabetes, cardiovascular risk, and treatment goals. Postgrad Med 2010; 122: 52–60.
9 Kieffer TJ, McIntosh CH, Pederson RA. Degradation of glucose-dependent insulinotropic polypeptide and truncated glucagon-like peptide 1 *in vitro* and *in vivo* by dipeptidyl peptidase IV. Endocrinology 1995; 136: 3585–96.
10 Amori RE, Lau J, Pittas AG. Efficacy and safety of incretin therapy in type 2 diabetes: systematic review and meta-analysis. JAMA 2007; 298: 194–206.
11 van Genugten RE, van Raalte DH, Diamant M. Does glucagon-like peptide-1 receptor agonist therapy add value in the treatment of type 2 diabetes? Focus on exenatide. Diabetes Res Clin Pract 2009; 86 Suppl 1: S26–34.
12 Liraglutide (Victoza) for type 2 diabetes. Med Lett Drugs Ther 2010; 52: 25–7.
13 Tiessen RG, Castaigne JP, Dreyfus JF, Nemansky M, Kruizinga HH, van Vliet AA. Pharmacokinetics and tolerability of a novel long-acting glucagon-like peptide-1 analog, CJC–1131, in healthy and diabetic subjects. Int J Clin Pharmacol Ther 2008; 46: 443–52.
14 A study of dose titration of LY2189265 in overweight patients with type 2 diabetes meellitus (EGO). http://clinicaltrials.gov/ct2/show/NCT00630825.
15 A study for patients with type 2 diabetes mellitus. http://clinicaltrials.gov/ct2/show/NCT00804986.
16 Baggio LL, Huang Q, Cao X, Drucker DJ. An albumin-exendin-4 conjugate engages central and peripheral circuits regulating murine energy and glucose homeostasis. Gastroenterology 2008; 134: 1137–47.
17 Roche moves investigational diabetes drug, Taspoglutide, into Phase III clinical trials. http://www.roche.com/med-cor-2008-06-10-e.pdf.
18 New diabetes compound AVE0010 showed clear dose response results with once — a day injection in phase IIb study. http://en.sanofi–aventis.com/press/press_releases/2008/ppc_19592.asp
19 New drug application for Galvus®, an innovative oral therapy for people with type 2 diabetes, accepted for review by FDA. http://www.glucagon.com/pdfs/VildagliptinUSNDAFilingpress%20release.pdf.
20 FDA approves new drug treatment for type 2 diabetes. http://www.fda.gov/NewsEvents/Newsroom/PressAnnouncements/ucm174780.htm.
21 Sitagliptin and Vildagliptin. http://www.npci.org.uk/therapeutics/cardio/diabetes2/resources/library_oth_future_medicines_sitagliptin_vildagliptin.pdf
22 Merrion announces license agreement with Novo Nordisk to develop oral formulation of GLP-1 receptor agonist(s). http://www.merrionpharma.com/archive/Merrion_NN_GLP–1.pdf
23 Hoare SR. Mechanisms of peptide and nonpeptide ligand binding to class B G-protein-coupled receptors. Drug Discov Today 2005; 10:

417–27.

24 Mjalli AM, Polisetti DR, Yokum TS, Kalpathy S, Guzei M, Behme C, *et al*. Oxadiazoanthracene compounds for the treatments of diabetes. WO Patent 2009111700. 2009; March 6.

25 Chen D, Liao J, Li N, Zhou C, Liu Q, Wang G, *et al*. A nonpeptidic agonist of glucagon-like peptide 1 receptors with efficacy in diabetic db/db mice. Proc Natl Acad Sci USA 2007; 104: 943–48.

26 Su H, He M, Li H, Liu Q, Wang J, Wang Y, et al. Boc5, a non-peptidic glucagon-like peptide-1 receptor agonist, invokes sustained glycemic control and weight loss in diabetic mice. PLoS One 2008; 3: e2892.

27 Kopin AS, Beinborn M. Methods and compositions for the treatment of metabolic disorders. WO Patent 2004103310. 2004; May 20.

28 Tibaduiza EC, Chen C, Beinborn M. A small molecule ligand of the glucagon-like peptide 1 receptor targets its amino–terminal hormone binding domain. J Biol Chem 2001; 276: 37787–93.

29 Nishimura T, Nagase T, Eiki J. Novel diallylmethylamine derivative. WO Patent 02067918. 2002; February 27.

30 Teng M, Truesdale LK, Bhumralkar D, Kiel D, Johnson MD, Thomas C, *et al*. Non-peptide GLP-1 agonists. WO Patent 0042026. 2000; January 14.

31 Knudsen LB, Kiel D, Teng M, Behrens C, Bhumralkar D, Kodra JT, *et al*. Small-molecule agonists for the glucagons-like peptide 1 receptor. Proc Natl Acad Sci USA 2007; 104: 937–42.

32 Teng M, Johnson MD, Thomas C, Kiel D, Lakis JN, Kercher T, *et al*. Small molecule ago-allosteric modulators of the human glucagons-like peptide-1 (hGLP-1R) receptor. Bioorg Med Chem Lett 2007; 17: 5472–8.

33 Wang MW, Yuan YY, Zhou L, Young AA, Zhang A, Gao LD, *et al*. A kind of receptor signaling transduction positive modulators, preparation methods and uses thereof. WO Patent 2009129696. 2009; April 27.

34 Runge S, Thogersen H, Madsen K, Lau J, Rudolph R. Crystal structure of the ligand-bound glucagon-like peptide-1 receptor extracellular domain. J Biol Chem 2008; 283: 11340–7.

35 Lin F, Wang R. Molecular modeling of the three-dimensional structure of GLP-1R and its interactions with several agonists. J Mol Model 2009; 15: 53–65.

36 Lipinski CA, Lombardo F, Dominy BW, Feeney PJ. Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. Adv Drug Deliv Rev 2001; 46: 3–26.

37 Murphy KG, Bloom SR. Nonpeptidic glucagon-like peptide 1 receptor agonists: a magic bullet for diabetes? Proc Natl Acad Sci USA 2007; 104: 689–90.