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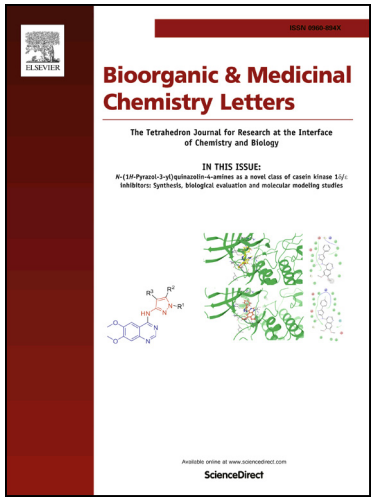

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Synthesis and *in vitro* biological evaluation of new pyrimidines as glucagon-like peptide-1 receptor agonists

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ABSTRACT: The therapeutic success of peptide glucagon-like peptide-1 (GLP-1) receptor agonists for the treatment of type 2 diabetes mellitus has inspired discovery efforts aimed at developing orally available small-molecule GLP-1 receptor agonists. In this study, two series of new pyrimidine derivatives were designed and synthesized using an efficient route, and were evaluated in terms of GLP-1 receptor agonist activity. In the first series, novel pyrimidines substituted at positions 2 and 4 with groups varying in size and electronic properties were synthesized in a good yield (78-90%). In the second series, the designed pyrimidine templates included both urea and Schiff base linkers, and these compounds were successfully produced with yields of 77-84%. *In vitro* experiments with cultured cells showed that compounds **3a** and **10a** (10^{-15} to 10^{-9} M) significantly increased insulin secretion compared to that of the control cells in both the absence and presence of 2.8 mM glucose; compound **8b** only demonstrated significance in the absence of glucose. These findings represent a valuable starting point for the design and discovery of small-molecule GLP-1 receptor agonists that can be administered orally.

Keywords: type 2 diabetes, GLP-1, pyrimidine analog, insulin secretion

The cellular uptake of blood glucose due to the action of insulin secreted from the endocrine pancreas is facilitated by gut hormones. The development of the incretin concept was based on the observation that enteral nutrition provided a more potent insulinotropic stimulus than an intravenous challenge did. [1] Glucose-dependent insulinotropic polypeptide (GIP) was the first incretin to be discovered. [2] GIP is a 42-amino acid hormone synthesized in duodenal and jejunal enteroendocrine K cells in the proximal small bowel. A second incretin hormone, glucagon-like peptide-1 (GLP-1), was identified later from proglucagon. GLP-1 is a 30-residue peptide hormone released from intestinal L cells following nutrient consumption. It potentiates the glucose-induced secretion of insulin from pancreatic β cells, increases insulin expression, inhibits beta-cell apoptosis, promotes beta-cell neogenesis, reduces glucagon secretion, delays gastric emptying, promotes satiety, and increases peripheral glucose disposal. These multiple effects have generated a great deal of interest in the discovery of long-lasting agonists of the GLP-1 receptor (GLP-1R) in order to treat type 2 diabetes. Both GIP and GLP-1 exert their actions by binding to distinct G-protein-coupled receptors (GPCRs). These receptors are members of the class B/II family of seven transmembrane GPCRs. [3] Activation of both incretin receptors on β cells leads to rapid increases in levels of cAMP and intracellular calcium, followed by insulin exocytosis, which occurs in a glucose-dependent manner. [4]

Whereas in healthy humans oral glucose elicits a considerably higher insulin secretory response than does intravenous glucose, this incretin effect is substantially reduced or absent in patients with type 2 diabetes. [5] In type 2 diabetes mellitus, the homeostasis of postprandial glucose is impaired by the dysregulation of insulin secretion and high glucagon levels. In addition, impairment of incretin results in reduced secretion of GLP-1 and subsequent progression of pancreatic islet dysfunction. [6]

Therefore, the development of incretin-based therapeutics may be an effective strategy to restore normal islet function in type 2 diabetes. Since GLP-1 activity remains relatively preserved in diabetes patients, most pharmaceutical efforts that are directed at potentiating the action of incretin to treat type 2 diabetes have focused on GLP-1R agonists. Recent reports have described activation of the GLP-1R by substituted quinoxalines [4-5, 7] and a cyclobutane derivative [8], suggesting the possibility of developing nonpeptide GLP-1R agonists.

Abbreviations
GLP-1, glucagon-like peptide-1; GLP-1R, glucagon-like peptide-1 receptor; SEM, standard error of the mean; DMSO, dimethyl sulfoxide; NMR, nuclear magnetic resonance; FT-IR, Fourier transform infrared (spectroscopy); DIPEA, N,N-diisopropyl ethylamine; GPCR, G-protein-coupled receptor; GIP, glucose-dependent insulinotropic polypeptide

Considering the above, we report the development of novel, nonpeptide pyrimidine analogs as GLP-1R agonists. These novel pyrimidines were synthesized and evaluated for their antidiabetic effects in comparison to those of the potent drug, exenatide. These molecules may offer a therapeutic advantage because of their ability to act alone or in combination with GLP-1. Pyrimidines compounds are likely to interacts with an allosteric site of the GLP-1 receptor. A similar hypothesis was proposed previously for the quinoxaline series of GLP-1 receptor agonists [9] and some reported antidiabetic pyrimidine-based compounds exert their agonistic effect on the GLP-1 receptor via an allosteric mechanism [10].

Our synthesis efforts focused on the development of a new template that can be used to generate novel pyrimidines, substituted at C-2 and C-4 positions with groups varying in size, steric properties, and electronic properties (Fig. 1).

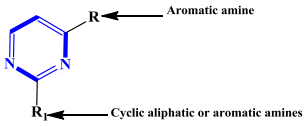
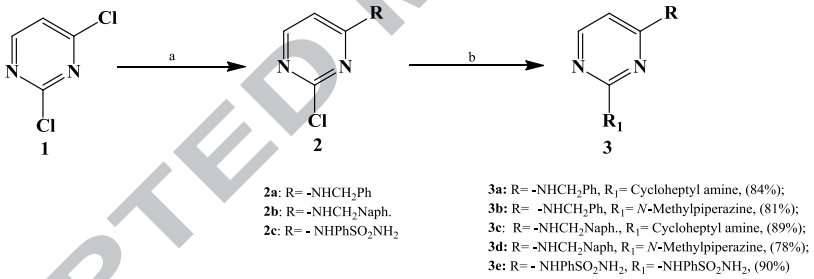


Figure 1. Template of the designed disubstituted pyrimidines **3a-e**

The synthetic routes to derivatives **3a-e** were relatively fast and efficient, requiring two steps to achieve the final products with a microwave heating protocol. In the first step, 2,4-dichloropyrimidine **1** was converted into 2-chloro-4-substituted-pyrimidine **2** in the presence of *N*,*N*-diisopropylethylamine (DIPEA) in approximately 8 min using microwave irradiation (yield: 85-93%). In toluene, the 4-arylaminopyrimidine intermediates **2** were then reacted with the second nucleophile using microwave irradiation for 10 min to produce the target products **3a-e** in relatively good yield (78-90%) (Scheme 1).



Scheme 1. The synthetic route of compounds **3a-e**. Reagents and conditions: (a) Ar-NH_2, *N*,*N*-diisopropylethylamine (DIPEA), ethanol, microwave, 100°C, 8 min; (b) Cyclic or aromatic amine, toluene, microwave, 80-100°C, 10 min.

The structures of the products **3a-e** were confirmed based on their elemental and spectroscopic properties (FT-IR, ^{1}H- and ^{13}C-NMR). The IR spectrum (KBr,υ, cm^{-1}) of compound **3a** showed a signal at υ = 3247 cm^{-1} representing (-NH). Another band appeared at υ = 3185 cm^{-1} corresponding to the C-H aromatic, while the C-H aliphatic appeared at υ = 2926 cm^{-1}. The ^{1}H-NMR (400 MHz, DMSO-d_6) spectrum of compound **3a** showed a multiplet at δ = 1.31-1.80 ppm, integrated for 12 protons and attributed to the corresponding cycloheptyl ring. The benzyl protons resonated as a doublet at δ = 4.42 ppm with coupling constant *J* = 4.7 *Hz*. Another signal appeared at δ = 5.68 ppm as a doublet, corresponding to the NH- proton exchanged with D_2O. The pyrimidine H-5 and H-6 appeared as two doublets at δ = 6.10 and 7.59 ppm, respectively, with equal coupling constants of *J* = 6.0 Hz. The ^{13}C-NMR spectrum of compound **3a** showed signals at δ = 24.4, 28.3, 34.9, 43.5, and 51.6 ppm that were designated as the cycloheptyl carbons. The pyrimidine carbons C-2, C-6, C-4, and C-5 resonated at δ = 161.6, 155.6, 162.9, and 95.3 ppm, respectively (see supplementary data).

The formation of **3e** was also confirmed using FT-IR spectroscopy, which revealed the appearance of NH_2 bands at υ = 3564 and 3357 cm^{-1}. The C=N stretching appeared at υ = 1455 cm^{-1}. Bands were assigned for –SO_2NH_2 at υ = 1207 and 1160 cm^{-1}. The ^{1}H-NMR (400 MHz, DMSO-d_6) spectrum of compound **3e** showed a broad signal at δ = 7.42 ppm, which was assigned to the NH_2 protons exchanged with D_2O. The phenyl protons appeared at δ = 7.75-7.91 ppm, while the pyrimidine H-5 and H-6 resonated as two doublets at δ = 6.71 and 8.13 ppm, respectively, with equal coupling constants of *J* = 8.0 Hz. The pyrimidine carbons C-4, C-6, C-2, and C-5

appeared at δ = 153.1, 145.3, 161.1, and 100.1 ppm, respectively. The phenyl carbons resonated at δ = 121.9 and 127.0-127.2 ppm (see supplementary data).

An unsymmetrically substituted urea is a common structural feature of many biologically active compounds, such as enzyme inhibitors and pseudopeptides. [11] Sulfonylureas were found to have applications as oral antidiabetic drugs and herbicides. [12] However, urea derivatives can also be used as glycation protectors, especially against advanced glycation end products. [13]

Moreover, a series of unsymmetrical phenylurenyl chalcone derivatives were synthesized and evaluated for *in vitro* antimalarial activity.[14] Encouraged by these facts, the present investigation involves designing new disubstituted pyrimidine templates with both urea and Schiff base linkers (Fig. 2).

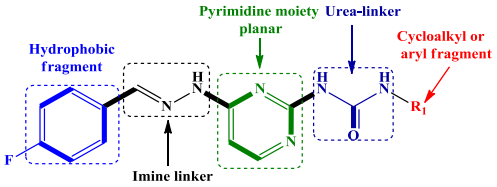
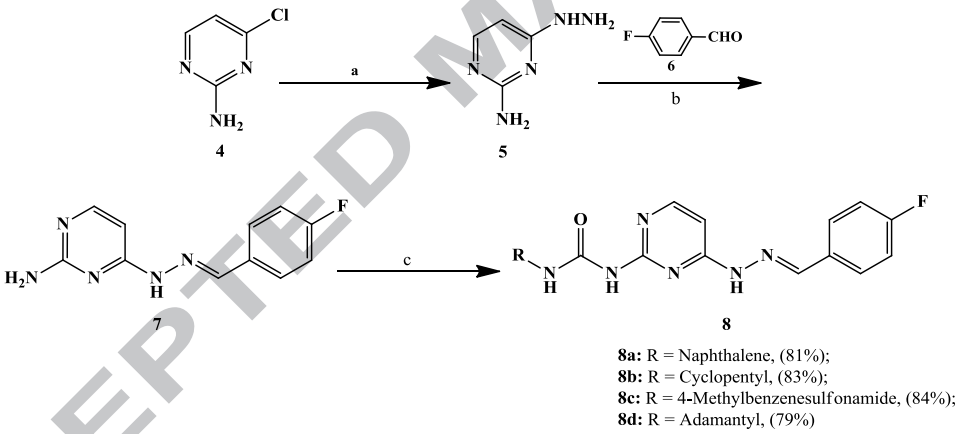


Figure 2. Disubstituted pyrimidine template for compounds **8a-d**

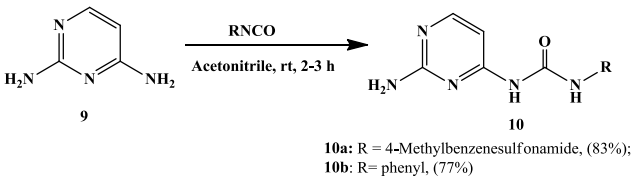
In the present study, the synthesis of disubstituted pyrimidines **8a-d** started with intermediate **5,** which was obtained by the reaction of 2-amino-4-chloropyrimidine **4** with hydrazine hydrate. The resultant hydrazopyrimidine **5** was reacted with *p*-fluoroaldehyde **6** in a Schiff condensation to form the 4-substituted 2-aminopyrimidine **7** with a 95% yield. In the final step, isocyanate was allowed to react with compound **7** at room temperature for 12 h under dry conditions to afford the target products **8a-d** in 79-84% yields (Scheme 2).



Scheme 2. The synthesis of compounds **8a-d**. Reagents and conditions: (a) $NH_2NH_2.H_2O$, ethanol, reflux, 30 min; (b) *p*-fluorobenzaldehyde, Ethanol, acetic acid, microwave, 15 min; (c) R-NCO, acetonitrile, 25°C, 12 h.

The formation of **8b** was confirmed using FT-IR spectroscopy, which revealed the appearance of the urea NH band at υ = 3442 cm^{-1}, while the Schiff-base NH- appeared at υ = 3360 cm^{-1}. The C=O stretching was observed at υ = 1682 cm^{-1}. The ^{1}H-NMR (400 MHz, DMSO-d_6) spectrum of compound **8b** showed multiplets at δ = 0.83-0.86 ppm, and δ = 1.25-1.34 ppm was assigned to the cyclopentyl ring protons. The phenyl protons resonated at δ = 7.96-7.99 ppm, while the pyrimidine H-5 and H-6 resonated as two doublets at δ = 6.24 and 7.82 ppm, respectively, with equal coupling constants (J = 4.0 Hz). The olefinic proton appeared at δ = 8.24 ppm. The NH proton of Schiff base appeared as a doublet at δ = 7.66 ppm, with a coupling constant J = 8.0 Hz exchanged with D_2O. The two NH protons of the urea linker resonated at δ = 8.35 ppm and δ = 8.58 ppm, exchanged with D_2O. The ^{13}C-NMR spectrum showed that C-4, C-6, C-2, and C-5 of the pyrimidine ring resonated at δ = 167.4, 162.6, 152.8, and 99.8 ppm, respectively, the carbonyl group resonated at δ = 145.9 ppm, and δ = 116.2-130.3 ppm represented the aromatic carbons of the fluorobenzene (see supplementary data).

Compounds **10a,b** were prepared by the reaction of 2,4-diaminopyrimidine **9** with isocyanate derivatives to form the urea linker at position 4 of the pyrimidine ring with a yield of 77-83% (Scheme 3).



Scheme 3. The synthetic route of compounds **10a,b**. Reagents and conditions: (a) R-NCO, acetonitrile, 25°C, 2-3 h.

The formation of **10b** was confirmed using FT-IR spectroscopy, which revealed the two NH_2 bands at υ = 3506 and 3370 cm^{-1}, while the NH group of the urea linker appeared at υ = 3170 cm^{-1}. The C=O stretching appeared at υ = 1700 cm^{-1}. The ^{1}H-NMR (400 MHz, DMSO-d_6) spectrum of compound **10b** showed that the pyrimidine H-5 and H-6 resonate as two doublets at δ = 6.08 and 7.91 ppm, respectively, with equal coupling constants (J = 6.0 Hz). The two NH groups in the urea linker resonated at δ = 9.48 and 11.77 ppm, exchanged with D_2O. The NH_2 group of pyrimidine C-2 appeared at δ = 7.61 ppm, exchanged with D_2O. The ^{13}C-NMR spectrum showed that pyrimidine C-4, C-6, C-2, and C-5 resonated at δ = 157.8, 155.2, 163.9, and 99.8 ppm, respectively. The carbonyl group resonated at δ = 152.4 ppm, and signals appeared at δ = 119.8, 123.2, 129.2, and 139.2 ppm that were assigned to the aromatic carbons of the phenyl substituent (see supplementary data).

Secretion of insulin by βTC6 cells was measured using the high-range insulin Sandwich ELISA kit described in the experimental section (see supplementary data). Fig. 3 shows the glucose response of the βTC6 cells in the absence of drugs. Glucose at 2.8 mM induced a mild insulin secretion of approximately 3000 pmol/L, which was used in subsequent testing of the novel pyrmidine analog compounds and the positive control, exenatide. Exenatide treatment caused a significant increase in insulin secretion compared to basal secretion from the βTC6 cells. Moreover, in the presence of 2.8 mM glucose, 10^{-12} and 10^{-5} M exenatide significantly increased insulin secretion compared to that of controls (i.e., 2.88 mM glucose alone) (Fig. 4a).

The *in vitro* effects of compound **3a** at 10^{-15}, 10^{-12}, and 10^{-9} M on insulin secretion in the absence and presence of 2.8 mM glucose are shown in Fig. 4b. In the absence of glucose, 10^{-15} and 10^{-12}, of compound **3a** significantly increased insulin secretion compared to that of the basal control. In the presence of 2.8 mM glucose, 10^{-15} M of **3a** significantly increased insulin secretion compared to that of the control (i.e., with 2.8 mM glucose alone, Fig. 4b). Regarding compound **8b**, Fig. 4c shows that, in the absence of glucose, 10^{-15} and 10^{-12} M of compound **8b** significantly increased insulin secretion compared to that of the basal control, while compound **8b** had no significant effect on insulin secretion in the presence of 2.8 mM glucose (Fig. 4c). In the absence of glucose, all tested concentrations of compound **10a** significantly increased insulin secretion compared to that of the basal control (Fig. 4d). In the presence of 2.8 mM glucose, insulin secretion with **10a** treatment increased in a concentration-dependent manner, but only 10^{-9} M compound **10a** was statistically different from the control value (i.e., with 2.8 mM glucose alone).

In this work, our compounds were evaluated as GLP-1R agonists. Compound **10a** showed similar effects as did exenatide in stimulating insulin secretion from βTC6 cells. The presence of the amino group at position 2 produced a significant increase in the magnitude of the response, suggesting that the hydrogen bond donor is preferred in this region and affects binding to the ago-allosteric site of the GLP-1R. On the other hand, replacement of the amino group at position 2 of the pyrimidine ring with other substitutes resulted in reduced antidiabetic potential. It can be deduced that the presence of any group at the 2-position of the pyrimidine could sterically hinder the interaction of the compound with its receptor.

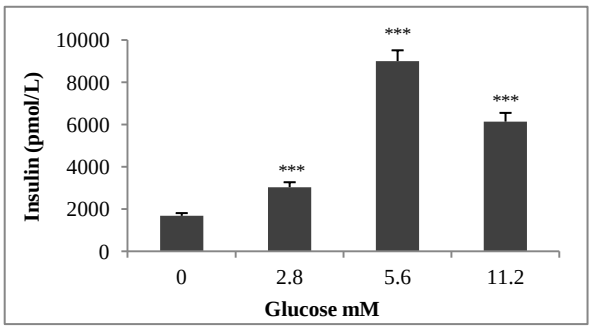


Figure 3. The glucose response of the βTC6 cells in the absence of drugs. Plotted values are means of triplicates ± SEM*; P < 0.05, ** P < 0.01, *** P < 0.001 versus 0 mM glucose.

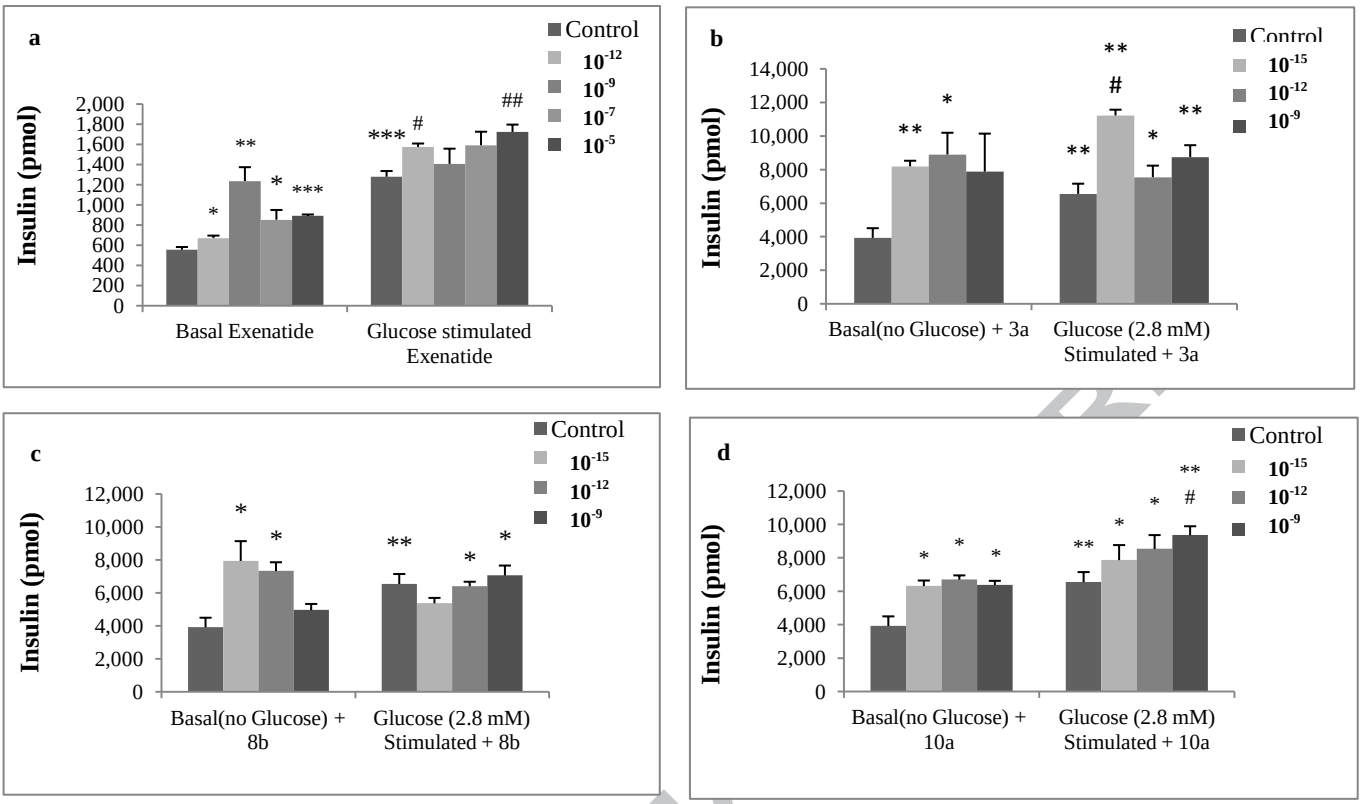


Figure 4. The effects of exenatide (10^{-12} –10^{-5} M, panel a) and test compounds **3a** (panel b)**, 8b** (panel c), and **10a** (panel d)(10^{-15} –10^{-9} M) on insulin secretion in βTC6 cells in the absence (Basal, left bars) and in the presence of 2.8 mM glucose concentration (right bars). Results are means of triplicates ± SEM; *P < 0.05, ** P < 0.01, *** P < 0.001 versus basal control, and #P < 0.05, ## P < 0.01, ### P < 0.001 versus 2.8 mM glucose alone.

In summary, our results with the pyrimidine-based GLP-1R agonists *in vitro* demonstrate that modulating glucose-dependent insulin secretion with low molecular weight compounds is technically feasible. A new series of substituted pyrimidine analogs, **3a-d**, **8a-d**, and **10a-b**, were synthesized using an economical and environmentally friendly procedure. The anti-diabetes activities of the newly synthesised compounds were evaluated in cultured cells. These results confirmed that compounds **3a,** and **10a** could significantly increase insulin secretion compared to that of the control cells in the absence and presence of 2.8 mM glucose; compound **8b** only demonstrated significance in the absence of glucose. Compound **10a** was similar to exenatide regarding insulin secretion from the βTC6 cells. Moreover, these findings provide pharmacological guidance for the discovery and characterization of small-molecule GLP-1R ligands as possible therapeutics. However, further work is needed to improve the potency and optimize the pharmacokinetic properties of these molecules to enable clinical development. Future studies will focus on characterizing the allosteric binding site and the ligand-mediated conformational changes that are induced by pyrimidine analogs to potentiate receptor signaling. A better understanding of the pocket and mechanism of activation will facilitate molecular modeling strategies to develop more potent small-molecule GLP-1R agonists.

Acknowledgements

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://1drv.ms/b/s!Aoxf511qpqQthgbZRPTfm4RqADK1

References and notes

1. Vilsbøll T, Krarup T, Deacon CF, Madsbad S, Holst JJ. Reduced postprandial concentrations of intact biologically active glucagon-like peptide 1 intype 2 diabetic patients. *Diabetes.* 2001; 50:609–613.

2. Toft-Nielsen MB, Damholt MB, Madsbad S, Hilsted LM, Hughes TE, Michelsen BK, Holst JJ. Determinants of the impaired secretion of glucagon-like peptide-1 in type 2 diabetic patients. *J Clin Endocrinol Metab.* 2001; 86:3717–3723.

3. (a) Laburthe M, Couvineau A, Gaudin P, Maoret JJ, Rouyer-Fessard C, Nicole P. Receptors for VIP, PACAP, secretin, GRF, glucagon, GLP-1, and other members of their new family of G protein-linked receptors: structure function relationship with special reference to the human VIP-1 receptor. *Ann N Y Acad Sci.* 1996; 805:94–109; (b) Fredriksson R, Lagerström MC, Lundin LG, Schioth HB. The G-protein coupled receptors in the human genome form five main families. Phylogenetic analysis, paralogon groups, and fingerprints. *Mol Pharmacol.* 2003; 63:1256–1272; (c) Lagerström MC, Schiöth HB. Structural diversity of G protein-coupled receptors and significance for drug discovery. *Nat Rev Drug Discov.* 2008; 7:339–357.

4. Knudsen LB, Kiel D, Teng M, Behrens C, Bhumralkar D, Kodra JT, Holst JJ, Jeppesen CB, Johnson MD, de Jong JC, Jorgensen AS, Kercher T, Kostrowicki J, Madsen P, Olesen PH, Petersen JS, Poulsen F, Sidelmann UG, Sturis J, Truesdale L, May J, Lau J. Small-molecule agonists for the glucagon-like peptide 1 receptor. *Proc Natl Acad Sci U S A.* 2007; 104:937–942.

5. Teng M, Johnson MD, Thomas C, Kiel D, Lakis JN, Kercher T, Aytes S, Kostrowicki J, Bhumralkar D, Truesdale L, May J, Sidelman U, Kodra JT, Jorgensen AS, Olesen PH, de Jong JC, Madsen P, Behrens C, Pettersson I, Knudsen LB, Holst JJ, Lau J. Small molecule ago-allosteric modulators of the human glucagon-like peptide-1 (hGLP-1) receptor. *Bioorg Med Chem Lett.* 2007;17:5472–5478.

6. Deng J, Peng L, Zhang G, Lan X, Li C, Chen F, Zhou Y, Lin Z, Chen L, Dai R, Xu H, Yang L, Zhang X, Hu W. The highly potent and selective dipeptidyl peptidase IV inhibitors bearing a thienopyrimidine scaffold effectively treat type 2 diabetes. *Eur J Med Chem.* 2010; 46:71–76.

7. Bahekar RH, Jain MR, Gupta AA, Goel A, Jadav PA, Patel DN, Prajapati VM, Patel PR. Synthesis and antidiabetic activity of 3,6,7-trisubstituted-2-(1H-imidazol-2-ylsulfanyl)quinoxalines and quinoxalin-2-yl isothioureas. *Arch Pharm (Weinheim).* 2007; 340:359–366.

8. Chen D, Liao J, Li N, Zhou C, Liu Q, Wang G, Zhang R, Zhang S, Lin L, Chen K, Xie X, Nan F, Young AA, Wang MW. A nonpeptidic agonist of glucagon-like peptide 1 receptors with efficacy in diabetic db/db mice. *Proc Natl Acad Sci U S A.* 2007; 104:943–948.

9. Irwin N, Flatt PR, Patterson S, Green BD. Insulin-releasing and metabolic effects of small molecule GLP-1 receptor agonist 6,7-dichloro-2-methylsulfonyl-3-N-tert-butylaminoquinoxaline. *Eur J Pharmacol.* 2010; 628:268–273.

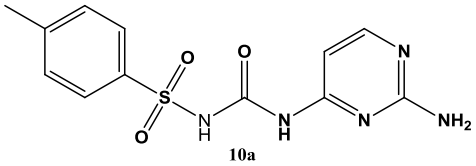
10. Sloop KW, Willard FS, Brenner MB, Ficorilli J, Valasek K, Showalter AD, Farb TB, Cao JXC, Cox AL, Michael MD, Gutierrez Sanfeliciano SM, Tebbe MJ, Coghlan MJ, Novel Small Molecule Glucagon-Like Peptide-1 Receptor Agonist Stimulates Insulin Secretion in Rodents and From Human Islets, *Diabetes*, 2010; 59: 3099-3107.

11. Bloom JD, Dushin RG, Curran KJ, Donahue F, Norton EB, Terefenko E, Jones TR, Ross AA, Feld B, Lang SA, DiGrandi MJ. Thiourea inhibitors of herpes viruses. Part 2: *N*-Benzyl-*N*'-arylthiourea inhibitors of CMV. *Bioorg Med Chem Lett.* 2004; 14:3401–3406.

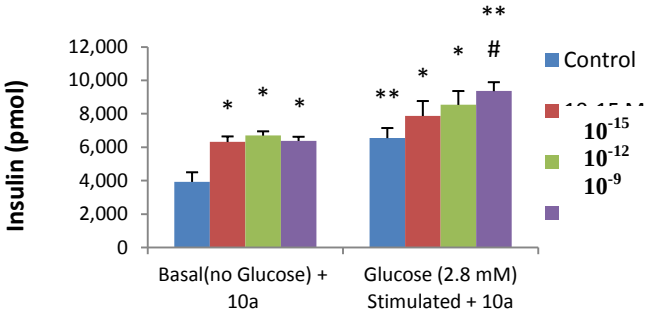
12. Venkatachalam TK, Sudbeck EA, Mao C, Uckun FM. Anti-HIV activity of aromatic and heterocyclic thiazolyl thiourea compounds. *Bioorg Med Chem Lett.* 2001; 11:523–528.

13. Shusheng Z, Tianrong Z, Kun C, Youfeng X, Bo Y. Simple and efficient synthesis of novel glycosyl thiourea derivatives as potential antitumor agents. *Eur J Med Chem.* 2008; 43:2778–2783.

14. Domínguez JN, León C, Rodrigues J, Gamboa de Domínguez N, Gut J, Rosenthal PJ. Synthesis and evaluation of new antimalarial phenylurenyl chalcone derivatives. *J Med Chem.* 2005; 48:3654–3658.



10a



In vitro biological evaluation of pyrimidine **10a** as glucagon-like peptide-1 receptor agonists