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# Synthesis of 5-chloro-*N*-aryl-1*H*-indole-2-carboxamide derivatives as inhibitors of human liver glycogen phosphorylase *a*

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**Abstract**—A series of 5-chloro-*N*-aryl-1*H*-indole-2-carboxamide derivatives were prepared and evaluated as inhibitors of human liver glycogen phosphorylase *a* (hLGPa). One compound, 5-chloro-*N*-[4-(1,2-dihydroxyethyl)phenyl]-1*H*-indole-2-carboxamide (**2f**), inhibited hLGPa with an $IC_{50}$ of 0.90 μM. The pyridine analogue of **2f** showed inhibitory activity of glucagon-induced glucose output in cultured primary hepatocytes with an $IC_{50}$ of 0.62 μM and oral hypoglycemic activity in diabetic db/db mice. Crystallographic determination of the complex of **2f** with hLGPa showed binding of the inhibitor in a solvent cavity at the dimer interface, with the two hydroxyl groups making favorable electrostatic interactions with hLGPa.


## 1. Introduction

Non-insulin-dependent diabetes mellitus (type 2 diabetes) is a severe and prevalent metabolic disorder. Tight control of blood glucose levels reduces the extent and progression of diabetic complications such as nerve and kidney damage, blindness, and heart disease,[1,2] but it is difficult to achieve exact glycemic control with oral hypoglycemic agents because of limited efficacy, tolerability, and hypoglycemic risk.[3] In type 2 diabetic patients, high blood glucose levels are in part caused by elevated hepatic glucose output (HGO) due to gluconeogenesis (de novo synthesis of glucose from 2- and 3-carbon precursors) and glycogenolysis (phosphorolysis of α-(1,4)-linkages within glycogen molecules),[4–7] and inhibition of glycogenolysis has recently emerged as an attractive therapeutic target for treatment of type 2 diabetes.[8–11] Glycogen phosphorylase (GP) catalyzes the first step of the breakdown of glycogen to yield glucose-1-phosphate. In the liver, phosphoglucomutase and glucose-6-phosphatase convert glucose-1-phosphate to glucose, which is then released into the blood to be supplied to the tissues (Fig. 1).[12–15] Therefore, inhibition of liver GP (LGP) is an alternative target for development of new drugs for type 2 diabetes.

GP is a homodimeric enzyme that exists in two conformational states (the GPa and GPb forms) that are interconverted by phosphorylation–dephosphorylation reactions at Ser14.[16] Only GPa has significant potency.[16] The activity of GP is regulated by ligand binding at several sites, including an allosteric site, a catalytic site and a caffeine-binding site.[8,17–22] Several studies have revealed a further site (a new allosteric site or dimer interface site) at which 5-chloroindole-2-carboxamide derivatives are able to interact as GP inhibitors.[23,24] Among this new class of inhibitors, CP-320626, which has 5-chloroindole and phenylalanine moieties, shows potent activity against human liver GPa (hLGPa) and may act synergistically with glucose.[10] As the blood glucose concentration decreases, the potency of GP inhibitors decreases, and so the risk of hypoglycemia seems to be minimized. This hypothesis prompted us to modify the structure of CP-320626 to identify novel and potent GP inhibitors.

A crystal structure of the CP-320626 inhibitor–GP complex shows that the planar 5-chloroindole moiety docks into a hydrophobic pocket, and suggests that there is little room for modification of this part of the structure.[23]

*Keywords*: Glycogen phosphorylase; Hepatic glucose output; Diabetes; Crystallographic determination.
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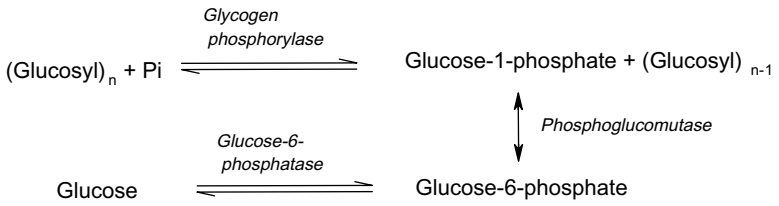



**Figure 1.** Hepatic pathways of glycogen metabolism.

However, the relatively flexible phenylalanine moiety is located close to a solvent-filled gap between the two subunits of the GP dimer and could be modified to maintain or improve the potency.[23–25] The hydroxyl group and the nitrogen atom of the 4-hydroxypiperidyl moiety form a hydrogen bond to GP through a water molecule.[26] Therefore, to discover more potent inhibitors, we focused on replacement of the phenylalanine of CP-320626 with substituents having hydroxyl group(s) that might form hydrophilic interactions with the enzyme (Fig. 2).

In this paper, we report the discovery of a novel class of GP inhibitors and discuss their binding site based on a crystal structure of one of the inhibitors with GP.

## 2. Chemistry

The 5-chloro-*N*-phenyl-1*H*-indole-2-carboxamide analogues **2a**–**i** were prepared as shown in Scheme 1.

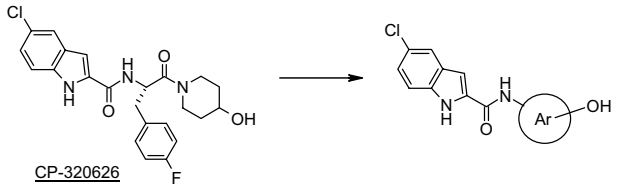



**Figure 2.** Design of *N*-aryl-5-chloroindolecarboxamides based on CP-320626.

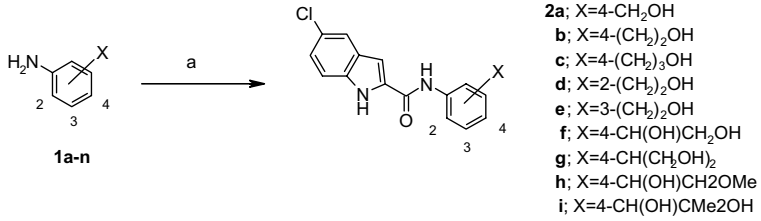
Coupling reactions of anilines **1a**–**n** with 5-chloro-1*H*-indole-2-carboxylic acid gave the corresponding products in moderate to high yield. In the case of branched diol derivative **2i**, the starting material **1i** was synthesized from 4-nitrobenzaldehyde through three steps, as shown in Scheme 2.[27]

Compound **1h** as starting material for **2h** was obtained by hydrogenation of 2-methoxy-1-(4-nitrophenyl)ethanol[28] under a hydrogen atmosphere. The substituted 4-(1,2-dihydroxyethyl) anilide derivatives **9a**–**j** were synthesized as shown in Scheme 3.

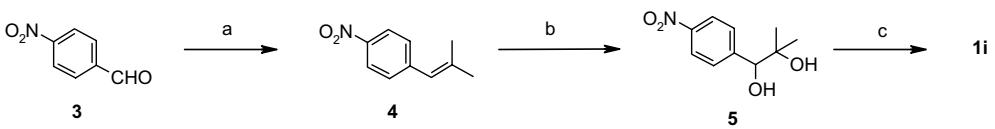
Coupling reactions of commercially available substituted 4-bromoanilines **6a**– **i** with 5-chloro-1*H*-indole-2-carboxylic acid or 5-chloro-1*H*-indole-2-carbonyl chloride gave intermediate bromides **7a**–**i** in moderate to high yield. The desired products **9a**–**i** were obtained by Stille's vinylation reaction[29] followed by dihydroxylation using osmium tetroxide and *N*-methylmorpholine-*N*-oxide. The 3-amino derivative **9j** was obtained from the corresponding nitro derivative **9i** by reduction using zinc dust.

Compounds **13a**–**c** with a pyridine or pyrazine ring instead of the benzene ring in **2f** were prepared from the corresponding arylamines **10a**–**c** by the same reactions as those described for compounds **9a**–**i** (Scheme 4).

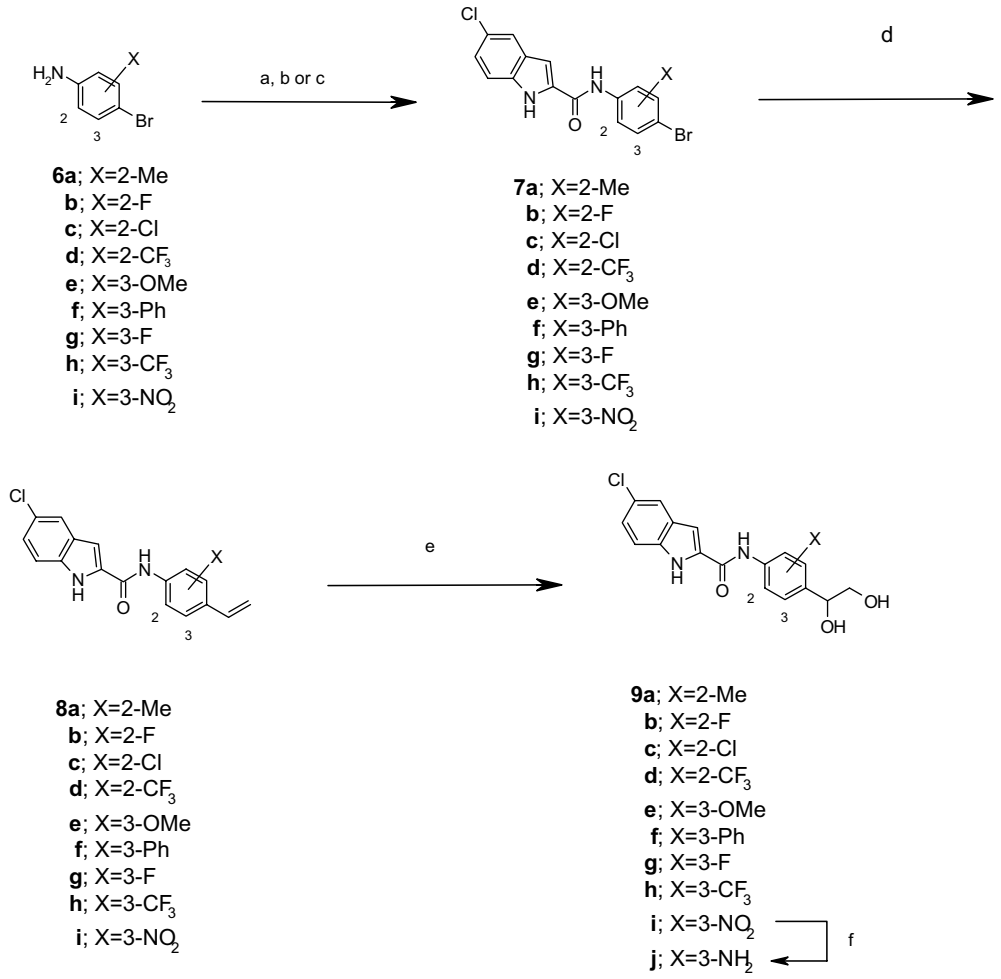
Thiazole derivatives **15a**,**b** were prepared from 1-(2-amino-1,3-thiazol-4-yl) ethane-1,2-diol (**14a**) or its isomer (**14b**) using the coupling reaction described above. Syn-



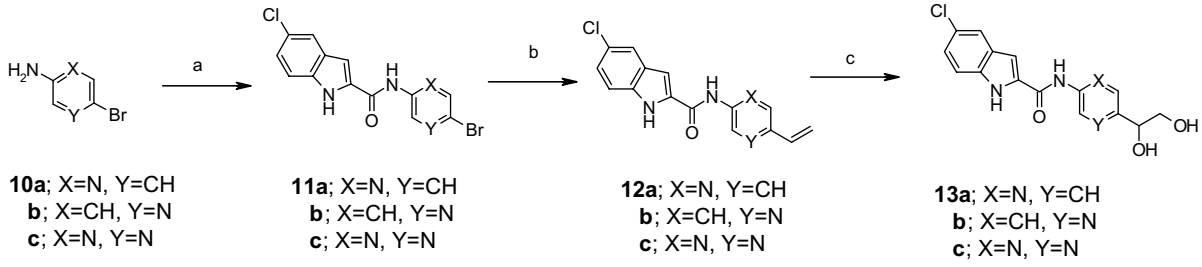

**Scheme 1.** Syntheses of compounds **2a**–**i**. Reagents: (a) 5-chloroindole-2-carboxylic acid, WSC·HCl, HOBt, DMF.




**Scheme 2.** Syntheses of compound **1i**. Reagents: (a) $Ph_3$(*i*-Pr)PI, *n*-BuLi, THF; (b) $OsO_4$, *N*-methylmorpholine-*N*-oxide, THF, $H_2O$; (c) $H_2$, 10% Pd–C, MeOH.




**Scheme 3.** Synthesis of compounds **9a**–**j**. Reagents and conditions: (a) 5-chloroindole-2-carboxylic acid, WSC·HCl, HOBt, DMF (for **7a**, **e**, **f**, and **k**); (b) 5-chloroindole-2-carbonyl chloride, py. (for **7b**, **c**, **d**, **g**, and **h**); (c) 5-chloroindole-2-carboxylic acid, $POCl_3$, py., −25 °C (for **7i**, and **j**); (d) *n*-$Bu_3SnCHCH_2$, $Pd(PPh_3)_4$, LiCl, DMF, THF, 80 °C; (e) $OsO_4$, *N*-methylmorpholine-*N*-oxide, THF, $H_2O$; (f) Zn, AcOH.

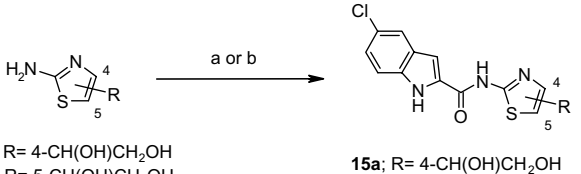



**Scheme 4.** Syntheses of compounds **13a**–**c**. Reagents and condition: (a) 5-chloroindole-2-carbonyl chloride, py.; (b) *n*-$Bu_3SnCHCH_2$, $Pd(PPh_3)_4$, LiCl, DMF, THF, 80 °C; (c) $OsO_4$, *N*-methylmorpholine-*N*-oxide, THF, $H_2O$.

theses of **14a** and **14b** were accomplished by a reduction of the corresponding α-hydroxy acid ester (Scheme 5).[30]

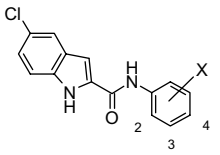
## 3. Results and discussion

The structure of the synthesized compounds and their hLGPa inhibitory activities in vitro are summarized in Tables 1–4. The 4-hydroxymethyl (**2a**) and 4-(2-hydroxyethyl) (**2b**) derivatives inhibited hLGPa with



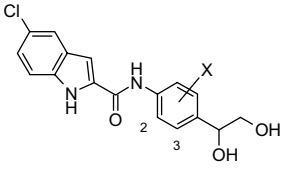

**Scheme 5.** Synthesis of compounds **15a**–**b**. Reagents: (a) 5-chloro-1*H*-indole-2-carboxylic acid, WSC·HCl, HOBt, DMF (for **15a**); (b) 5-chloro-1*H*-indole-2-carbonyl chloride, TEA, MeCN (for **15b**).

**Table 1.** SAR of *N*-aryl-5-chloroindolecarboxamides



| Entry | Compound | Position | X | hLGPa $IC_{50}$ (μM) |
|---|---|---|---|---|
| 1 | **2a** | 4 | $CH_2OH$ | 1.6 |
| 2 | **2b** | 4 | $(CH_2)_2OH$ | 1.2 |
| 3 | **2c** | 4 | $(CH_2)_3OH$ | >10 |
| 4 | **2d** | 2 | $(CH_2)_2OH$ | >10 |
| 5 | **2e** | 3 | $(CH_2)_2OH$ | 6.2 |
| 6 | **2f** | 4 | $CH(OH)CH_2OH$ | 0.90 |
| 7 | **2g** | 4 | $CH(CH_2OH)_2$ | 5.8 |
| 8 | **2h** | 4 | $CH(OH)CH_2OMe$ | 3.6 |
| 9 | **2i** | 4 | $CH(OH)CMe_2OH$ | >10 |
| 10 | CP-320626 | | | 0.92 |

**Table 2.** SAR of *N*-aryl-5-chloroindolecarboxamides with a 1,2-dihydroxyethyl moiety

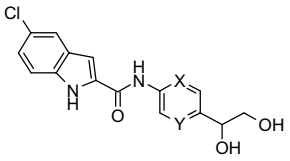


**9a**; X=2-Me
**b**; X=2-CF3
**c**; X=2-F
**d**; X=2-Cl
**e**; X=3-OMe
**f**; X=3-Ph
**g**; X=3-$CF_3$
**h**; X=3-F
**i**; X=3-$NO_2$
**j**; X=3-$NH_2$

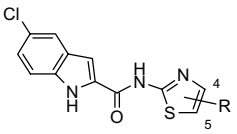
| Entry | Compound | Position | X | hLGPa $IC_{50}$ (μM) |
|---|---|---|---|---|
| 1 | **9a** | 2 | Me | 2.6 |
| 2 | **9b** | 2 | $CF_3$ | 3.2 |
| 3 | **9c** | 2 | F | 0.42 |
| 4 | **9d** | 2 | Cl | 1.1 |
| 5 | **9e** | 3 | OMe | 2.5 |
| 6 | **9f** | 3 | Ph | 7.4 |
| 7 | **9g** | 3 | $CF_3$ | 3.3 |
| 8 | **9h** | 3 | F | 0.34 |
| 9 | **9i** | 3 | $NO_2$ | 2.1 |
| 10 | **9j** | 3 | $NH_2$ | 1.7 |
| 11 | **2f** | — | H | 0.90 |
| 12 | CP-320626 | | | 0.92 |

$IC_{50}$ values of 1.6 and 1.2 μM, respectively, whereas the 4-(3-hydroxypropyl) derivative (**2c**) did not show strong inhibitory activity. For 2-hydroxyethyl substitution, the three positions showed different activities and the 4-position was the most effective (**2d**, **2e** vs **2b**). We then synthesized the 4-(1,2-dihydroxyethyl) derivative (**2f**) with a combination of the hydroxy groups of **2d** and **2e** at the 4-position. Interestingly, **2f** inhibited hLGPa with an $IC_{50}$ of 0.90 μM, which is similar to the activity of CP-320626.

**Table 3.** SAR of *N*-heteroaryl-5-chloroindolecarboxamides



**13a**; X=N, Y=CH
**b**; X=CH, Y=N
**c**; X=N, Y=N



**15a**; R= 4-$CH(OH)CH_2OH$
**b**; R= 5-$CH(OH)CH_2OH$

| Entry | Compound | hLGPa $IC_{50}$ (μM) |
|---|---|---|
| 1 | **13a** | 0.25 |
| 2 | **13b** | 4.4 |
| 3 | **13c** | 0.44 |
| 4 | **15a** | >10 |
| 5 | **15b** | 3.1 |
| 6 | **2f** | 0.90 |
| 7 | CP-320626 | 0.92 |

Lengthening the distance between the benzylic hydroxy group and the benzene ring in **2f** by addition of a one-carbon unit and changing the primary hydroxy group into a methoxy group resulted in a moderate decrease in inhibitory activity (**2f** vs **2g**, **2h**). The presence of branches in the 1,2-dihydroxyethyl chain (**2i**) was detrimental to inhibitory activity. These results suggested that the hydroxy group(s) and their appropriate position on the side chain played an important role in inhibitory activity against hLGPa, and that the introduction of substituents on the side chain did not seem to be well tolerated, probably due to steric factors. We then examined the effects of substituents on the benzene ring of **2f** (Table 2).

Introduction of a methyl or trifluoromethyl group on the 2-position of the benzene ring led to a slight decrease of inhibitory activity (**9a**,**b**). In introduction of a halogen atom to benzene ring, the chloro analogue (**9d**) was equipotent compared to parent compound **2f** and the fluoro analogue (**9c**) showed improved inhibitory activity. Substitution at the 3-position with a methoxy, phenyl or trifluoromethyl group led to a slight decrease in inhibitory activity (**9e**–**g**), but a fluoro group (**9h**) conferred potent activity, as at the 2-position. Introduction of a nitro or amino group resulted in a slight decrease of inhibitory activity (**9i**,**j**). From these results, it appears that the steric properties of substituents are more important than the electronic character.

Next, changing the benzene ring of **2f** into other aromatic rings was attempted (Table 3). Among the compounds with 6-membered aromatic rings containing nitrogen, the 3-pyridine derivative (**13b**) had lower activity whereas the 2-pyridine derivative (**13a**) showed the best in vitro activity ($IC_{50}$ = 0.25 μM). The pyrazine derivative (**13c**) was approximately two times more potent than **2f**. These results suggest that the nitrogen atom in the pyridine ring of **13a** might form an appropriate electrostatic interaction with hLGPa. On the other

**Table 4.** Inhibition of glucagon-induced glucose output in cultured primary hepatocytes and oral hypoglycemic activity in diabetic db/db mice

| Entry | Compound | hLGPa $IC_{50}$ (μM) | HGO inh. $IC_{50}$ (μM) | db/db Mice hypoglycemic activity[a] | |
|---|---|---|---|---|---|
| | | | | Dose (mg/kg, po) | % Glucose lowering |
| 1 | **13a** | 0.25 | 0.62 | 50 | 26[b] |
| 2 | | | | 30 | 13 |
| 3 | CP-320626 | 0.92 | 1.7 | 50 | 20[b] |

[a] Percentage decrease in drug-treated blood glucose concentration at 2 h postdose in db/db mice, relative to vehicle-treated controls.
[b] $P < 0.05$ versus control.

hand, the corresponding nitrogen atom in **13b** might not be properly located to give potent activity because of undesirable interactions with hydroxyl groups on **13b** itself or on hLGPa. For 5-membered rings, the 2,5-substituted thiazole derivative (**15b**) slightly decreased inhibitory activity whereas the corresponding 2,4-substituted analogue (**15a**) showed a complete loss of inhibitory activity. We suspect that these effects were caused by changing the distance and/or angle between the indole ring and the diol moiety, both of which are thought to play important roles in the inhibitory activity.

Compound **13a**, which was the most potent hLGPa inhibitor in vitro, was examined for inhibition of glucagon-induced glucose output in cultured primary hepatocytes and for oral hypoglycemic activity in diabetic db/db mice (Table 4).

Compound **13a** inhibited glucose output dose-dependently with an $IC_{50}$ value of 0.62 μM, showing approximately three times greater potency than CP-320626. A single oral administration of **13a** at a dose of 50 mg/kg significantly reduced the blood glucose level at 2 h postdose.

To understand the enzyme binding of the 5-chloro-1*H*-indole-2-carboxamides with a 1,2-dihydroxyethyl moiety, we determined the crystal structure of the hLGPa–glucose–**2f** complex at 2.45 Å resolution (Fig. 3).

As expected, the crystal structure showed that two molecules of **2f** bind at the dimer interface of the enzyme (Fig. 3A). The 5-chloroindole group is buried in a hydrophobic pocket composed of lipophilic side chains of amino acids including Leu63, Val64, and Trp67, and the indole NH moiety acts as a hydrogen bond donor to the backbone of Glu190. Furthermore, the amide moiety establishes a hydrogen bond from NH to the backbone of Thr38 and the carbonyl O makes two polar contacts with the backbone oxygen of Glu190 and a water molecule. This water molecule mediates two additional polar contacts between the carbonyl oxygen and the backbone oxygens of Asn187 and Glu190. The benzene ring appears to act as a junction between the 5-chloro-1*H*-indole-2-carboxamide and the 1,2-dihydroxyethyl side chain, because no significant interaction of the ring with the enzyme was observed. The two hydroxyl groups of **2f** have direct electrostatic interactions with the imidazole ring of His57 and the backbone of Tyr185, respectively. This supports the observation that both these two hydroxyl groups are both important for inhibitory activity (Table 1). At the medium resolution of the X-ray structure, the dominant configuration of the asymmetric carbon of **2f** was indistinguishable, but

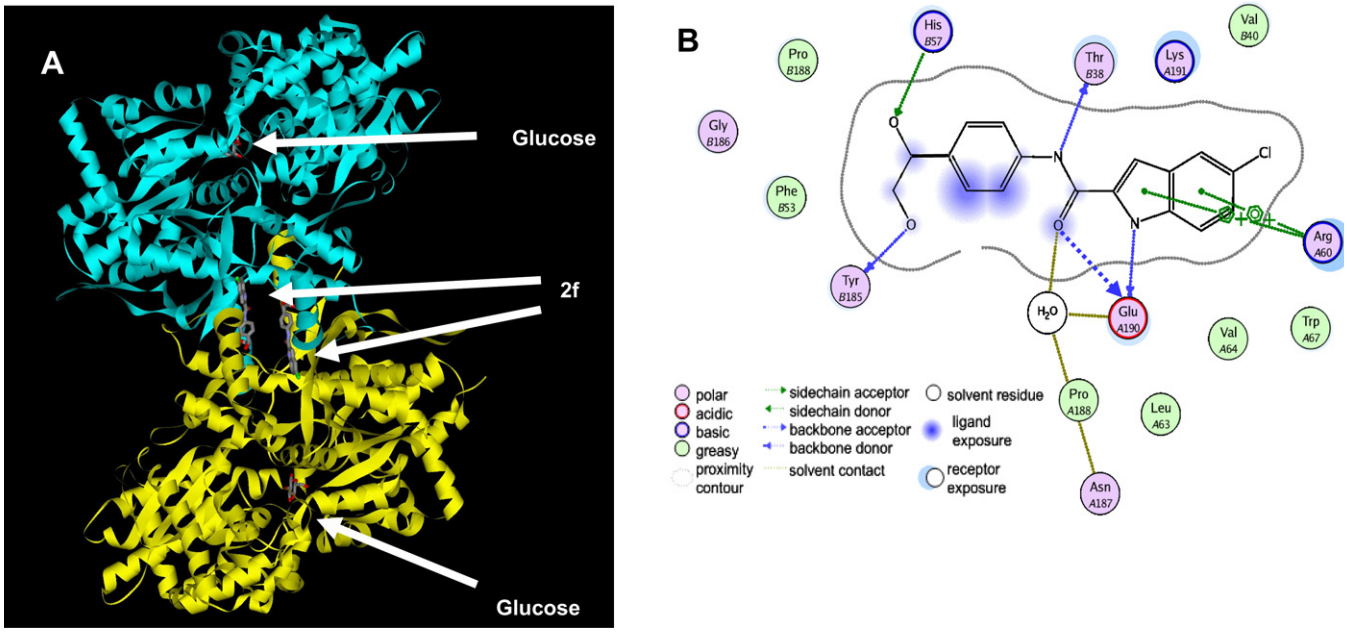



**Figure 3.** (A) Crystal structure of **2f** bound to the hLGPa dimer interface. Glucose is situated at the catalytic site. (B) Schematic diagram of the interactions made by **2f** with hLGPa revealed by crystallographic analysis. Protein residues of the two hLGPa molecules in the dimer are distinguished with labels (A) and (B), respectively. The figure was prepared using the ligand interactions application in MOE.[31]

an electron density map showed that both enantiomers are able to bind to the enzyme (Fig. 3B).

To study differences in the binding mode of **2f** and CP-320626, we overlaid the crystal structures of the two compounds (Fig. 4). The 5-chloroindole group and the amide moiety of **2f** are situated in almost the same position as those in the CP-320626 complex with muscle glycogen phosphorylase *b* (MGPb) (PDB: 1H5U).[26] Interestingly, the 1,2-dihydroxyethyl moiety of **2f** occupies a similar position to the 4-fluorophenyl moiety of CP-320626, with van der Waals interactions with a hydrophobic pocket containing Phe53, Pro188, and His57, indicating that this pocket can accept both hydrophilic and hydrophobic moieties. In contrast, the hydroxyl group of the 4-hydroxypiperidyl moiety of CP-320626 makes an indirect water-mediated hydrogen bond with rMGPb.[9] Neither of the hydroxyl groups in **2f** mimics the role of the hydroxyl group in CP-320626, but both play an important role in the enzyme binding of **2f**. Collectively, the results indicate that hydrophobic and hydrophilic interactions observed in the crystal structure stabilize the less active conformational state of the enzyme, which leads to inhibitory activity.

## 4. Conclusion

In conclusion, we synthesized a series of *N*-aryl-1*H*-indole-2-carboxamide derivatives with a 1,2-dihydroxyethyl moiety as a new class of hLGPa inhibitors. The inhibitory activity of these compounds was explained using the structure of the hLGPa–**2f** complex. Compound **13a**, which was the most potent in vitro hLGPa inhibitor in the study, inhibited glucose output from hepatocytes and reduced blood glucose in a db/db mouse model of diabetes.

## 5. Experimental

### 5.1. Chemistry

$^1$H NMR spectra were recorded on a JEOL JNM-LA300 or a JEOL JNM-EX400 spectrometer and the chemical shifts are expressed in $\delta$ (ppm) values with tetramethylsilane as an internal standard (in NMR description, s, singlet; d, doublet; dd, double doublet; t, triplet; q, quartet; m, multiplet; and br, broad peak). Mass spectra were recorded on a JEOL JMS-700T or micromass Q-Tof Ultima API spectrometer. The elemental analyses were performed with a Yanaco MT-5 microanalyzer (C, H, N) and were within ±0.4% of theoretical values. Drying of organic solutions during work-up was done over anhydrous $MgSO_4$ or $Na_2SO_4$.

**5.1.1. 5-Chloro-*N*-[4-(hydroxymethyl)phenyl]-1*H*-indole-2-carboxamide (2a).** To a solution of 4-hydroxymethylaniline (**1a**, 182 mg, 1.48 mmol) and 5-chloro-1*H*-indole-2-carboxylic acid (290 mg, 1.48 mmol) in DMF (10 mL) were added 1-ethyl-3-(3′-dimethylaminopropyl)carbodiimide hydrochloride (340 mg, 1.77 mmol) and 1-hydroxybenzotriazole hydrate (272 mg, 1.77 mmol), and the mixture was stirred at room temperature for 14 h. The resulting mixture was concentrated in vacuo and the residue was washed with $H_2O$ (60 mL) to give **2a** (385 mg, 96%) as colorless solid: $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$: 4.48 (2H, d, $J$ = 5.4 Hz), 5.14 (1H, t, $J$ = 5.4 Hz), 7.22 (1H, dd, $J$ = 8.8, 2.0 Hz), 7.28 (2H, d, $J$ = 8.3 Hz), 7.41 (1H, s), 7.47 (1H, d, $J$ = 8.3 Hz), 7.74–7.81 (3H, m), 10.27 (1H, s), 11.94 (1H, s). FAB-MS *m*/*z*: 301 $(M+1)^+$. Anal. Calcd for $C_{16}H_{13}Cl\ N_2O_2{\cdot}0.15H_2O$: C, 63.33; H, 4.42; N, 9.23. Found: C, 63.20; H, 4.26; N, 9.57.

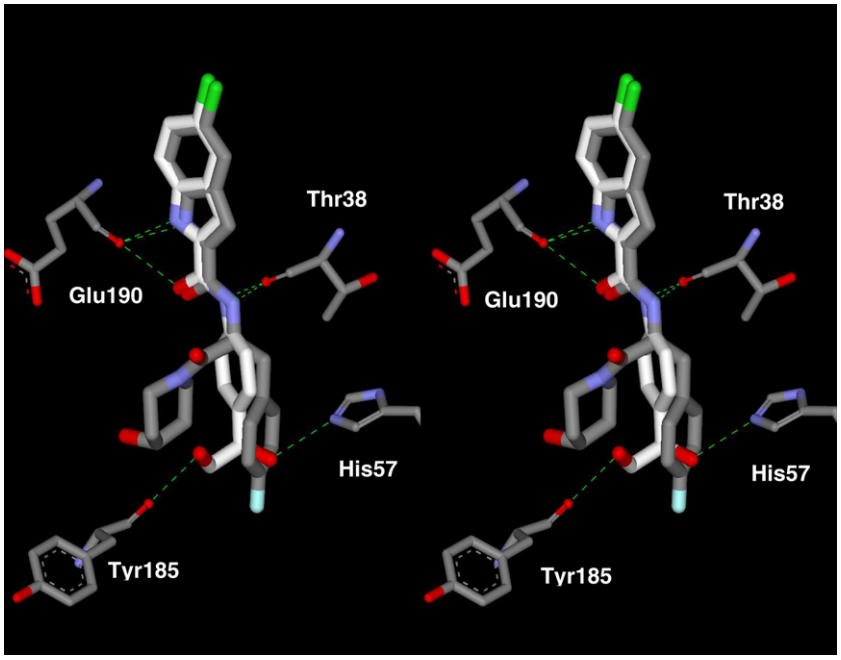



**Figure 4.** A stereoview of a docking model of **2f** with CP-320626.

**5.1.2. 5-Chloro-*N*-[4-(2-hydroxyethyl)phenyl]-1*H*-indole-2-carboxamide (2b).** The title compound was prepared in the same manner as described for **2a** using **1b** instead of **1a**, in 96% yield. $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$: 2.71 (2H, t, $J$ = 7.1 Hz), 3.55–3.68 (2H, m), 4.63 (1H, t, $J$ = 5.2 Hz), 7.15–7.28 (3H, m), 7.40 (1H, d, $J$ = 1.8 Hz), 7.47 (1H, d, $J$ = 8.8 Hz), 7.69 (2H, d, $J$ = 8.3 Hz), 7.77 (1H, d, $J$ = 1.8 Hz), 10.23 (1H, s), 11.93 (1H, s). FAB-MS $m/z$: 315 $(M+1)^+$. Anal. Calcd for $C_{17}H_{15}ClN_2O_2$: C, 64.87; H, 4.80; N, 8.90. Found: C, 64.61; H, 4.75; N, 8.92.

**5.1.3. 5-Chloro-*N*-[4-(3-hydroxypropyl)phenyl]-1*H*-indole-2-carboxamide (2c).** The title compound was prepared in the same manner as described for **2a** using **1c** instead of **1a**, in 40% yield. $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$: 1.65–1.75 (2H, m), 2.59 (2H, t, $J$ = 7.8 Hz), 3.43 (2H, q, $J$ = 5.8 Hz), 4.46 (1H, t, $J$ = 5.4 Hz), 7.15–7.25 (3H, m), 7.39 (1H, s), 7.46 (1H, d, $J$ = 8.8 Hz), 7.68 (2H, d, $J$ = 8.3 Hz), 7.76 (1H, s), 10.21 (1H, s), 11.92 (1H, s). FAB-MS $m/z$: 329 $(M+1)^+$. Anal. Calcd for $C_{18}H_{17}ClN_2O_2$: C, 65.75; H, 5.21; N, 8.52. Found: C, 65.82; H, 5.36; N, 8.54.

**5.1.4. 5-Chloro-*N*-[2-(2-hydroxyethyl)phenyl]-1*H*-indole-2-carboxamide (2d).** The title compound was prepared in the same manner as described for **2a** using **1d** instead of **1a**, in 94% yield. $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$: 2.83 (2H, t, $J$ = 6.1 Hz), 3.64–3.78 (2H, m), 5.44 (1H, t, $J$ = 4.2 Hz), 7.10–7.36 (5H, m), 7.47 (1H, d, $J$ = 8.8 Hz), 7.64 (1H, d, $J$ = 7.8 Hz), 7.73 (1H, s), 10.39 (1H, s), 11.99 (1H, s). FAB-MS $m/z$: 315 $(M+1)^+$. Anal. Calcd for $C_{17}H_{15}ClN_2O_2$: C, 64.87; H, 4.80; N, 8.90. Found: C, 64.53; H, 4.73; N, 9.02.

**5.1.5. 5-Chloro-*N*-[3-(2-hydroxyethyl)phenyl]-1*H*-indole-2-carboxamide (2e).** The title compound was prepared in the same manner as described for **2a** using **1e** instead of **1a**, in 97% yield. $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$: 2.74 (2H, t, $J$ = 7.1 Hz), 3.57–3.69 (2H, m), 4.68 (1H, t, $J$ = 5.2 Hz), 6.98 (1H, d, $J$ = 7.9 Hz), 7.23 (1H, dd, $J$ = 8.7, 2.3 Hz), 7.28 (1H, d, $J$ = 7.9 Hz), 7.42 (1H, d, $J$ = 1.7 Hz), 7.48 (1H, d, $J$ = 8.7 Hz), 7.62 (1H, s), 7.65–7.73 (1H, m), 7.78 (1H, d, $J$ = 1.7 Hz), 10.22 (1H, s), 11.92 (1H, s). FAB-MS $m/z$: 315 $(M+1)^+$. Anal. Calcd for $C_{17}H_{15}ClN_2O_2 \cdot 0.25H_2O$: C, 63.95; H, 4.89; N, 8.77. Found: C, 63.91; H, 4.89; N, 8.87.

**5.1.6. 5-Chloro-*N*-[4-(1,2-dihydroxyethyl)phenyl]-1*H*-indole-2-carboxamide (2f).** The title compound was prepared in the same manner as described for **2a** using **1f** instead of **1a**, in 76% yield. $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$: 3.40–3.47 (2H, m), 4.53 (1H, dd, $J$ = 5.8, 4.4 Hz), 4.69 (1H, t, $J$ = 5.8 Hz), 5.18 (1H, d, $J$ = 4.4 Hz), 7.22 (1H, dd, $J$ = 8.8, 2.0 Hz), 7.32 (2H, d, $J$ = 8.8 Hz), 7.40 (1H, d, $J$ = 1.5 Hz), 7.46 (1H, d, $J$ = 8.8 Hz), 7.73 (2H, d, $J$ = 8.8 Hz), 7.77 (1H, d, $J$ = 2.0 Hz), 10.25 (1H, s), 11.93 (1H, s). FAB-MS $m/z$: 331 $(M+1)^+$. Anal. Calcd for $C_{17}H_{15}ClN_2O_3$: C, 61.73; H, 4.57; N, 8.47. Found: C, 61.63; H, 4.41; N, 8.54.

**5.1.7. 5-Chloro-*N*-{4-[2-hydroxy-1-(hydroxymethyl)ethyl]-phenyl}-1*H*-indole-2-carboxamide (2g).** The title compound was prepared in the same manner as described for **2a** using **1g** instead of **1a**, in 59% yield. $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$: 2.75–2.82 (1H, m), 3.56–3.61 (2H, m), 3.66–3.72 (2H, m), 4.52 (2H, t, $J$ = 4.6 Hz), 7.21–7.23 (3H, m), 7.40 (1H, d, $J$ = 1.7 Hz), 7.47 (1H, d, $J$ = 8.8 Hz), 7.67–7.69 (2H, m), 7.76 (1H, d, $J$ = 1.7 Hz), 10.21 (1H, s), 11.93 (1H, br s). FAB-MS $m/z$: 345 $(M+1)^+$. Anal. Calcd for $C_{18}H_{17}ClN_2O_3 \cdot 0.04CHCl_3$: C, 61.98; H, 4.91; N, 8.01. Found: C, 61.76; H, 4.80; N, 7.94.

**5.1.8. 5-Chloro-*N*-[4-(1-hydroxy-2-methoxyethyl)phenyl]-1*H*-indole-2-carboxamide (2h).** The title compound was prepared in the same manner as described for **2a** using **1h** instead of **1a**, in 56% yield. $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$: 3.27 (3H, s), 3.35–3.44 (2H, m), 4.66–4.70 (1H, m), 5.31 (1H, d, $J$ = 3.4 Hz), 7.22 (1H, dd, $J$ = 8.8, 2.0 Hz), 7.34 (2H, d, $J$ = 8.3 Hz), 7.41 (1H, s), 7.47 (1H, d, $J$ = 8.8 Hz), 7.73 (2H, d, $J$ = 8.3 Hz), 7.77 (1H, d, $J$ = 2.0 Hz), 10.26 (1H, br s), 11.94 (1H, br s). FAB-MS $m/z$: 345 $(M+1)^+$. Anal. Calcd for $C_{18}H_{17}ClN_2O_3$: C, 62.70; H, 4.97; N, 8.12. Found: C, 62.32; H, 4.83; N, 8.02.

**5.1.9. 5-Chloro-*N*-[4-(1,2-dihydroxy-2-methylpropyl)-phenyl]-1*H*-indole-2-carboxamide (2i).** The title compound was prepared in the same manner as described for **2a** using **1i** instead of **1a**, in 74% yield. $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$: 0.98 (3H, s), 1.07 (3H, s), 4.21 (1H, s), 4.31 (1H, d, $J$ = 4.4 Hz), 5.13 (1H, d, $J$ = 4.4 Hz), 7.22 (1H, dd, $J$ = 8.8, 2.4 Hz), 7.33 (2H, d, $J$ = 8.8 Hz), 7.38–7.50 (2H, m), 7.69 (2H, d, $J$ = 8.8 Hz), 7.77 (1H, d, $J$ = 2.0 Hz), 10.23 (1H, s), 11.93 (1H, s). FAB-MS $m/z$: 359 $(M+1)^+$. Anal. Calcd for $C_{19}H_{19}ClN_2O_3$: C, 63.60; H, 5.34; N, 7.81. Found: C, 63.33; H, 5.28; N, 7.73.

**5.1.10. 2-Methyl-1-(4-nitrophenyl)propane-1,2-diol (5).** To a mixture of isopropyltriphenylphosphonium iodide (9.43 g, 21.8 mmol) and THF (60 mL) was added *n*-BuLi (1.55 M in *n*-hexane, 14.0 mL, 21.7 mmol) at −20 °C, then the mixture was stirred at same temperature for 45 min. To this reaction mixture was added to a solution of 4-nitrobenzaldehyde (2.64 g, 17.5 mmol) in THF (10.0 mL), then the mixture was stirred at room temperature for 1 h, and then under reflux for 1.5 h. The resulting mixture was concentrated in vacuo and the residue was partitioned between AcOEt (2× 100 mL) and satd $NH_4Cl$ (100 mL), and the AcOEt layer was washed with satd NaCl, then dried and concentrated in vacuo. The residue was purified by column chromatography on silica gel (*n*-hexane/AcOEt = 5:1) to give a reddish oil (900 mg). To a solution of this reddish oil in THF/$H_2O$ (6:1, 7.0 mL) were added osmium tetroxide (0.08 Mt-*t*-BuOH solution; 5.0 mL, 0.4 mmol) and *N*-methylmorpholine-*N*-oxide (860 mg, 7.34 mmol), and the mixture was stirred at room temperature for 72 h. The resulting mixture was concentrated in vacuo and the residue was partitioned between AcOEt (60 mL) and 10% $NaHSO_3$ (30 mL), and the AcOEt layer was washed with satd NaCl, then dried and concentrated in vacuo. The residue was purified by column chromatography on silica gel ($CHCl_3$/MeOH = 50:1) to give **5**

(325 mg, 9% for 2 steps) as a colorless solid. $^1$H NMR (300 MHz, $CDCl_3$) $\delta$: 1.09 (3H, s), 1.28 (3H, s), 2.00 (1H, s), 2.83 (1H, s), 4.64 (1H, s), 7.57 (2H, d, $J$ = 9.0 Hz), and 8.19 (2H, d, $J$ = 10.1 Hz). FAB-MS $m/z$: 211 $(M)^+$.

**5.1.11. 1-(4-Aminophenyl)-2-methylpropane-1,2-diol (1i).** To a solution of **5** (316 mg, 1.50 mmol) in EtOH/THF (5:1, 12.0 mL) was added Pd/C (10 w/w %, 60 mg), and the mixture was stirred under hydrogen atmosphere at room temperature for 2.5 h. The catalyst was filtrated on Celite and the filtrate was concentrated in vacuo to give **1i** (259 mg, 95%) as a colorless solid. $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$: 0.93 (3H, s), 0.98 (3H, s), 4.01 (1H, s), 4.13 (1H, d, $J$ = 3.4 Hz), 4.78 (1H, d, $J$ = 3.9 Hz), 4.84 (2H, s), 6.46 (2H, d, $J$ = 8.3 Hz), and 6.97 (2H, d, $J$ = 8.3 Hz). EI-MS $m/z$: 181 $(M)^+$.

**5.1.12. *N*-(4-Bromo-2-methylphenyl)-5-chloro-1*H*-indole-2-carboxamide (7a).** The title compound was prepared in the same manner as described for **2a** using **6a** instead of **1a**, in 72% yield. $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$: 2.27 (3H, s), 7.23 (1H, dd, $J$ = 8.8, 1.5 Hz), 7.32–7.40 (2H, m), 7.44 (1H, dd, $J$ = 8.3, 1.5 Hz), 7.47 (1H, d, $J$ = 8.7 Hz), 7.53 (1H, d, $J$ = 1.9 Hz), 7.76 (1H, d, $J$ = 1.4 Hz), 9.99 (1H, s), and 11.96 (1H, s). FAB-MS $m/z$: 364 $(M+1)^+$.

**5.1.13. *N*-(4-Bromo-2-fluorophenyl)-5-chloro-1*H*-indole-2-carboxamide (7b).** To a mixture of 5-chloro-1*H*-indole-2-carbonyl chloride (428 mg, 2.00 mmol) and 4-bromo-2-fluoroaniline (**6b**, 380 mg, 2.00 mmol) was added pyridine (20 mL) and the mixture was stirred at room temperature for 21 h. The resulting mixture was concentrated in vacuo and the residue was washed with $H_2O$, 0.5 M HCl, and 0.5 M NaOH, then ether to give **7b** (453 mg, 62%) as an orange solid. $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$: 7.24 (1H, dd, $J$ = 8.8, 2.0 Hz), 7.39 (1H, d, $J$ = 1.5 Hz), 7.47 (2H, d, $J$ = 8.8 Hz), 7.61–7.69 (2H, m), 7.77 (1H, d, $J$ = 1.5 Hz), 10.25 (1H, s), and 11.99 (1H, s). FAB-MS $m/z$: 368 $(M+1)^+$.

**5.1.14. *N*-(4-Bromo-2-chlorophenyl)-5-chloro-1*H*-indole-2-carboxamide (7c).** The title compound was prepared in the same manner as described for **7b** using **6c** instead of **6b**, in 76% yield. $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$: 7.23 (1H, dd, $J$ = 8.8, 1.0 Hz), 7.38 (1H, d, $J$ = 2.0 Hz), 7.47 (1H, d, $J$ = 8.8 Hz), 7.56–7.65 (2H, m), 7.77 (1H, d, $J$ = 2.0 Hz), 7.86 (1H, d, $J$ = 2.0 Hz), 10.18 (1H, s), and 12.00 (1H, s). FAB-MS $m/z$: 384 $(M+1)^+$.

**5.1.15. *N*-[4-Bromo-2-(trifluoromethyl)phenyl]-5-chloro-1*H*-indole-2-carboxamide (7d).** The title compound was prepared in the same manner as described for **7b** using **6d** instead of **6b**, in 61% yield. $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$: 7.20–7.22 (1H, m), 7.34 (1H, s), 7.45 (1H, d, $J$ = 8.4 Hz), 7.55 (1H, d, $J$ = 8.4 Hz), 7.73–8.20 (3H, m), 10.24 (1H, s), and 11.98 (1H, s). FAB-MS $m/z$: 418 $(M+1)^+$.

**5.1.16. *N*-(4-Bromo-3-methoxyphenyl)-5-chloro-1*H*-indole-2-carboxamide (7e).** The title compound was prepared in the same manner as described for **2a** using **6e** instead of **6b**, in 96% yield. $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$: 3.87 (3H, s), 7.24 (1H, dd, $J$ = 8.3, 2.0 Hz), 7.40–7.50 (3H, m), 7.55 (1H, d, $J$ = 8.3 Hz), 7.63 (1H, d, $J$ = 2.0 Hz), 7.79 (1H, d, $J$ = 2.0 Hz), 10.38 (1H, s), and 11.97 (1H, s). FAB-MS $m/z$: 380 $(M+1)^+$.

**5.1.17. *N*-(6-Bromobiphenyl-3-yl)-5-chloro-1*H*-indole-2-carboxamide (7f).** The title compound was prepared in the same manner as described for **2a** using **6f** instead of **6b**, in 84% yield. $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$: 7.22 (1H, dd, $J$ = 8.8, 2.0 Hz), 7.40–7.53 (7H, m), 7.74–7.85 (4H, m), 10.43 (1H, s), and 11.95 (1H, s). FAB-MS $m/z$: 426 $(M+1)^+$.

**5.1.18. *N*-(4-Bromo-3-fluorophenyl)-5-chloro-1*H*-indole-2-carboxamide (7g).** The title compound was prepared in the same manner as described for **7b** using **6g** instead of **6b**, in 66% yield. $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$: 7.25 (1H, dd, $J$ = 8.8, 2.0 Hz), 7.43 (1H, s), 7.49 (1H, d, $J$ = 8.8 Hz), 7.58 (1H, dd, $J$ = 8.8, 2.0 Hz), 7.70 (1H, t, $J$ = 8.3 Hz), 7.80 (1H, d, $J$ = 1.5 Hz), 7.95 (1H, dd, $J$ = 11.2, 2.0 Hz), 10.56 (1H, s), 12.00 (1H, s). FAB-MS $m/z$: 368 $(M+1)^+$.

**5.1.19. *N*-[4-Bromo-3-(trifluoromethyl)phenyl]-5-chloro-1*H*-indole-2-carboxamide (7h).** The title compound was prepared in the same manner as described for **7b** using **6h** instead of **6b**, in 57% yield. $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$: 7.25 (1H, dd, $J$ = 8.8, 2.0 Hz), 7.44 (1H, d, $J$ = 1.6 Hz), 7.48 (1H, d, $J$ = 8.8 Hz), 7.81 (1H, d, $J$ = 2.0 Hz), 7.89 (1H, d, $J$ = 8.8 Hz), 8.08 (1H, dd, $J$ = 8.8, 2.4 Hz), 8.35 (1H, d, $J$ = 2.4 Hz), 10.66 (1H, s), and 12.03 (1H, s). FAB-MS $m/z$: 418 $(M+1)^+$.

**5.1.20. *N*-(4-Bromo-3-nitrophenyl)-5-chloro-1*H*-indole-2-carboxamide (7i).** To a solution of 5-chloro-1*H*-indole-2-carboxylic acid (780 mg, 3.99 mmol) and 4-bromo-3-nitroaniline (830 mg, 4.00 mmol) in pyridine (15 mL) was added phosphoric trichloride (0.41 mL, 4.41 mmol) at −25 °C, then the mixture was stirred at room temperature for 30 min. To this reaction mixture was added ice-water (150 mL) and extracted with AcOEt (200 mL). The organic layer was washed with 0.5 M HCl (100 mL) and satd NaCl then dried and concentrated in vacuo. The obtained solid was washed with ether and MeCN, then dried to give **7i** (390 mg, 25%) as a reddish powder: $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$: 7.26 (1H, dd, $J$ = 8.8, 2.0 Hz), 7.45–7.51 (2H, m), 7.80–8.07 (3H, m), 8.57 (1H, d, $J$ = 2.4 Hz), 10.86 (1H, s), 12.08 (1H, s). FAB-MS $m/z$: 395 $(M+1)^+$.

**5.1.21. 5-Chloro-*N*-(2-methyl-4-vinylphenyl)-1*H*-indole-2-carboxamide (8a).** To a mixture of *N*-(4-bromo-2-methylphenyl)-5-chloro-1*H*-indole-2-carboxamide **7a** (436 mg, 1.20 mmol) and tributyl(vinyl)stannane (1.14 g, 3.60 mmol) in DMF-THF (1:1, 8 mL) were added $Pd(PPh_3)_4$ (70 mg, 0.061 mmol) and LiCl (153 mg, 3.60 mmol), then the mixture was refluxed for 8 h under Ar atmosphere. The resulting mixture was concentrated in vacuo and the residue was dissolved in satd NaCl, then extracted with AcOEt (80 mL). The extract was dried and concentrated in vacuo. The obtained solid was washed with ether to give **8a** (168 mg, 45%) as a col-

orless powder. $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$: 2.27 (3H, s), 5.25 (1H, d, $J$ = 11.2 Hz), 5.82 (1H, d, $J$ = 17.6 Hz), 6.72 (1H, dd, $J$ = 17.6, 11.2 Hz), 7.22 (1H, dd, $J$ = 8.8, 2.0 Hz), 7.30–7.68 (5H, m), 7.45 (1H, d, $J$ = 1.9 Hz), 9.94 (1H, s), and 11.93 (1H, s). FAB-MS $m/z$: 311 $(M+1)^+$.

**5.1.22. 5-Chloro-*N*-(2-fluoro-4-vinylphenyl)-1*H*-indole-2-carboxamide (8b).** The title compound was prepared in the same manner as described for **8a** using **7b** instead of **7a**, in 91% yield. $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$: 5.33 (1H, d, $J$ = 10.8 Hz), 5.91 (1H, d, $J$ = 17.6 Hz), 6.74 (1H, dd, $J$ = 17.6, 10.8 Hz), 7.23 (1H, dd, $J$ = 8.8, 2.0 Hz), 7.32–7.77 (6H, m), 10.19 (1H, s), and 11.98 (1H, s). FAB-MS $m/z$: 315 $(M+1)^+$.

**5.1.23. 5-Chloro-*N*-(2-chloro-4-vinylphenyl)-1*H*-indole-2-carboxamide (8c).** The title compound was prepared in the same manner as described for **8a** using **7c** instead of **7a**, in 68% yield. $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$: 5.35 (1H, d, $J$ = 11.2 Hz), 5.94 (1H, d, $J$ = 17.6 Hz), 6.76 (1H, dd, $J$ = 17.6, 11.2 Hz), 7.23 (1H, dd, $J$ = 8.8, 2.0 Hz), 7.39 (1H, d, $J$ = 2.0 Hz), 7.47 (1H, d, $J$ = 8.8 Hz), 7.52 (1H, dd, $J$ = 8.3, 2.0 Hz), 7.62 (1H, d, $J$ = 8.3 Hz), 7.70 (1H, d, $J$ = 2.0 Hz), 7.77 (1H, d, $J$ = 1.5 Hz), 10.11 (1H, s), and 11.99 (1H, s). FAB-MS $m/z$: 331 $(M+1)^+$.

**5.1.24. 5-Chloro-*N*-[2-(trifluoromethyl)-4-vinylphenyl]-1*H*-indole-2-carboxamide (8d).** The title compound was prepared in the same manner as described for **8a** using **7d** instead of **7a**, in 54% yield. $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$: 5.43 (1H, d, $J$ = 10.8 Hz), 6.02 (1H, d, $J$ = 18.0 Hz), 6.83 (1H, dd, $J$ = 18.0, 10.8 Hz), 7.23 (1H, dd, $J$ = 8.8, 1.9 Hz), 7.33 (1H, s), 7.47 (1H, d, $J$ = 8.8 Hz), 7.57 (1H, d, $J$ = 8.3 Hz), 7.77 (1H, d, $J$ = 1.9 Hz), 7.87–7.89 (2H, m), 10.18 (1H, s), and 11.97 (1H, s). FAB-MS $m/z$: 365 $(M+1)^+$.

**5.1.25. 5-Chloro-*N*-(3-methoxy-4-vinylphenyl)-1*H*-indole-2-carboxamide (8e).** The title compound was prepared in the same manner as described for **8a** using **7e** instead of **7a**, in 33% yield. $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$: 3.83 (3H, s), 5.19 (1H, d, $J$ = 10.8 Hz), 5.74 (1H, d, $J$ = 18.0 Hz), 6.91 (1H, dd, $J$ = 18.0, 10.8 Hz), 7.23 (1H, dd, $J$ = 8.8, 2.0 Hz), 7.41–7.56 (5H, m), 7.78 (1H, d, $J$ = 1.6 Hz), 10.32 (1H, s), and 11.95 (1H, s). FAB-MS $m/z$: 327 $(M+1)^+$.

**5.1.26. 5-Chloro-*N*-(6-vinylbiphenyl-3-yl)-1*H*-indole-2-carboxamide (8f).** The title compound was prepared in the same manner as described for **8a** using **7f** instead of **7a**, in 57% yield. $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$: 5.19 (1H, d, $J$ = 11.2 Hz), 5.75 (1H, d, $J$ = 17.5 Hz), 6.59 (1H, dd, $J$ = 17.6, 10.7 Hz), 7.20–7.98 (12H, m), 10.39 (1H, s), and 11.94 (1H, s). FAB-MS $m/z$: 371 $(M-1)^-$.

**5.1.27. 5-Chloro-*N*-(3-fluoro-4-vinylphenyl)-1*H*-indole-2-carboxamide (8g).** The title compound was prepared in the same manner as described for **8a** using **7g** instead of **7a**, in 65% yield. $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$: 5.37 (1H, d, $J$ = 11.5 Hz), 5.86 (1H, d, $J$ = 17.9 Hz), 6.80 (1H, dd, $J$ = 17.9, 11,5 Hz), 7.24 (1H, dd, $J$ = 8.8, 1.9 Hz), 7.43 (1H, s), 7.48 (1H, d, $J$ = 8.8 Hz), 7.58 (1H, dd, $J$ = 8.8, 2.0 Hz), 7.66 (1H, t, $J$ = 8.8 Hz), 7.79–7.83 (2H, m), 10.50 (1H, s), and 12.00 (1H, s). FAB-MS $m/z$: 313 $(M-1)^-$.

**5.1.28. 5-Chloro-*N*-[3-(trifluoromethyl)-4-vinylphenyl]-1*H*-indole-2-carboxamide (8h).** The title compound was prepared in the same manner as described for **8a** using **7h** instead of **7a**. This compound was an inseparable mixture with **7h**, therefore further purification was not attempted.

**5.1.29. 5-Chloro-*N*-(3-nitro-4-vinylphenyl)-1*H*-indole-2-carboxamide (8i).** The title compound was prepared in the same manner as described for **8a** using **7i** instead of **7a**, in 46% yield. $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$: 5.47 (1H, d, $J$ = 11.7 Hz), 5.87 (1H, d, $J$ = 17.4 Hz), 7.00 (1H, dd, $J$ = 17.2, 11.7 Hz), 7.25 (1H, dd, $J$ = 17.4, 2.2 Hz), 7.45–7.52 (2H, m), 7.81–7.88 (2H, m), 8.12 (1H, dd, $J$ = 8.8, 2.4 Hz), 8.52 (1H, d, $J$ = 2.2 Hz), 10.73 (1H, s), and 12.03 (1H, s). FAB-MS $m/z$: 340 $(M-1)^-$.

**5.1.30. 5-Chloro-*N*-[4-(1,2-dihydroxyethyl)-2-methylphenyl]-1*H*-indole-2-carboxamide (9a).** To a solution of **8a** (160 mg, 0.52 mmol) in THF/$H_2O$ (4:1, 7.5 mL) were added osmium tetroxide (0.08 Mt-*t*-BuOH solution; 0.65 mL, 0.052 mmol) and *N*-methylmorpholine-*N*-oxide (90 mg, 0.77 mmol), and the mixture was stirred at room temperature for 22 h. The resulting mixture was concentrated in vacuo and the residue was partitioned between AcOEt (30 mL) and 10% $NaHSO_3$ (10 mL), and the AcOEt layer was washed with satd NaCl, then dried and concentrated in vacuo. The residue was purified by column chromatography on silica gel ($CHCl_3$/MeOH = 95:5) to give **9a** (45 mg, 25%) as a colorless solid. $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$: 2.25 (3H, s), 3.45 (2H, t, $J$ = 5.8 Hz), 4.50–4.56 (1H, m), 4.72 (1H, t, $J$ = 5.9 Hz), 5.20 (1H, d, $J$ = 4.4 Hz), 7.17–7.36 (5H, m), 7.46 (1H, d, $J$ = 8.8 Hz), 7.75 (1H, d, $J$ = 1.5 Hz), 9.92 (1H, s), and 11.92 (1H, s). FAB-MS $m/z$: 345 $(M+1)^+$. Anal. Calcd for $C_{18}H_{17}ClN_2O_3$: C, 62.70; H, 4.97; N, 8.12. Found: C, 62.39; H, 5.02; N, 8.10.

**5.1.31. 5-Chloro-*N*-[4-(1,2-dihydroxyethyl)-2-fluorophenyl]-1*H*-indole-2-carboxamide (9b).** The title compound was prepared in the same manner as described for **9a** using **8b** instead of **8a**, in 34% yield. $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$: 3.45–3.49 (2H, m), 4.57 (1H, dt, $J$ = 5.8, 5.4 Hz), 4.77 (1H, dd, $J$ = 5.8, 5.4 Hz), 5.38 (1H, d, $J$ = 4.9 Hz), 7.18–7.27 (3H, m), 7.38 (1H, d, $J$ = 0.9 Hz), 7.46 (1H, d, $J$ = 8.8 Hz), 7.51–7.56 (1H, m), 7.76 (1H, d, $J$ = 1.9 Hz), 10.15 (1H, s), and 11.96 (1H, s). FAB-MS $m/z$: 349 $(M+1)^+$. Anal. Calcd for $C_{17}H_{14}ClFN_2O_3 \cdot 0.2H_2O \cdot 0.02CHCl_3$: C, 57.62; H, 4.10; N, 7.90. Found: C, 57.65; H, 3.86; N, 7.89.

**5.1.32. 5-Chloro-*N*-[2-chloro-4-(1,2-dihydroxyethyl)-phenyl]-1*H*-indole-2-carboxamide (9c).** The title compound was prepared in the same manner as described for **9a** using **8c** instead of **8a**, in 63% yield. $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$: 3.45–3.52 (2H, m), 4.57 (1H, dt, $J$ = 5.9, 5.3 Hz), 4.79 (1H, dd, $J$ = 5.9, 5.4 Hz), 5.41 (1H, d, $J$ = 4.4 Hz), 7.23 (1H, dd, $J$ = 8.8, 2.0 Hz), 7.35

(1H, dd, $J$ = 8.3, 1.5 Hz), 7.37 (1H, s), 7.46 (1H, d, $J$ = 8.8 Hz), 7.51 (1H, d, $J$ = 1.5 Hz), 7.53 (1H, d, $J$ = 8.3 Hz), 7.76 (1H, d, $J$ = 1.9 Hz), 10.09 (1H, s), and 11.97 (1H, s). FAB-MS $m/z$: 365 $(M+1)^+$. Anal. Calcd for $C_{17}H_{14}Cl_2N_2O_3$: C, 54.56; H, 4.04; N, 7.49. Found: C, 54.35; H, 3.92; N, 7.44.

**5.1.33. 5-Chloro-*N*-[4-(1,2-dihydroxyethyl)-2-(trifluoromethyl)phenyl]-1*H*-indole-2-carboxamide (9d).** The title compound was prepared in the same manner as described for **9a** using **8d** instead of **8a**, in 87% yield. $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$: 3.46–3.58 (2H, m), 4.67 (1H, q, $J$ = 5.4 Hz), 4.84 (1H, dd, $J$ = 5.8, 5.4 Hz), 5.52 (1H, d, $J$ = 4.9 Hz), 7.23 (1H, dd, $J$ = 8.8, 2.0 Hz), 7.34 (1H, d, $J$ = 1.4 Hz), 7.46 (1H, d, $J$ = 8.8 Hz), 7.50 (1H, d, $J$ = 8.3 Hz), 7.69 (1H, d, $J$ = 8.3 Hz), 7.76–7.78 (2H, m), 10.14 (1H, s), and 11.96 (1H, s). FAB-MS $m/z$: 399 $(M+1)^+$. Anal. Calcd for $C_{18}H_{14}ClF_3N_2O_3{\cdot}0.1H_2O{\cdot}0.02CHCl_3$: C, 53.71; H, 3.56; N, 6.95. Found: C, 53.36; H, 3.41; N, 6.99.

**5.1.34. 5-Chloro-*N*-[4-(1,2-dihydroxyethyl)-3-methoxyphenyl]-1*H*-indole-2-carboxamide (9e).** The title compound was prepared in the same manner as described for **9a** using **8e** instead of **8a**, in 24% yield. $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$: 3.20–3.50 (2H, m), 3.80 (3H, s), 4.63 (1H, t, $J$ = 5.9 Hz), 4.83–4.89 (1H, m), 5.00 (1H, d, $J$ = 4.9 Hz), 7.23 (1H, dd, $J$ = 8.8, 2.0 Hz), 7.34–7.49 (5H, m), 7.77 (1H, d, $J$ = 1.5 Hz), 10.23 (1H, s), and 11.93 (1H, s). FAB-MS $m/z$: 361 $(M+1)^+$. Anal. Calcd for $C_{18}H_{17}ClN_2O_4$: C, 59.92; H, 4.75; N, 7.76. Found: C, 60.01; H, 4.82; N, 7.86.

**5.1.35. 5-Chloro-*N*-[6-(1,2-dihydroxyethyl)biphenyl-3-yl]-1*H*-indole-2-carboxamide (9f).** The title compound was prepared in the same manner as described for **9a** using **8f** instead of **8a**, in 4% yield. $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$: 3.34–3.43 (2H, m), 4.58–4.63 (2H, m), 5.06 (1H, d, $J$ = 3.9 Hz), 7.21 (1H, dd, $J$ = 8.8, 1.9 Hz), 7.37–7.50 (7H, m), 7.54 (1H, d, $J$ = 8.8 Hz), 7.61 (1H, d, $J$ = 2.4 Hz), 7.77 (1H, d, $J$ = 2.0 Hz), 7.84 (1H, dd, $J$ = 8.8, 2.0 Hz), 10.24 (1H, s), and 11.91 (1H, s). FAB-MS $m/z$: 407 $(M+1)^+$. Anal. Calcd for $C_{23}H_{19}ClN_2O_3$: C, 67.90; H, 4.71; N, 6.89. Found: C, 67.80; H, 4.75; N, 6.92.

**5.1.36. 5-Chloro-*N*-[4-(1,2-dihydroxyethyl)-3-fluorophenyl]-1*H*-indole-2-carboxamide (9g).** The title compound was prepared in the same manner as described for **9a** using **8g** instead of **8a**, in 68% yield. $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$: 3.40–3.51 (2H, m), 4.77–4.83 (2H, m), 5.33 (1H, d, $J$ = 4.4 Hz), 7.23 (1H, dd, $J$ = 8.8, 2.0 Hz), 7.41–7.48 (3H, m), 7.54 (1H, dd, $J$ = 8.8, 2.0 Hz), 7.71 (1H, dd, $J$ = 13.0, 1.9 Hz), 7.79 (1H, d, $J$ = 2.0 Hz), 10.41 (1H, s), and 11.98 (1H, s). FAB-MS $m/z$: 349 $(M+1)^+$. Anal. Calcd for $C_{17}H_{14}ClFN_2O_3{\cdot}0.2H_2O{\cdot}0.05CHCl_3$: C, 57.15; H, 4.06; N, 7.82. Found: C, 57.45; H, 4.27; N, 7.73.

**5.1.37. 5-Chloro-*N*-[4-(1,2-dihydroxyethyl)-3-(trifluoromethyl)phenyl]-1*H*-indole-2-carboxamide (9h).** The title compound was prepared in the same manner as described for **9a** using **8h** instead of **8a**, in 29% yield for two steps. $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$: 3.37–3.46 (2H, m), 4.84–4.90 (2H, m), 5.50 (1H, d, $J$ = 4.3 Hz), 7.24 (1H, dd, $J$ = 8.6, 2.2 Hz), 7.44 (1H, d, $J$ = 1.6 Hz), 7.48 (1H, d, $J$ = 8.6 Hz), 7.76 (1H, d, $J$ = 8.6 Hz), 7.80 (1H, d, $J$ = 2.2 Hz), 8.09 (1H, dd, $J$ = 8.6, 1.6 Hz), 8.17 (1H, d, $J$ = 2.2 Hz), 10.54 (1H, s), and 11.99 (1H, s). FAB-MS $m/z$: 399 $(M+1)^+$. Anal. Calcd for $C_{18}H_{14}ClF_3N_2O_3{\cdot}0.4H_2O$: C, 53.25; H, 3.67; N, 6.90. Found: C, 53.25; H, 3.36; N, 6.98.

**5.1.38. 5-Chloro-*N*-[4-(1,2-dihydroxyethyl)-3-nitrophenyl]-1*H*-indole-2-carboxamide (9i).** The title compound was prepared in the same manner as described for **9a** using **8i** instead of **8a**, in 62% yield. $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$: 3.42–3.50 (2H, m), 4.88 (1H, t, $J$ = 5.9 Hz), 5.09 (1H, q, $J$ = 5.4 Hz), 5.59 (1H, d, $J$ = 4.4 Hz), 7.25 (1H, dd, $J$ = 8.8, 2.0 Hz), 7.44–7.50 (2H, m), 7.77–7.81 (2H, m), 8.08 (1H, dd, $J$ = 8.8, 2.0 Hz), 8.41 (1H, d, $J$ = 1.9 Hz), 10.65 (1H, s), and 12.01 (1H, s). ESI-MS $m/z$: 376 $(M+1)^+$. Anal. Calcd for $C_{17}H_{14}ClN_3O_5{\cdot}H_2O$: C, 51.85; H, 4.10; N, 10.67. Found: C, 51.87; H, 3.85; N, 10.58.

**5.1.39. *N*-[3-Amino-4-(1,2-dihydroxyethyl)phenyl]-5-chloro-1*H*-indole-2-carboxamide (9j).** To a solution of 5-chloro-*N*-[4-(1,2-dihydroxyethyl)-3-nitrophenyl]-1*H*-indole-2-carboxamide **9i** (137 mg, 0.36 mmol) in AcOH (5 mL) were added Zn powder (240 mg, 3.67 mmol), and the mixture was stirred at room temperature for 1.5 h. The excess Zn was removed by filtration and the filtrate was concentrated in vacuo. The residue was purified by column chromatography on silica gel ($CHCl_3$/MeOH/$NH_4OH$ = 100:10:1) to give **9j** (37 mg, 30%) as slightly yellow solid. $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$: 3.43–3.50 (2H, m), 4.55–4.65 (2H, m), 5.03 (2H, s), 5.16 (1H, d, $J$ = 3.9 Hz), 6.92 (1H, dd, $J$ = 8.0, 2.0 Hz), 7.04 (1H, d, $J$ = 8.3 Hz), 7.10 (1H, d, $J$ = 2.0 Hz), 7.20 (1H, dd, $J$ = 8.8, 1.9 Hz), 7.37 (1H, d, $J$ = 1.5 Hz), 7.47 (1H, d, $J$ = 8.8 Hz), 7.74 (1H, d, $J$ = 2.0 Hz), 9.99 (1H, s), and 11.85 (1H, s). FAB-MS $m/z$: 346 $(M+1)^+$. Anal. Calcd for $C_{17}H_{16}ClN_3O_3$: C, 59.05; H, 4.66; N, 12.15. Found: C, 58.85; H, 4.67; N, 12.14.

**5.1.40. *N*-(5-Bromopyridin-2-yl)-5-chloro-1*H*-indole-2-carboxamide (11a).** The title compound was prepared in the same manner as described for **7b** using **10a** instead of **6b**, in 70% yield. $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$: 7.24 (1H, dd, $J$ = 8.8, 1.9 Hz), 7.48 (1H, d, $J$ = 8.8 Hz), 7.62 (1H, d, $J$ = 1.5 Hz), 7.74 (1H, d, $J$ = 2.0 Hz), 8.09 (1H, dd, $J$ = 8.8, 2.5 Hz), 8.22 (1H, d, $J$ = 8.8 Hz), 8.54 (1H, d, $J$ = 2.0 Hz), 11.09 (1H, s), and 12.01 (1H, s). FAB-MS $m/z$: 351 $(M+1)^+$.

**5.1.41. *N*-(6-bromopyridin-3-yl)-5-chloro-1*H*-indole-2-carboxamide (11b).** The title compound was prepared in the same manner as described for **7b** using **10b** instead of **6b**, in 74% yield. $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$: 7.25 (1H, dd, $J$ = 8.7, 1.4 Hz), 7.43 (1H, s), 7.48 (1H, d, $J$ = 8.7 Hz), 7.66 (1H, d, $J$ = 8.3 Hz), 7.81 (1H, d, $J$ = 1.0 Hz), 8.18 (1H, dd, $J$ = 8.3, 2.4 Hz), 8.81 (1H, d, $J$ = 2.4 Hz), 10.62 (1H, s), and 12.04 (1H, s). FAB-MS $m/z$: 351 $(M+1)^+$.

**5.1.42. *N*-(5-Bromopyrazin-2-yl)-5-chloro-1*H*-indole-2-carboxamide (11c).** The title compound was prepared in the same manner as described for **7b** using **10c** instead of **6b**,

in 47% yield. $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$: 7.26 (1H, dd, $J$ = 8.8, 2.0 Hz), 7.48 (1H, $J$ = 8.8 Hz), 7.65 (1H, d, $J$ = 1.6 Hz), 7.77 (1H, d, $J$ = 1.6 Hz), 8.71 (1H, d, $J$ = 1.2 Hz), 9.29 (1H, d, $J$ = 1.2 Hz), 11.43 (1H, s), and 12.08 (1H, s). FAB-MS $m/z$: 352 $(M+1)^+$.

**5.1.43. 5-Chloro-*N*-(5-vinylpyridin-2-yl)-1*H*-indole-2-carboxamide (12a).** The title compound was prepared in the same manner as described for **8a** using **11a** instead of **7a**, in 69% yield. $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$: 5.34 (1H, d, $J$ = 10.8 Hz), 5.92 (1H, d, $J$ = 17.6 Hz), 6.77 (1H, dd, $J$ = 17.6, 10.8 Hz), 7.24 (1H, dd, $J$ = 8.8, 2.4 Hz), 7.48 (1H, d, $J$ = 8.3 Hz), 7.62 (1H, d, $J$ = 1.4 Hz), 7.73 (1H, d, $J$ = 1.9 Hz), 8.03 (1H, dd, $J$ = 8.8, 2.4 Hz), 8.22 (1H, d, $J$ = 8.3 Hz), 8.48 (1H, d, $J$ = 2.4 Hz), 10.99 (1H, s), and 11.99 (1H, s). FAB-MS $m/z$: 298 $(M+1)^+$.

**5.1.44. 5-Chloro-*N*-(6-vinylpyridin-3-yl)-1*H*-indole-2-carboxamide (12b).** The title compound was prepared in the same manner as described for **8a** using **11b** instead of **7a**, in 72% yield. $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$: 5.41 (1H, dd, $J$ = 10.8, 1.6 Hz), 6.15 (1H, dd, $J$ = 17.6, 1.2 Hz), 6.79 (1H, dd, $J$ = 17.6, 10.8 Hz), 7.24 (1H, dd, $J$ = 8.8, 2.0 Hz), 7.44 (1H, s), 7.48 (1H, d, $J$ = 8.4 Hz), 7.54 (1H, d, $J$ = 8.4 Hz), 7.80 (1H, d, $J$ = 2.0 Hz), 8.21 (1H, dd, $J$ = 8.4, 2.4 Hz), 8.93(1H, d, $J$ = 2.4 Hz), 10.54 (1H, s), and 12.02 (1H, s). FAB-MS $m/z$: 298 $(M+1)^+$.

**5.1.45. 5-Chloro-*N*-(5-vinylpyrazin-2-yl)-1*H*-indole-2-carboxamide (12c).** The title compound was prepared in the same manner as described for **8a** using **11c** instead of **7a**, in 70% yield. $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$: 5.54 (1H, d, $J$ = 10.8 Hz), 6.28 (1H, d, $J$ = 17.6 Hz), 6.88 (1H, dd, $J$ = 17.8, 11.0 Hz), 7.25 (1H, dd, $J$ = 8.8, 2.0 Hz), 7.49 (1H, d, $J$ = 9.0 Hz), 7.64–7.80 (2H, m), 8.59 (1H, s), 9.44 (1H, s), 11.30 (1H, s), and 12.07 (1H, s). FAB-MS $m/z$: 299 $(M+1)^+$.

**5.1.46. 5-Chloro-*N*-[5-(1,2-dihydroxyethyl)pyridin-2-yl]-1*H*-indole-2-carboxamide (13a).** The title compound was prepared in the same manner as described for **9a** using **12a** instead of **8a**, in 78% yield. $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$: 3.43–3.49 (1H, m), 3.50–3.56 (1H, m), 4.58 (1H, t, $J$ = 5.7 Hz), 4.79 (1H, s), 5.38 (1H, s), 7.23 (1H, d, $J$ = 7.4 Hz), 7.47 (1H, d, $J$ = 8.3 Hz), 7.59 (1H, s), 7.72 (1H, s), 7.79 (1H, dd, $J$ = 8.8, 2.4 Hz), 8.16 (1H, d, $J$ = 8.3 Hz), 8.35 (1H, d, $J$ = 1.9 Hz), 10.88 (1H, s), and 11.98 (1H, s). ESI-MS $m/z$: 332 $(M+1)^+$. Anal. Calcd for $C_{16}H_{14}ClN_3O_3 \cdot 0.1H_2O$: C, 57.61; H, 4.29; N, 12.60. Found: C, 57.63; H, 4.41; N, 12.26.

**5.1.47. 5-Chloro-*N*-[6-(1,2-dihydroxyethyl)pyridin-3-yl]-1*H*-indole-2-carboxamide (13b).** The title compoundwas prepared in the same manner as described for **9a** using **12b** instead of **8a**, in 20% yield. $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$: 3.46–3.53 (1H, m), 3.64–3.72 (1H, m), 4.56–4.61 (1H, m), 4.67 (1H, s), 5.35 (1H, s), 7.24 (1H, dd, $J$ = 8.3, 2.0), 7.42 (1H, s), 7.46–7.52 (2H, m), 7.79 (1H, d, $J$ = 1.9 Hz), 8.16 (1H, dd, $J$ = 8.3, 2.4 Hz), 8.87 (1H, d, $J$ = 2.4 Hz), 11.72 (1H, s), and 12.00 (1H, s). FAB-MS $m/z$: 332 $(M+1)^+$. Anal. Calcd for $C_{16}H_{14}ClN_3O_3$: C, 57.93; H, 4.25; N, 12.67. Found: C, 57.55; H, 4.46; N, 12.46.

**5.1.48. 5-Chloro-*N*-[5-(1,2-dihydroxyethyl)pyrazin-2-yl]-1*H*-indole-2-carboxamide (13c).** The title compound was prepared in the same manner as described for **9a** using **12c** instead of **8a**, in 35% yield. $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$: 3.57–3.63 (1H, m), 3.70–3.74 (1H, m), 4.68 (1H, q, $J$ = 4.9 Hz), 4.75 (1H, t, $J$ = 5.9 Hz), 5.57 (1H, d, $J$ = 5.3 Hz), 7.25 (1H, dd, $J$ = 8.8, 1.9 Hz), 7.48 (1H, d, $J$ = 8.3 Hz), 7.64 (1H, d, $J$ = 1.2 Hz), 7.76 (1H, d, $J$ = 2.0 Hz), 8.52 (1H, d, $J$ = 1.5 Hz), 9.36 (1H, d, $J$ = 1.4 Hz), 11.23 (1H, s), and 12.06 (1H, s). FAB-MS $m/z$: 333 $(M+1)^+$. Anal. Calcd for $C_{15}H_{13}ClN_4O_3 \cdot 0.1H_2O \cdot 0.12CHCl_3$: C, 52.05; H, 3.85; N, 16.06. Found: C, 52.10; H, 3.75; N, 16.00.

**5.1.49. 5-Chloro-*N*-[4-(1,2-dihydroxyethyl)-1,3-thiazol-2-yl]-1*H*-indole-2-carboxamide (15a).** The title compound was prepared in the same manner as described for **2a** using **14a** instead of aniline, in 27% yield. $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$: 3.47–3.55 (1H, m), 3.60–3.75 (1H, m), 4.58–4.64 (1H, m), 4.64–4.72 (1H, m), 5.25–5.32 (1H, m), 7.01 (1H, s), 7.25 (1H, dd, $J$ = 8.8, 1.9 Hz), 7.47 (1H, d, $J$ = 8.8 Hz), 7.62 (1H, s), 7.76 (1H, d, $J$ = 1.9 Hz), 12.07 (1H, s), and 12.76 (1H, s). FAB-MS $m/z$: 338 $(M+1)^+$. Anal. Calcd for $C_{14}H_{12}ClN_3O_3S$: C, 49.78; H, 3.58; N, 12.44. Found: C, 49.81; H, 3.83; N, 12.43.

**5.1.50. 5-Chloro-*N*-[5-(1,2-dihydroxyethyl)-1,3-thiazol-2-yl]-1*H*-indole-2-carboxamide (15b).** The title compound was prepared in the same manner as described for **7b** using **14b** instead of **6b**, in 51% yield. $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$: 3.48–3.64 (2H, m), 4.70–4.80 (1H, m), 4.85–4.97 (1H, m), 5.63 (1H, d, $J$ = 3.9 Hz), 7.25 (1H, dd, $J$ = 8.8, 2.0 Hz), 7.38 (1H, s), 7.47 (1H, d, $J$ = 8.3 Hz), 7.60 (1H, s), 7.75 (1H, d, $J$ = 2.0 Hz), 12.08 (1H, s), and 12.59 (1H, s). FAB-MS $m/z$: 338 $(M+1)^+$. Anal. Calcd for $C_{14}H_{12}ClN_3O_3S$: C, 49.78; H, 3.58; N, 12.44. Found: C, 49.85; H, 3.92; N, 12.47.

### 5.2. Pharmacology

**5.2.1. Glycogen phosphorylase activity.** The activity of recombinant hLGPa in the forward direction was measured by determination of NADPH according to the methods of Pesce et al. modified.[32] The enzyme activity was assayed at pH 6.8 in a phosphate buffer containing $KH_2PO_4$ (45 mM), β-NADP (22.5 μM), α-glucose 1,6-bisphosphate ($4 \times 10^{-4}$%), glucose-6-phosphate dehydrogenase (385 U/L), phosphoglucomutase (77 U/L), glycogen (0.24%), and glucose (10.85 mM). The test compounds were added as 10 μL of solution in DMSO prior to the addition of the enzyme. The basal rate of hLGPa enzyme activity in the absence of inhibitors (Control) is determined by adding 10 μL of DMSO and a fully inhibited rate of hLGPa enzyme activity is obtained by adding 10 μL of 200 mM of the positive control test substance, caffeine. Following, the phosphate buffer was added as 216.5 μL, and the reaction was started by addition 23.5 μL hLGPa solution

(40 mM β-glycerophosphate, 80 mM cystein, pH 6.8). The reaction was followed at room temperature by measuring the conversion of oxidized β-NADP to reduced β-NADPH at 340 nm for 2 h. The hLGPa inhibition $IC_{50}$ values were calculated using the logistic regression method with SAS software.

**5.2.2. Isolation and culturing of hepatocytes.** Male Sprague–Dawley rats (150–200 g, purchased from Japan CLEA, Inc., Tokyo, Japan) were anesthtized with a freshly prepared pentobarbital intraperitoneally (65 mg/kg). Hepatocytes were isolated from rats fed ad libtum as described in the literature.[33] Cell viability, assessed by Trypan blue exclusion, was consistently greater than 85%. Cells were plated on to collagen-coated 24-well plates in basal medium (William's medium E containing 100 U/mL penicillin/streptomycin, 1 nM insulin, 1 nM dexamethazone, and 2.2 g/L $NaHCO_3$) supplemented with 10 % FBS at a cell density of $2.5 \times 10^5$ cells/well.

**5.2.3. Glucagon-induced glycogenolysis in primary hepatocytes.** After attachment, the cells were washed two times with pre-warmed, oxygen-saturated, glucose-free/calcium-free Hanks buffer (137 mM NaCl, 5.4 mM KCl, 0.5 mM $NaH_2PO_4$, 0.42 mM $Na_2HPO_4$, 10 mM Hepes, 0.017 mM phenol red, and 4.17 mM $NaHCO_3$) and subsequently incubated for 30 min at 37 °C in a final volume of 500 μL in glucose-free/calcium-free Hanks buffer, 0.1% DMSO, and respective inhibitor at a concentration within the range 0.1–10 μM in the absence (basal condition) or presence of 10 nM glucagon (stimulated condition). Medium (100 μL) was removed, and glucagon-induced glucose release into the medium was determined using glucose assay reagent (0.1 M potassium phosphate solution containing 2.8 U/mL glucoseoxydase, 1 U/mL peroxydase, 0.25 mM 4-aminoantipyrine, and 1.5 μL/mL dimethylaniline, pH 5.6). The $IC_{50}$ values of glucagon-induced glucose output were calculated using the logistic regression method with SAS software.

**5.2.4. Hypoglycemic activity of glycogen phosphorylase inhibitor.** The acute hypoglycemic activities of glycogen phosphorylase inhibitor compounds were determined using 6 week old male C57BL/KsJ Jcl-dbm +db/+db mice (purchased from Japan CLEA, Inc., Tokyo, Japan) housed under conventional conditions with controlled temperature, humidity, and lighting, and provided with a commercial diet (CE-2; Oriental Yeast, Tokyo, Japan) and water ad libitum. All procedures were conducted according to the Yamanouchi Animal care Committee's Guideline. After a one-week acclimation period, the animals were weighted and 10 μL of blood was collected from the tail veins of mice. The blood was mixed with 0.33 M perchloric acid solution (100 μL) in tubes and stirred. The mixtures were centrifuged at 3000 rpm for 10 min, and the glucose levels in the supernatants were measured using glucose CII-Test reagent (Wako Pure Chemical Industries, Ltd, Osaka, Japan). Animals were then grouped to make the blood glucose levels and body weight uniform for all groups ($n = 6$). Next day, blood glucose levels (pre) were measured in non-fasted mice. Either the vehicle or test compounds, dissolved in 5% propylenglycol–5% Tween 80–water solution, were orally administered to mice at the rate of 10 mL/kg. After 2 h, blood glucose levels (2 h) were measured. Hypoglycemic activity of the test compounds was determined by statistical analysis (unpaired *t*-test) of the mean blood glucose levels between the test compound group and vehicle group.

### 5.3. Crystallization and data collection

Crystal of hLGP in complex with **2f** was obtained at 4 °C by the hanging drop vapor diffusion method. The hanging drop consisted of equal volume of the concentrated protein solution (20 mg/mL) and reservoir solution containing 100 mM NaMES (pH 6), 22.5% MPD, 60 mM D-glucose, and 0.9 mM **2f**. The crystal belongs to the trigonal space group $P3_1$, with cell dimensions of $a = b = 123.8$ Å and $c = 123.5$ Å. The diffraction data set is 99% complete to 2.4 Å. The structure was solved with molecular replacement method using published hLGP/CP-403700 complex structure (PDB Code 1EXV) as the starting model,[18] and refined using the program CNX[34] to 2.45 Å resolution and a final *R*-factor of 0.261 ($R_{free}$ 0.307). The coordinate for the complex described in the paper has been deposited in the PDB (Accession Code 2ZB2) for immediate release on publication.

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