Synthesis and biological evaluation of glucagon-like peptide-1 receptor agonists

Yu-Juan Zhang · Liu-Lan Shen · Hyae-Gyeong Cheon · Yong-Nan Xu · Jin-Hyun Jeong

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Abstract In this study, a series of fused-heterocyclic derivatives were systematically designed and synthesized using an efficient route, and evaluated in terms of GLP-1R agonist activity. We employed short synthetic steps and reactions that are tolerant of the presence of various functional groups and suitable for parallel operations to enable the rapid generation of libraries of diverse and structurally complex small molecules. Of the compounds synthesized, 3-(8-chloro-6-(trifluoromethyl)imidazo[1,2-a] pyridin-2-yl)phenyl methanesulfonate (8e) was the most potent agonist with an EC50 of 7.89 μM, and thus is the compound with the greatest potential for application. These findings represent a valuable starting point for the design and discovery of small-molecule GLP-1R agonists that can be administered orally.

Keywords Small molecule agonists · GLP-1R · Heterocycles · Structure–activity relationships · Synthesis

Introduction

Type 2 diabetes mellitus (DM2), a state of hormonal disruption and incretin deficiency, is increasingly becoming a worldwide epidemic (Kwak and Ha 2013). Current drugs utilized in the treatment of DM2 have well-established shortcomings: (1) increasing body weight and (2) increasing loss of β-cell function (Whitehouse 1997; Giugliano et al. 2009). However, the recent emergence of incretin-based therapies, which focus on glucagon-like peptide-1 (GLP-1), has attracted much interest.

GLP-1 is a peptide hormone of 30 amino acid residues. As a peptide, it has a very short half-life (2 min) (Deacon et al. 1995). Such a short half-life has limited the utility of native GLP-1 in the treatment of DM2. The effort to identify GLP-1 analogues has resulted in the development of the drugs exenatide (Sennik et al. 2011; Buse et al. 2004) and liraglutide (Sjöholm 2010; Hribal and Sesti 2010). However, the requirement for injection limits the clinical utility of these peptide drugs. Therefore, orally active, small-molecule agonists of the GLP-1 receptor (GLP-1R) are highly sought after (Murphy and Bloom 2007).

Figure 1 shows synthetic small molecule agonists reported by several groups (Teng et al. 2000; Wang et al. 2009; Teng et al. 2007; Kopin 2004; Gong et al. 2010). Compound 6b, characterized by a novel imidazopyridine hit core, was identified from a library of 10,000 heterocyclic small molecules (Gong et al. 2010). As a small and drug-like active molecule, it represents an interesting starting point for the development of novel drugs. Therefore, we selected this compound as a model. In an effort to move away from the labile ester group of the phenol, we planned a synthetic pathway of new derivatives of imidazo[1,2-a]pyridine-based molecules (Fig. 2). To evaluate the structure–activity relationship, we designed and synthesized a series of
heterocyclic derivatives containing a ring-junction nitrogen using a three-dimensional (3D) pharmacophore model reported previously (Gong et al. 2010) (Fig. 2). For the first stage, only combinations of five- and six-membered rings are considered, including imidazo[1,5-a]pyridine, imidazo[1,2-a]pyrimidine and imidazo[1,2-a]pyrazine. We employed short synthetic steps and reactions that are tolerant of the presence of various functional groups and suitable for parallel operations to enable the rapid generation of libraries of diverse, structurally complex, small molecules.

Materials and methods

Chemistry

All the chemicals used in synthesis were supplied by Aldrich and TCI, and were used without further purification. All solvents were purified and stored in a dry condition. Reaction progress was determined by thin-layer chromatography (TLC) on Merck TLC Silica gel 60 F245 plates. Column chromatography was carried out using a silica gel 60 (63–200 mesh, Merck). NMR spectra were recorded on Agilent 400 instruments operating at 400 MHz for 1H and 100 MHz for 13C, and Agilent 500 instruments operating at 500 MHz for 1H and 125 MHz for 13C. Chemical shifts are expressed as parts per million (ppm) with tetramethylsilane as the internal standard. MS spectra were recorded on an Agilent G6530A Q-TOF.

General synthetic procedure for (6a–b)

To a stirred solution of bromomethylketone 3 (1.21 g, 4.7 mmol) and 2-amino-5-trifluoromethylpyridine 4 (0.61 g, 4.7 mmol) or 2-amino-3-chloro-5-trifluoromethylpyridine 5 (0.92 g, 4.7 mmol) in EtOH (50 mL) was added NaHCO3 (0.31 g, 4.7 mmol) at room temperature. The reaction mixture was heated to reflux and monitored by TLC (hexane/ethyl acetate: 2/1) until completion. After removing EtOH, the residue was extracted with ethyl acetate and water. The combined organic phases were washed with water, 1 N HCl, and brine, dried, and filtered and concentrated in vacuo. The residue was purified by silica gel column chromatography (ethyl acetate/hexane = 10–20%, Rf = 0.23).

3-(6-(Trifluoromethyl)imidazo[1,2-a]pyridin-2-yl)phenyl acetate (6a)

Pale yellow solid; Yield: 64 %; 1H NMR (500 MHz, CDCl3): δ 2.33 (s, 3H), 7.10 (d, J = 10.1 Hz, 1H), 7.32 (d, J = 11.9 Hz, 1H), 7.45 (t, J = 10.0 Hz, 1H), 7.70–7.73 (m, 2H), 7.80 (d, J = 9.7 Hz, 1H), 7.94 (s, 1H); 13C NMR (125 MHz, CDCl3); δ 21.1, 109.8, 117.0, 117.3, 118.0, 119.5, 121.1, 121.8, 123.6, 124.8, 129.9, 134.2, 145.2, 146.2, 151.2, 169.6; EI-HRMS calculated for (C16H11F3N2O2) + 321.0851, found 321.0860.

3-(8-Chloro-6-(trifluoromethyl)imidazo[1,2-a]pyridin-2-yl)phenyl acetate (6b)

Pale yellow solid; Yield: 29 %; 1H NMR (500 MHz, CDCl3); δ 2.34 (s, 3H), 7.10 (d, J = 10.1 Hz, 1H), 7.41–7.47 (m, 2H), 7.76 (t, J = 2.2 Hz, 1H), 7.81 (d, J = 10.2 Hz, 1H), 7.98 (s, 1H); 13C NMR (125 MHz, CDCl3); δ 21.2, 111.2, 119.62, 119.65, 119.68, 122.0, 123.3, 123.4, 123.7, 124.3, 129.8, 134.0, 142.7, 147.0, 151.1, 169.6; EI-HRMS calculated for (C16H10ClF3N2O2) + 355.0461, found 355.0470.

Fig. 2 Structures of synthesized compounds. a Synthesized imidazo[1,2-a]pyridine-based molecules. b Other synthesized heterocycle-series compounds

Fig. 1 Known ago-allosteric modulators of GLP-1R

(0.92 g, 4.7 mmol) in EtOH (50 mL) was added NaHCO3 (0.31 g, 4.7 mmol) at room temperature. The reaction mixture was heated to reflux and monitored by TLC (hexane/ethyl acetate: 2/1) until completion. After removing EtOH, the residue was extracted with ethyl acetate and water. The combined organic phases were washed with water, 1 N HCl, and brine, dried, and filtered and concentrated in vacuo. The residue was purified by silica gel column chromatography (ethyl acetate/hexane = 10–20 %, Rf = 0.23).

General synthetic procedure for (8a and 8d)

To a mixture of 7a (99 mg, 0.36 mmol) or 7b (113 mg, 0.36 mmol) and K2CO3 (250 mg, 1.81 mmol) in acetone...
(10 mL) was added 1-chloroacetone (1 mL, 34.83 mmol) at room temperature. The reaction mixture was heated to reflux for 6 h. After removing acetone and 1-chloroacetone, the residue was extracted with ethyl acetate and water. The combined organic phases were washed with water and brine, dried, and filtered and concentrated in vacuo. The residue was purified by silica gel column chromatography (ethyl acetate/hexane = 10–20 %, Rf = 0.25).

General synthetic procedure for (8b and 8e)

To a solution of 7a (98 mg, 0.35 mmol) or 7b (121 mg, 0.45 mmol) in pyridine (5 mL) was added methanesulfonyl chloride (66 mg, 0.60 mmol) dropwise with stirring over 0.45 mmol) in pyridine (5 mL) was added methanesulfonyl chloride (80 mg, 0.42 mmol) dropwise in an ice bath.

After stirring for 2 h at room temperature, the reaction mixture was quenched with water in an ice bath and then reflux for 6 h. After removing acetone and 1-chloroacetone, the residue was extracted with ethyl acetate and CDCl3: δ 2.32 (s, 3H), 4.64 (s, 2H), 6.89 (dd, J = 8.2, 2.7 Hz, 1H), 7.35 (t, J = 7.9 Hz, 1H), 7.40 (s, 1H), 7.54–7.57 (m, 2H), 7.97 (s, 1H), 8.43 (s, 1H); 13CN M R (125 MHz, CDCl3): δ 26.7, 73.0, 110.3, 112.6, 115.0, 116.5, 116.9, 119.6, 119.7, 123.3, 124.3, 130.1, 134.1, 142.7, 147.6, 158.1, 205.5; EI-HRMS calculated for (C15H15F3N2O3S Na)+ 455.0653, found 455.0656.

I-(3-(8-Chloro-6-(trifluoromethyl)imidazo[1,2-a]pyridin-2-yl)phenox)propan-2-one (8d)

Pale yellow solid; Yield: 50 %; 1H NMR (500 MHz, CDCl3): δ 2.32 (s, 3H), 3.20 (s, 3H), 3.23 (s, 3H), 3.26 (s, 3H), 6.91 (d, J = 8.2 Hz, 1H), 7.29–7.35 (m, 4H), 7.62 (s, 1H), 7.67 (m, 1H), 7.75 (d, J = 7.4 Hz, 2H), 7.84–7.89 (m, 2H), 8.48(s, 1H); 13CN M R (125 MHz, CDCl3): δ 37.6, 109.9, 118.3, 119.7, 121.09, 121.11, 122.0, 124.77, 124.81, 125.0, 130.5, 135.3, 145.3, 145.9, 149.8; EI-HRMS calculated for (C15H11F3N2O3S Na)+ 379.0340, found 379.0360.

3-(6-(Trifluoromethyl)imidazol[1,2-a]pyridin-2-yl)phenyl 4-methylbenzenesulfonate (8f)

Pale yellow solid; Yield: 38 %; 1H NMR (500 MHz, CDCl3): δ 2.43 (s, 3H), 6.91 (d, J = 8.2 Hz, 1H), 7.27–7.33 (m, 3H), 7.39 (m, 1H), 7.61 (s, 1H), 7.74 (d, J = 8.2 Hz, 2H), 7.87 (d, J = 7.8 Hz, 1H), 7.94 (s, 1H); 13CN M R (125 MHz, CDCl3): δ 21.7, 111.5, 119.8, 120.3, 122.2, 123.5, 124.3, 125.1, 126.9, 128.5, 130.0, 132.3, 134.4, 138.6, 145.6, 145.8, 146.5, 149.8,
General synthetic procedure for (12a–c)

Pyridium bromide perbromide (1.79 g, 5.60 mmol) was added to a solution of 10a–c (0.9 g, 5.08 mmol) in AcOH (100 mL) with stirring for 3 h at room temperature. The reaction mixture was poured into ice-cold water and then extracted with ethyl acetate (3 × 50 mL). The combined organic phases were washed with saturated aqueous NaHCO₃, water, and brine, dried, and filtered and concentrated in vacuo to give crude 11a–c as a yellow oil (1.29 g, 98%). The resulting crude 11a–c could be used without further purification. To a stirred solution of bromomethylketone 11a–c (1.30 g, 5.1 mmol) and amino-pyridine 4 (0.82 g, 5.1 mmol) in EtOH (80 mL) was added NaHCO₃ (0.43 g, 5.1 mmol) at room temperature. The reaction mixture was heated to reflux for 8 h. After removing EtOH, the residue was extracted with ethyl acetate and water. The combined organic phases were washed with water, 1 N HCl, water, and brine, dried, and filtered and concentrated in vacuo. The residue was purified by silica gel column chromatography (hexane/ethyl acetate = 1/1, Rf = 0.24; 12a, hexane/ethyl acetate = 4/1, Rf = 0.22).

N-(3-(6-(Trifluoromethyl)imidazo[1,2-a]pyridin-2-yl)phényl)acetamide (12a)

Pale yellow solid; Yield: 39%; ¹H NMR (500 MHz, CDCl₃): δ 2.19 (s, 3H), 7.31 (d, J = 11.6 Hz, 1H), 7.39 (t, J = 9.8 Hz, 1H), 7.51 (s, NH), 7.60 (d, J = 9.8 Hz, 1H), 7.68 (d, J = 11.1 Hz, 2H), 7.93 (s, 1H), 8.07 (s, 1H), 8.47(s, 1H); ¹³C NMR (125 MHz, CDCl₃): δ 24.7, 109.6, 117.4, 113.4, 119.9, 120.7, 120.9, 124.6, 124.7, 129.6, 133.7, 138.5, 145.2, 147.1, 168.5; EI-HRMS calculated for (C₁₆H₁₀ClF₃N₂O₂)⁺ 342.0835, found 342.0830.

N-(3-(6-(Trifluoromethyl)imidazo[1,2-a]pyridin-2-yl)phényl)methanesulfonamide (12b)

Pale yellow solid; Yield: 20%; ¹H NMR (500 MHz, CDCl₃): δ 3.05 (s, 3H), 7.30 (dt, J = 7.0, 1.3 Hz, 1H), 7.34 (dd, J = 9.5, 1.8 Hz, 1H), 7.40 (t, J = 7.9 Hz, 1H), 7.69–7.74 (m, 3H), 7.82 (t, J = 1.9 Hz, 1H), 7.99 (s, 1H), 8.51(s, 1H); ¹³C NMR (125 MHz, CDCl₃): δ 39.4, 110.0, 118.0, 118.5, 120.7, 121.3, 123.0, 124.8, 124.9, 130.2, 130.3, 134.2, 137.6, 145.2, 146.2; EI-HRMS calculated for (C₁₆H₁₀ClF₃N₂O₂S+H)⁺ 356.0705, found 356.0705.

4-Methyl-N-(3-(6-(trifluoromethyl)imidazo[1,2-a]pyridin-2-yl)phenyl)benzene sulfonamide (12c)

Pale yellow solid; Yield: 21%; ¹H NMR (500 MHz, CDCl₃): δ 2.30 (s, 3H), 7.14–7.19 (m, 3H), 7.26–7.29 (m, 2H), 7.63 (dd, J = 7.7, 0.9 Hz, 1H), 7.66–7.70 (m, 4H), 7.85 (brs, NH), 7.89 (s, 1H), 8.45(s, 1H); ¹³C NMR (125 MHz, CDCl₃): δ 21.5, 109.9, 118.0, 118.8, 120.7, 121.0, 122.7, 124.7, 127.3, 129.7, 130.9, 134.0, 136.0, 137.5, 143.9, 145.2, 146.5; EI-HRMS calculated for (C₂₁H₁₄ClF₃N₂O₃S+Na)⁺ 489.0274, found 489.0263.

3-(8-Chloro-6-(trifluoromethyl)imidazo[1,5-a]pyridin-3-yl)phenyl acetate (19)

To a solution of 18 (300 mg, 0.80 mmol) in benzene (10 mL) was added POCl₃ (1.2 mL, 13.04 mmol) dropwise at room temperature. The reaction mixture was heated to reflux for 6 h. After cooling to room temperature, the mixture was poured into iced-water and then extracted with ethyl acetate (3 × 50 mL). The combined organic phases were washed with water and brine, dried, and filtered and concentrated in vacuo. The residue was purified by silica gel column chromatography (hexane/ethyl acetate = 12/1, Rf = 0.25). White solid; Yield: 90%; ¹H NMR (400 MHz, CDCl₃): δ 2.35 (s, 3H), 6.92 (s, 1H), 7.25 (d, J = 8.6 Hz, 1H), 7.54–7.61 (m, 3H), 7.77 (s, 1H), 8.51(s, 1H); ¹³C NMR (100 MHz, CDCl₃): δ 21.1, 113.9, 117.6, 117.9, 119.6, 121.5, 121.8, 122.5, 122.9, 124.9, 125.3, 126.5, 126.8, 129.7, 130.1, 130.5, 151.3, 169.2; EI-HRMS calculated for (C₁₆H₁₆ClF₃N₂O₂+H)⁺ 355.0461, found 355.0474.

3-(8-Chloro-6-(trifluoromethyl)imidazo[1,5-a]pyridin-3-yl)cyclohexane carboxylate (21a)

To a solution of 19 (257 mg, 0.72 mmol) in THF (20 mL) was added a solution of NaOH (50 mg, 1.25 mmol) in water (10 mL) with stirring for 3 h at room temperature. After removing THF, the resulting mixture was extracted with ethyl acetate. The combined organic phases were washed with water and brine, dried, and filtered and concentrated in vacuo. The resulting crude 20 could be used without further purification. Cyclohexanecarboxylic chloride (28 mg, 0.19 mmol) was added to a solution of 20 (50 mg, 0.16 mmol), TEA (19 mg, 0.19 mmol), and DMAP (4 mg, 0.03 mmol) in anhydrous CH₂Cl₂ (20 mL) slowly in an ice bath. After stirring for 3 h at room temperature, the reaction mixture was poured into ice water and then extracted with CH₂Cl₂ (3 × 20 mL). The combined organic phases were washed with 1 N HCl, water, and brine, dried, and filtered and concentrated in vacuo.
The residue was purified by silica gel column chromatography (hexane/ethyl acetate = 20/1, Rf = 0.23) to afford 21a as a pale yellow solid (64 mg, 94%). 1H NMR (400 MHz, CDCl3): δ 1.25–1.39 (m, 4H), 1.57–1.65 (m, 2H), 1.81–1.84 (m, 2H), 2.06–2.09 (m, 2H), 2.58 (t, J = 10.1 Hz, 1H), 6.92 (s, 1H), 7.24 (m, 1H), 7.52–7.59 (m, 3H), 7.78 (s, 1H). 13C NMR (100 MHz, CDCl3): δ 25.3, 25.6, 28.8, 43.1, 113.9, 117.7, 119.6, 121.4; EI-HRMS calculated for (C17H15ClF3N3O2 + Na)+ 369.0618, found 369.0670.

I-(3-(8-Chloro-6-(trifluoromethyl)imidazo[1,5-a]pyridin-3-yl)phenoxy)propan-2-one (21b)

Using the same method as for the preparation of 8a. Starting with 20 (73 mg, 0.23 mmol), 1-chloroacetone (0.5 mL, 17.41 mmol) and K2CO3 (161 mg, 1.17 mmol), POCl3 (0.3 mL, 3.40 mmol) was added to a mixture of 32a (73 mg, 0.23 mmol), 1-chloroacetone (0.5 mL, 17.41 mmol) and K2CO3 (161 mg, 1.17 mmol), tert-Butyl (3-(8-chloro-6-(trifluoromethyl)imidazo[1,5-a]pyridin-3-yl)phenyl)cyclohexane carboxamide (27a)

Pale yellow solid; Yield: 13%; 1H NMR (400 MHz, CDCl3): δ 1.53 (s, 9H), 6.71 (s, 1H), 6.90 (s, 1H), 7.41–7.48 (m, 3H), 7.76 (s, 1H), 7.88 (s, 1H), 8.58 (s, 1H); 13C NMR (100 MHz, CDCl3): δ 28.2, 113.7, 117.3, 117.6, 118.3, 119.7, 119.9, 122.3, 122.5, 124.2, 126.3, 129.5, 130.0, 139.4, 141.6, 152.6; EI-HRMS calculated for (C19H17ClF3N3O2 + Na)+ 434.0859, found 434.0865.

N-(3-(8-Chloro-6-(trifluoromethyl)imidazo[1,2-a]pyridin-3-yl)phenyl)cyclohexane carboxamide (27c)

Pale yellow solid; Yield: 32%; 1H NMR (400 MHz, CDCl3): δ 1.28–1.35 (m, 2H), 1.50–1.59 (m, 2H), 1.71 (m, 2H), 1.83–1.85 (m, 2H), 1.94–1.97 (m, 2H), 2.26 (t, J = 11.6 Hz, 1H), 6.90 (s, 1H), 7.44–7.52 (m, 2H), 7.55 (s, 1H), 7.71 (d, J = 7.6 Hz, 1H), 7.75 (s, 1H), 7.95 (s, 1H), 8.54 (s, 1H); 13C NMR (100 MHz, CDCl3): δ 25.6, 29.6, 46.5, 113.8, 117.4, 117.8, 119.7, 119.8, 121.0, 122.2, 123.3, 126.3, 129.4, 129.6, 130.0, 139.1, 141.5, 174.6; EI-HRMS calculated for (C21H16ClF3N3O + Na)+ 444.1066, found 444.1075.

tert-Butyl (3-(8-chloro-6-(trifluoromethyl)imidazo[1,5-a]pyridin-3-yl)phenyl)pyrrolidine-2,5-dione (27d)

Pale yellow solid; Yield: 28%; 1H NMR (400 MHz, CDCl3): δ 2.95 (s, 4H), 6.93 (s, 1H), 7.52 (d, J = 7.9 Hz, 1H), 7.69 (t, J = 7.8 Hz, 1H), 7.76–7.82 (m, 3H), 8.63 (s, 1H); 13C NMR (100 MHz, CDCl3): δ 28.4, 114.0, 117.7, 118.0, 119.7, 121.5, 122.6, 124.2, 125.7, 126.4, 127.1, 128.2, 129.8, 130.3, 132.7, 140.7, 175.8; EI-HRMS calculated for (C18H13ClF3N3O2+H)+ 394.0570, found 394.0608.

N-(3-(8-Chloro-6-(trifluoromethyl)imidazo[1,2-a]pyridin-2-yl)phenyl)acetamide (33a)

Acetic anhydride (20 mg, 0.20 mmol) was added to a mixture of 32 (50 mg, 0.16 mmol) and DMAP (3 mg, 0.02 mmol) in anhydrous CH2Cl2 (10 mL) with stirring for 1 h at room temperature. After removing the solvent, the residue was extracted with ethyl acetate and water. The combined organic phases were washed with 1 N HCl, water, and brine, dried, and filtered and concentrated in vacuo. The residue was purified by silica gel column chromatography (hexane/ethyl acetate = 20/1, Rf = 0.23). Pale yellow solid; Yield: 50%; 1H NMR (400 MHz, CDCl3): δ 2.19 (s, 3H), 7.35–7.39 (m, 2H), 7.62–7.66 (m, 2H), 7.72 (s, 1H), 7.94 (s, 1H), 8.06 (s,
1H), 8.39 (s, 1H); 13C NMR (100 MHz, CDCl3): δ 24.6, 111.3, 116.6, 117.6, 119.6, 120.3, 121.5, 122.2, 123.4, 124.2, 129.5, 133.1, 138.5, 142.6, 147.5, 168.8; EI-HRMS calculated for (C₁₈H₁₄ClF₃N₃O + Na)⁺ 376.0440, found 376.0453.

General synthetic procedure for (33b–c)

Cyclohexanecarboxylic chloride (28 mg, 0.19 mmol) or toluenesulfonyl chloride (39 mg, 0.21 mmol) was added to a solution of 32 (50 mg, 0.16 mmol), TEA (19 mg, 0.19 mmol), and DMAP (4 mg, 0.03 mmol) in anhydrous CH₂Cl₂ (10 mL) slowly in an ice bath. After stirring for 3 h at room temperature, the reaction mixture was poured into ice water and then extracted with CH₂Cl₂ (3 × 20 mL). The combined organic phases were washed with 1 N HCl, water, and brine, dried, and filtered and concentrated in vacuo. The residue was purified by silica gel column chromatography (hexane/ethyl acetate = 9/1, RF = 0.22).

N-(3-(6-Chloro-6-(trifluoromethyl)imidazo[1,2-a]pyridin-2-yl)phenyl)cyclohexane carboxamide (33b)

Pale yellow solid; Yield: 54 %; ¹H NMR (400 MHz, CDCl3): δ 1.28–1.33 (m, 2H), 1.51–1.60 (m, 2H), 1.72 (m, 2H), 1.84–1.86 (m, 2H), 1.96–1.99 (m, 2H), 2.25 (m, 1H), 7.35–7.39 (m, 2H), 7.48 (s, 1H), 7.66 (d, J = 7.0 Hz, 2H), 7.98 (s, 1H), 8.13 (s, 1H), 8.40 (s, 1H); ¹³C NMR (100 MHz, CDCl3): δ 25.6, 25.7, 29.7, 46.6, 111.3, 116.6, 116.9, 117.5, 119.6, 120.2, 122.0, 123.3, 124.2, 129.5, 133.0, 138.7, 142.7, 147.6, 174.7; EI-HRMS calculated for (C₂₃H₁₉ClF₃N₃O⁺) 422.12470, found 422.12446.

N-(3-(6-Chloro-6-(trifluoromethyl)imidazo[1,2-a]pyridin-2-yl)phenyl)-4-toluenesulfonamide (33c)

Pale yellow solid; Yield: 68 %; ¹H NMR (400 MHz, CDCl3): δ 2.46 (s, 3H), 7.00 (d, J = 7.5 Hz, 1H), 7.33–7.35 (m, 3H), 7.40 (s, 1H), 7.58 (s, 1H), 7.83–7.87 (m, 4H), 8.10 (d, J = 7.4 Hz, 1H), 8.40 (s, 1H); ¹³C NMR (100 MHz, CDCl3): δ 21.7, 111.3, 116.6, 117.0, 119.7, 123.3, 124.4, 128.2, 128.5, 128.6, 129.4, 129.6, 129.7, 129.8, 131.5, 133.9, 134.9, 136.5, 142.7, 145.1, 146.6; EI-HRMS calculated for (C₂₃H₁₉ClF₃N₃O₂S⁺) 466.06038, found 466.07034.

General synthetic procedure for (37a–c)

A mixture of 5-bromopyrimidin-2-amine 35–36 (222 mg, 1.27 mmol) and bromoacetonitrile 3 (257 mg, 1.0 mmol) in dioxane (10 mL) with or without NaHCO₃ (84 mg, 1.00 mmol) was stirred until reflux for 7 h. After cooling to room temperature, ethyl acetate was added, washed with water and brine, dried over calcium oxide, and filtered and concentrated in vacuo. The residue was purified by silica gel column chromatography (hexane/ethyl acetate = 20–50 %, RF = 0.21).

3-(6-Bromoimidazo[1,2-a]pyrimidin-2-yl)phenyl acetate (37a)

White solid; Yield: 11 %; ¹H NMR (400 MHz, CDCl3): δ 2.33 (s, 3H), 7.10 (d, J = 7.9 Hz, 1H), 7.45 (t, J = 7.9 Hz, 1H), 7.75 (s, 1H), 7.77 (s, 1H), 7.84 (d, J = 7.6 Hz, 1H), 8.51 (s, 1H), 8.55 (s, 1H); ¹³C NMR (100 MHz, CDCl3): δ 21.2, 104.8, 106.6, 119.6, 122.1, 123.7, 129.8, 132.5, 134.2, 150.7, 151.2, 158.6, 169.5; EI-HRMS calculated for (C₁₄H₁₀BrN₃O₂H⁺) 332.00346, found 332.00372.

3-(Imidazo[1,2-a]pyrimidin-2-yl)phenyl acetate (37b)

Pale yellow solid; Yield: 21 %; ¹H NMR (400 MHz, CDCl3): δ 2.32 (s, 3H), 6.82 (t, J = 5.6 Hz, 1H), 7.07 (d, J = 7.7 Hz, 1H), 7.42 (t, J = 7.8 Hz, 1H), 7.76 (m, 1H), 7.83 (d, J = 7.7 Hz, 1H), 8.39 (d, J = 6.3 Hz, 1H), 8.49 (s, 1H); ¹³C NMR (100 MHz, CDCl3): δ 21.2, 106.5, 108.9, 119.5, 121.7, 123.6, 129.7, 133.1, 134.7, 146.2, 148.6, 150.1, 151.2, 169.5; EI-HRMS calculated for (C₁₄H₁₁N₃O₂⁺) 254.09295, found 254.09322.

3-(Imidazo[1,2-a]pyrazin-2-yl)phenyl acetate (37c)

Pale yellow solid; Yield: 11 %; ¹H NMR (400 MHz, CDCl3): δ 2.34 (s, 3H), 7.12 (d, J = 7.6 Hz, 1H), 7.47 (t, J = 7.5 Hz, 1H), 7.74 (s, 1H), 7.83 (d, J = 7.3 Hz, 1H), 7.89 (s, 1H), 7.95 (s, 1H), 8.07 (s, 1H), 9.10 (s, 1H); ¹³C NMR (100 MHz, CDCl3): δ 21.1, 109.4, 118.6, 119.6, 122.0, 123.7, 129.8, 134.4, 140.9, 143.8, 146.8, 151.2, 169.4; EI-HRMS calculated for (C₁₄H₁₁N₃O₂⁺) 254.09295, found 254.09288.

2-(3-Nitrophenyl)imidazo[1,2-a]pyrimidine (37d)

A mixture of 2-aminopyrazine 34 (195 mg, 2.05 mmol) and bromoacetonitrile 29 (660 mg, 2.70 mmol) in ethanol (20 mL) was stirred until reflux for 3 h. After cooling to room temperature, the mixture was concentrated. The residue was dissolved in ethyl acetate, washed with 1 N HCl, water, and brine, dried over calcium oxide, and filtered and concentrated in vacuo. The residue was purified by silica gel column chromatography (methanol/methylene chloride = 0–1 %, RF = 0.21) to afford 37d as a yellow solid (24 mg, 5 %). ¹H NMR (400 MHz, CDCl3): δ 6.95 (t, J = 1.7 Hz, 1H), 7.65 (t, J = 8.0 Hz, 1H), 7.97 (s, 1H), 8.21 (d, J = 7.9 Hz, 1H), 8.45 (d, J = 7.6 Hz, 1H), 8.50
A mixture of 2-aminopyrazine 36 (95 mg, 1.0 mmol) and bromoacetone 29 (488 mg, 2.0 mmol) in ethanol (10 mL) was stirred until reflux for 3 h. After cooling to room temperature, the mixture was concentrated. Then the residue was dissolved in ethyl acetate and washed with water. The combined aqueous phase was extracted with ethyl acetate. The combined organic phase was washed with brine, dried over calcium oxide, and filtered and concentrated in vacuo. The residue was purified by silica gel column chromatography (methanol/chloroform = 100/1; Rf = 0.21) to afford 37e as a yellow solid (40 mg, 17%).

Biology

In vitro GLP-1R activation assay (Chen et al. 2007)

CHO-K1 cells (4 × 10^6/100 mm dish) were transiently transfected with the pCMV6-GLP-1R (Origene #SC124060) and pCRE-Luc (Promega #631911) plasmids using Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA). After 24 h incubation at 37°C, cells were seeded into 96-well culture plates (2 × 10^4/well), and further incubated at 37°C overnight. At the time of assay, GLP-1 (7–37) (Sigma, St. Louis, MO) or test compounds in DMSO were added to the plate. After 8 h incubation, cells were lysed and luciferase activity quantified using the Steady-Glo luciferase assay system (Promega #E2550).

Data were analyzed in Excel and EC_{50} values were determined graphically from dose–response curves in OriginPro.

Results and discussion

Chemistry

The general synthetic pathway yielding the novel derivatives 6a, 6b, and 8a–8f is outlined in Scheme 1. 3-Hydroxy acetophenone 1 was converted into 3-acetoxy acetophenone 2 by acetylation. Treatment of substituted acetophenone 2 with bromine in the presence of AlCl_{3} in Et_{2}O (Bunders et al. 2010) afforded α-bromomethylketone 3. Subsequently, 3 and the substituted 2-aminopyridines 4, 5 were allowed to react in the presence of sodium bicarbonate in refluxing ethanol (Fookes et al. 2008), resulting in the generation of compounds 6a and 6b. The deacetylation of compounds 6a and 6b with sodium hydroxide afforded compounds 7a and 7b, respectively. The akylation or acylation of 7a and 7b furnished the target compounds 8a–8f.

The derivatives 12a–12c were readily prepared in three steps, as illustrated in Scheme 2. 3-Aminooacetophenone 9 was first acylated with acyl chloride or sulfonyl chloride, as appropriate, to produce compounds 10a–10c. Subsequent bromination of 10a–10c with PBB (pyridinium bromide perbromide) in acetic acid (Yu et al. 2008) afforded compounds 11a–11c, which were then cyclized with 5-trifluoro-2-aminopyridine in refluxing ethanol to give the desired derivatives 12a–12c.

Next we synthesized the imidazo[1,5-a]pyridine derivatives 19, 21a–21b, and 27a–27d, as detailed in Scheme 3. The intermediate 2-aminomethylpyridine 15, prepared from 2,3-dichloro-5-trifluoropyridine 14 in two steps using a method reported elsewhere (Stolting 2004), was treated...
with 3-acetoxybenzoic acid 17, followed by cyclization in the presence of POCl₃ in refluxing benzene (Bower and Ramage 1955) to give intermediate 19. Then, deacylation of 19 and subsequent acylation or alkylation of the resulting compound 20 with appropriate acyl chloride or 1-chloroacetone resulted in the generation of the desired compounds 21a–21b. For the synthesis of derivatives 27a–27d, the first step is the amidation of intermediate 15 with benzoic acid 23 in the presence of DCC and DMAP in dichloromethane, resulting in the generation of compound 24. Then, deprotection of 24 with trifluoroacetic acid (Mu 2001) followed by acylation of the resulting compound 25 afforded the compounds 26a–26c. Finally, the amides 24 and 26a–26c were cyclized in the presence of POCl₃ and pyridine in refluxing dichloroethane (Cookson et al. 1986), resulting in the generation of the target derivatives 27a–27d, respectively.

Scheme 4 describes the synthesis of derivatives 33a–33c. The formation of η-diazoketone intermediate 30 was achieved from η-bromomethylketone 28 by treatment with N,N'-ditosylhydrazine and DBU (Toma et al. 2007). Subsequent coupling of η-diazoketone with 3-chloro-5-trifluoro-2-aminopyridine in the presence of 10 mol % Cu(OTf)₂ in dichloroethane (DCE) (Yadav et al. 2007) afforded substituted 2-arylimidazo[1,2-α]pyridine 31.
Reduction of 31 with stannous chloride in a refluxing mixture of ethanol and concentrated hydrochloride (Denora et al. 2008) resulted in the generation of compound 32. Finally, the acylation of 32 with the corresponding acyl chloride afforded derivatives 33a–33c.

The syntheses of compounds 37a–37e are detailed in Scheme 5. The preparation of intermediate 35 was achieved by bromination of 2-aminopyrimidine 34 with NBS in refluxing acetonitrile. The subsequent cyclization of 35, 2-aminopirimidine 34, and 2-aminopyrazine 36 with intermediate 3 yielded the target derivatives 37a–37c, respectively. The cyclization of 2-aminopirimidine 34 and 2-aminopyrazine 36 with intermediate 29 yielded the target derivatives 37d–37e, respectively.

Biology

The compounds prepared in this study were evaluated in terms of GLP-1R agonist activity using an in vitro activation efficacy assay in CHO-K1 cells (Chen et al. 2007), and the magnitude of the responses have been compared at two concentrations of compounds used. GLP-1 (7–37) was used as the positive control and DMSO (0.1 %) was used as the negative control. Induction values represent luciferase activities driven by CRE (cAMP response element). Compounds were grouped into three series according to fused-heterocyclic ring type.

In general, the first series of compounds, 6a–6b, 8a–8f, 12a–12c, and 33a–33c (Fig. 3), based on the imidazo[1,2-α]pyridine structure and containing various substituted groups, generated higher responses than those of the second series. Compound 6b is the model compound, in which replacement of the acetyl group with propanyl-2-one 8d, mesyl 8e, or tosyl 8f resulted in a significant increase in magnitude of the response, suggesting that the hydrogen-bond donor is preferred to be this region and that the length of linker affects binding to the ago-allosteric binding site of GLP-1R. To determine whether the chlorine in imidazo[1,2-α]pyridine is essential for its activity, compounds 6a and 8a–8c were synthesized. Compounds 8a and 8b showed good responses similar to that of compounds 8d and 8e at 10 μM. However, a loss of response was observed for compounds 6a and 8c.

In the second series of compounds, 19, 21a–21b, and 27a–27d (Fig. 4), the imidazo[1,2-α]pyridine structure was changed to an imidazo[1,5-α]pyridine structure. Unfortunately, all derivatives generated low responses at a low concentration (10 μM). Surprisingly, compound 8e, which generated the highest response at 10 μM, also exhibited the greatest response at a high concentration (100 μM).
groups showed higher responses than those with a substituted amide group.

Finally, a nitrogen atom was introduced into the six-membered fused-heterocyclic ring to evaluate the effect of electron density on activity (Fig. 4). The majority of the compounds 37a–37e thus generated showed loss of responses compared to the first series. We speculated that the loss of responses might be attributable to a decreased interaction between the π-electron and the receptor.

Over the half of the synthesized compounds, the effects did not appear concentration-dependent. However the effects of the compounds on coupling the GLP-1R to the signaling way may well be concentration-dependent, but the responses measured did not appear concentration-dependent due to cytotoxicity.

In addition, selected compounds 8a, 8b and 8e which showed >2.5-fold increases at 10 μM were assayed further to determine concentration–response curves (Fig. 5) and calculate EC50 values (Table 1). Compound 8e, bearing chlorine substitution imidazo[1,2-α]pyridine ring and mesyl group of benzene ring, was found to be a potent GLP-1R agonist exhibiting an EC50 of 7.89 μM. Compounds 8a and 8b, without chlorine substitution of imidazo[1,2-α]pyridine ring, were about threefold less potent, with EC50 values of 20 μM and 17 μM, respectively (Table 1). Concentration–response curves of selected compounds are shown in Fig. 5. The concentration are in a range from 1 μM to 100 μM. Compounds 8b and 8e showed above 50 % response (8b, 52 %; 8e, 58 %) at their EC50 values, while compound 8a showed lower response 43 % at EC50 value (Fig. 5). Thus, compound 8e may serve as a GLP-1R agonist with potential for application.

In conclusion, these new compounds, synthetic methodology developed and preliminary biological evaluation results could be helpful in further design and discovery of more potent GLP-1R agonists for the treatment of DM2.

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Fig. 5 Concentration-response curves of agonists 8a, 8b and 8e of GLP-1R. Experimental procedures were performed as in Fig. 3. Vertical axes show the response percentage of GLP-1 response. Values shown are mean ± SD of three independent experiments. For determined EC50 values see Table 1.

Table 1 Potency of agonists 8a, 8b and 8e at GLP-1R

| Compounds | X   | Y   | EC50 (µM)a |
|-----------|-----|-----|------------|
| 8a        | H   |     | 19.75 ± 0.64 |
| 8b        | H   |     | 16.96 ± 0.16 |
| 8e        | Cl  |     | 7.89 ± 2.26  |

a Values are reported as mean ± SD

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