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SYNTHESIS AND EVALUATIONS OF GLP-1 SECRETION AND ANTI-DIABETIC EFFECT IN KKAY MICE OF NEW TRICYCLIC COMPOUNDS

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Abstract – Glucagon-like peptide-1 (GLP-1), which belongs to the family of incretins, plays important role for the regulation of plasma glucose. Accordingly, GLP-1-based therapies for type 2 diabetes have recognized as one of the most interesting target. In this study, we have found the new tricyclic compounds having strong GLP-1 secretion from human intestinal L cells, and anti-diabetic properties in spontaneously obese and diabetic KKAy mice. The most potent compound **5ka** was obtained as the unexpected product, and we would like to report the details of the synthesis, structure elucidations, pharmacological activities on secretion of GLP-1, and anti-diabetic effects using diabetic KKAy mice.

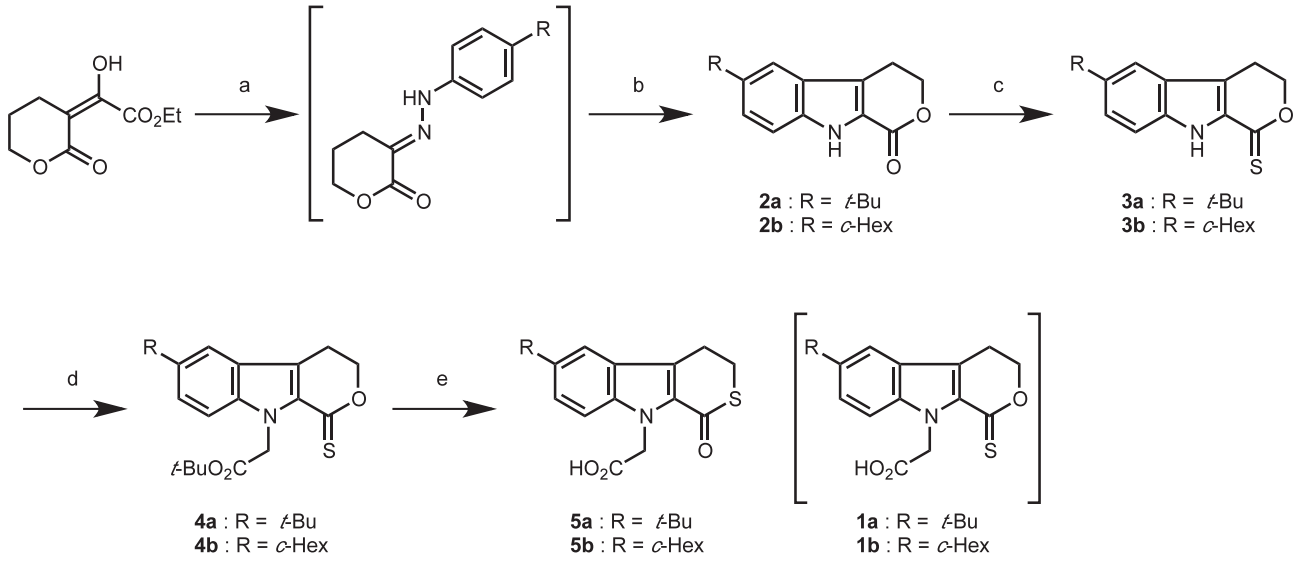
INTRODUCTION

Glucagon-like peptide-1 (GLP-1) belongs to the family of incretins, and it is secreted from enteroendocrine L cells following the stimulation by nutrients.[1,2] It promotes glucose-stimulated insulin secretion and suppresses postprandial hyperglycemia.[3] Furthermore, GLP-1 has also been reported to have various other beneficial effects such as suppressing glucagon release,[4] promoting β-cells proliferation,[5] suppressing food intake,[6] slowing gastric emptying,[7] improving fatty liver,[8] and cardiovascular protection.[9] However, the incretin effect is reduced in patients with type 2 diabetes, mainly due to defective GLP-1 secretion in response to stimulation by various nutrients.[10,11] For this reason, although clinically available GLP-1-based therapies, especially DPP-IV inhibitors are currently widely used for treatment of patients with type 2 diabetes, their monotherapy might be insufficient for achievement of treatment goals for blood glucose control. Therefore, restoration of GLP-1 secretion by intestinal L cells could be an important new therapeutic option for the management of metabolic syndrome. Various mechanisms, such as KATP channel,[12] Na^+/glucose co-transporter 1,[13] glucose transporter 2,[14] sweet taste receptor,[15] TGR-5,[16] G protein-coupled receptors,[17] and 5-hydroxytryptamine (5-HT)4 receptor,[18] have been reported to be involved in inducing GLP-1 secretion. But none of the compounds interacting with these targets are currently clinically available. In this study, we tried to discover a novel small molecule inducing GLP-1 secretion from intestinal L cells. As part of a program directed at studying the discovery of new drug candidate based upon the structure of rhetsinine, a minor alkaloidal component of *Evodia rutaecarpa*,[19,20] we found the new tricyclic compounds having strong GLP-1 secretion from human intestinal L cells, and anti-diabetic properties in spontaneously obese and diabetic KKAy mice. We report herein the synthesis and evaluations of GLP-1 secretion and anti-diabetic effect of these tricyclic compounds.

RESULTS AND DISCUSSION

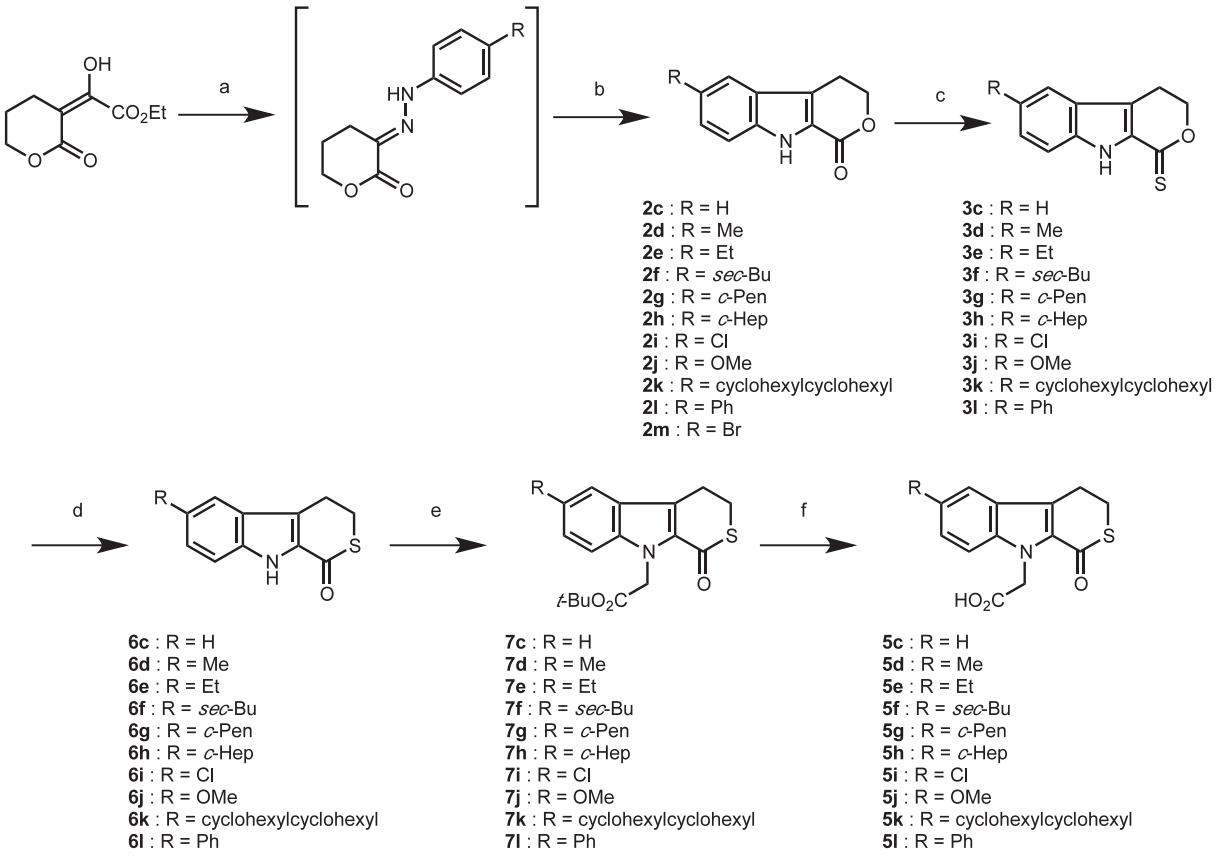
CHEMISTRY

We planned the synthesis of new tricyclic carboxylic acid **1** as the first target compound. The synthesis began with known lactone **2a**,[21] which was converted to thiolactone **3a** with Lawesson's reagent. The thiolactone **3a** was converted to the ester **4a**, which was treated with TMSI to afford the S-lactone **5a** as the major product along with **1a**. The separation of **5a** and **1a** was difficult, and repeated column chromatography of the above mixture provided pure **5a** in 66% yield. In the case of the ester **4b**, derived from **3b** via **2b**, we got the same result, and the S-lactone **5b** was isolated in 64% yield as the major product again (Scheme 1).



Scheme 1. *Reagents and conditions*: (a) ArN_2Cl, 2.5% NaOH aq; (b) HCl, AcOH, reflux; (c) Lawesson's reagent, toluene, reflux; (d) NaH, then $BrCH_2CO_2t$-Bu, DMF; (e) TMSI, $ClCH_2CH_2Cl$, reflux

At this time, the first target compound **1a** or **1b** was obtained as a minor product, we tried to synthesize **5** as new target compound. To avoid the tedious separation of **1** and **5** in the final step, we explored another synthetic route as shown in Scheme 2. The lactones **2c-k** and **2m** were prepared using the same procedure as in Scheme 1. The lactone **2m** was transformed into the lactone **2l** by the Suzuki-Miyaura coupling reaction.[22] The lactones **2c-l** were treated with Lawesson's reagent to afford the thiolactones **3c-l**. Treatment of the thiolactones **3c-l** with TMSI gave rise to S-lactone **6c-l**, which was transformed into the target acids **5c-l** via the corresponding esters **7c-l**.



Scheme 2. *Reagents and conditions*: (a) ArN_2Cl, 2.5% NaOH aq; (b) HCl, AcOH, reflux; (c) Lawesson's reagent, toluene, reflux; (d) TMSI, $ClCH_2CH_2Cl$, reflux; (e) NaH, then $BrCH_2CO_2t$-Bu, DMF; (f) TMSI, $ClCH_2CH_2Cl$, reflux

PHARMACOLOGICAL ACTIVITY: EFFECTS ON SECRETION OF GLP-1

We investigated the direct effect of the compounds on GLP-1 secretion by intestinal L cells using a human intestinal L cell line (NCI-H716). In this cell line, glucose has been reported to stimulate GLP-1 release at concentrations above 50 mM.[15, 23] In this assay system, we found that **5k** had a strong activity to induce GLP-1 secretion under normal glucose concentration (5.6 mM) without any leakage of LDH in the culture medium (Figure 1). Their EC_{50} values for GLP-1 secretion is 100 μM for **5k**. In addition to the lowest EC_{50} value for **5k**, Emax value of this compound is the highest (ca. 1300 pM) among all tested compounds. Therefore, we investigated the in vivo effect of **5k** on blood glucose both in normal and diabetic mice.

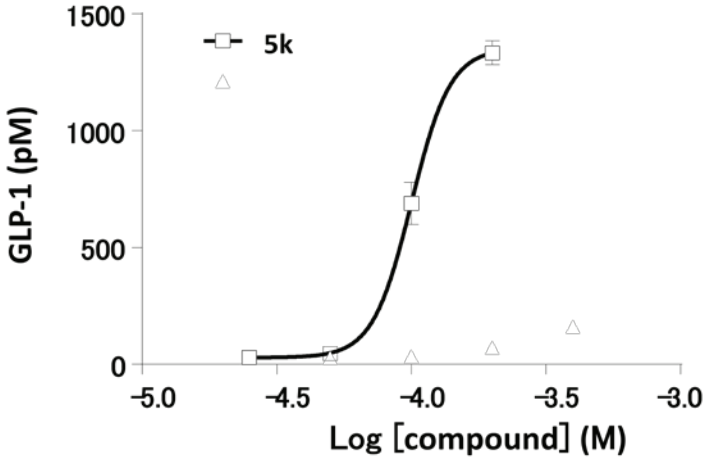


Figure 1. Effect of **5k** on GLP-1 release by NCI-H716 cells

NCI-H716 cells ($1x10^5$/well) were incubated with **5k** for 1 h, after which the GLP-1 level in the culture medium was measured. Mean ± SEM., n=3.

HYPOGLYCEMIC EFFECT OF 5K AFTER AN ORAL GLUCOSE LOAD AND THE INFLUENCE OF A GLP-1 ANTAGONIST (EXENDIN 9-39) ON IT IN C57BL/6J MICE

Acute effect of **5k** after an oral glucose load on blood glucose was investigated in normal mice. An oral administration of **5k** (100 mg/kg) just before glucose loading suppressed the increase of blood glucose

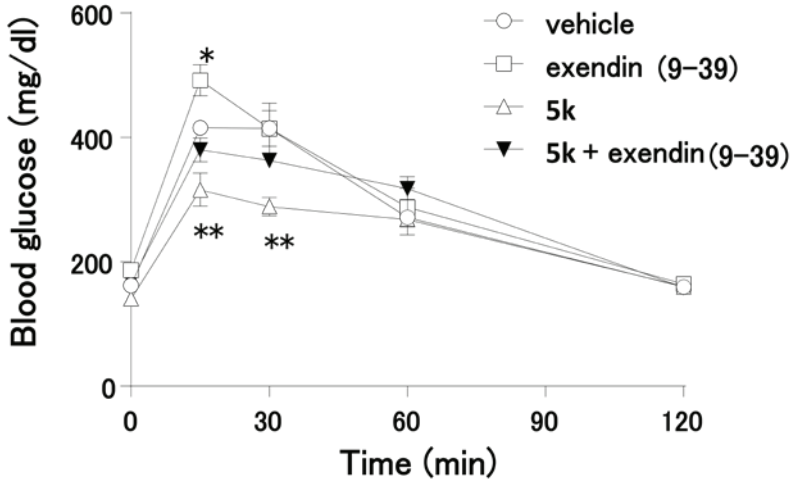


Figure 2. Hypoglycemic effect of **5k** after an oral glucose load and the influence of a GLP-1 antagonist (Exendin (9-39)) on it in C57BL/6J mice. After an overnight fast, either the vehicle, or 100 mg/kg of TMAJ 47 was administered by oral gavage with or without subcutaneous injection of exendin (9-39) (24 nmol/kg). Then, 2 g/kg of glucose was given orally immediately after **5k** administration. Blood samples were collected from the tail vein, and the blood glucose levels were measured. Mean ± SEM., n=6. *$p<0.05$, **$p<0.01$ vs vehicle (Dunnett's test).

(Figure 2). Since exendin (9-39) is often used to confirm the involvement of GLP-1 in the hypoglycemic effect of the compounds *in vivo*,[23,24] we investigated the influence of exendin (9-39) on hypoglycemic effect of **5k**. The acute hypoglycemic effect of **5k** after an oral glucose load is cancelled by subcutaneous injection of exendin (9-39) (24 nmol/kg) prior to administration of **5k**. These data indicate that **5k** suppresses the increase of blood glucose via stimulation of GLP-1 secretion *in vivo*. Then, we investigated the anti-diabetic effect of **5k** in diabetic mice.

PHARMACOLOGICAL ACTIVITY: ANTI-DIABETIC EFFECTS OF 5K

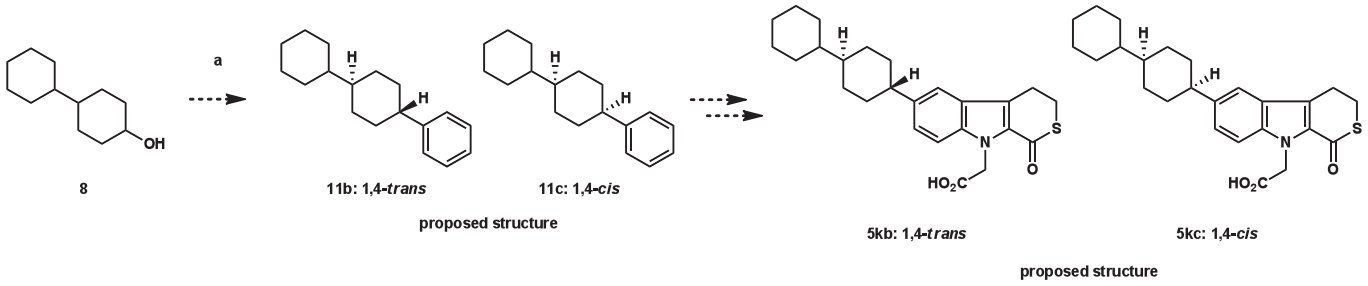
Effect of long-term treatment with **5k** on blood glucose control was investigated using obese and diabetic KKAy mice. Data are summarized in Table 1. Treatment with **5k** for 3 weeks had no impact on food intake and body weight, but it lowered both fasting and non-fasting blood glucose. As a result, HbA1c in **5k**-treated mice was decreased. In addition, fasting plasma insulin level and fasting plasma glucagon level tended to be lowered after 3 weeks treatment with **5k**. The anti-diabetic effect of **5k** was almost comparable to that of pioglitazone. On the other hand, although a DPP-IV inhibitor sitagliptin is known to increase plasma GLP-1 level by inhibiting degradation of GLP-1 by DPP-IV, in this study, 3 weeks treatment with sitagliptin in KKAy mice showed no effect on blood glucose and HbA1c. Taken together, stimulation of GLP-1 secretion by **5k** may be a new therapeutic option for patients with type 2 diabetes.

Table 1. Long-term effect of **5k** treatment for 3 weeks on blood glucose control in KKAy mice

	Body weight (g)	Fasting blood glucose (mg/dl)	Non-fasting blood glucose (mg/dl)	HbA1c (%)	Fasting plasma insulin (ng/mL)	Fasting plasma glucagon (pg/mL)
Normal	**25.5±0.3**	**166.0±12.9**	**156.0±6.4**	**3.9±0.1**	**2.9±0.4**	**155.7±17.9**
Vehicle	**39.5±1.8**	**204.8±16.5**	**353.2±37.5**	**7.5±0.3**	**20.6±10.4**	**257.8±34.5**
5k	**41.0±1.9**	**124.6±6.6****	**231.6±13.5**	**6.3±0.3**	**8.7±3.3**	**217.1±38.5**
Sitagliptin	**39.9±1.4**	**194.6±18.5**	**457.6±35.1**	**7.9±0.3**	**5.8±0.4**	**249.8±33.2**
Pioglitazone	**42.6±1.1**	**151.6±11.1**	**256.4±49.4**	**6.8±0.4**	**7.7±1.4**	**241.6±29.4**

STRUCTURE OF THE MOST POTENT COMPOUND 5K

As shown in 2.2, the most potent compound for the secretion of GLP-1 was **5k**, which was a 5:1 mixture of 2 compounds. We thought these two compounds would be the stereoisomers **5kb** and **5kc** produced in the Friedel-Crafts reaction of the commercially available alcohol **8** (Scheme 3).



Scheme 3. *Reagents and conditions*: (a) $AlCl_3$, PhH

So we planned more selective synthesis of the major compound **5kc**. The silane reduction of the tertiary alcohol **10**, derived from the alcohol **8** via the ketone **9**, afforded the two products **11b** and **11c** in the ratio of 3:1 (Scheme 4.). Although the product **11b** was identical with the minor product of the Friedel-Crafts reaction the other product **11c** was not identical with the major product of the Friedel-Crafts reaction product (Figure 3).

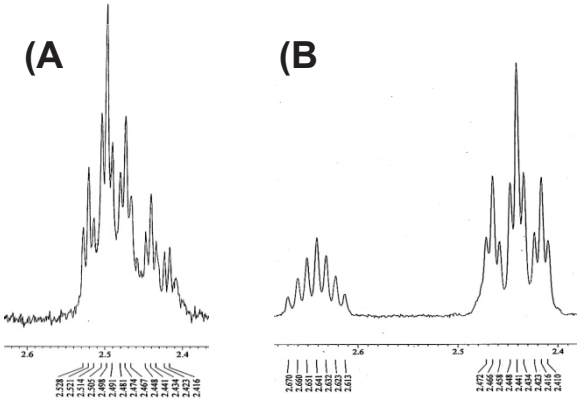
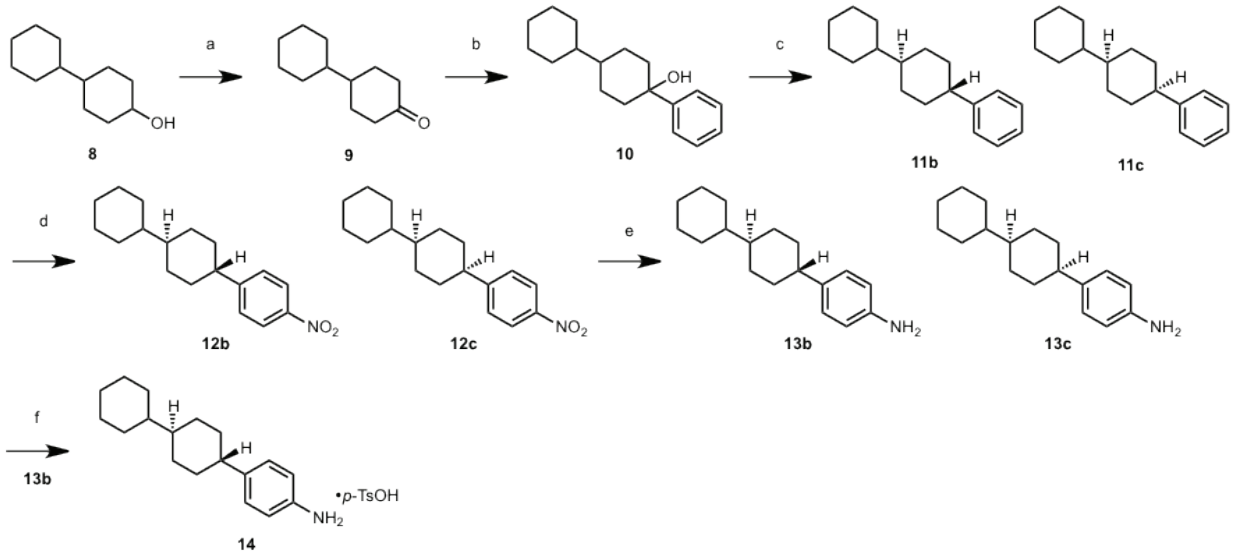


Figure 3. ^{1}H NMR spectrum on the benzylic position **(A** indicates the product from Friedel-Crafts reaction. **(B** indicates the product from silane reduction.

These results suggested that the major product in the Friedel-Crafts reaction was not the stereoisomer **11c** but the rearranged product. To determine the structure of the major and rearranged product in the Friedel-Crafts reaction, first we prepared the *p*-toluenesulfonic acid salt **14** of the major aniline derivative **13b** in the silane reduction of **10** (Scheme 4). The X-ray analysis of **14** revealed that this product was the 1,4-*trans*-substituted compound (Figure 4).



Scheme 4. *Reagents and conditions*: (a) PCC, Celite, CH_2Cl_2 (96%); (b) PhMgBr, THF (93%); (c) Et_3SiH, $BF_3 \cdot OEt_2$, CH_2Cl_2, -40 °C (92%); (d) Ac_2O, HNO_3, $CHCl_3$ (52%); (e) H_2, Pd/C, EtOAc (**13b** : 58%, **13c** : 20%); (f), *p*-TsOH・H_2O, MeOH (quant.)

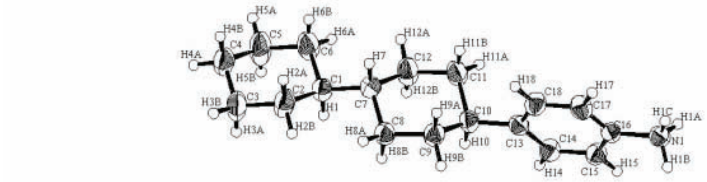
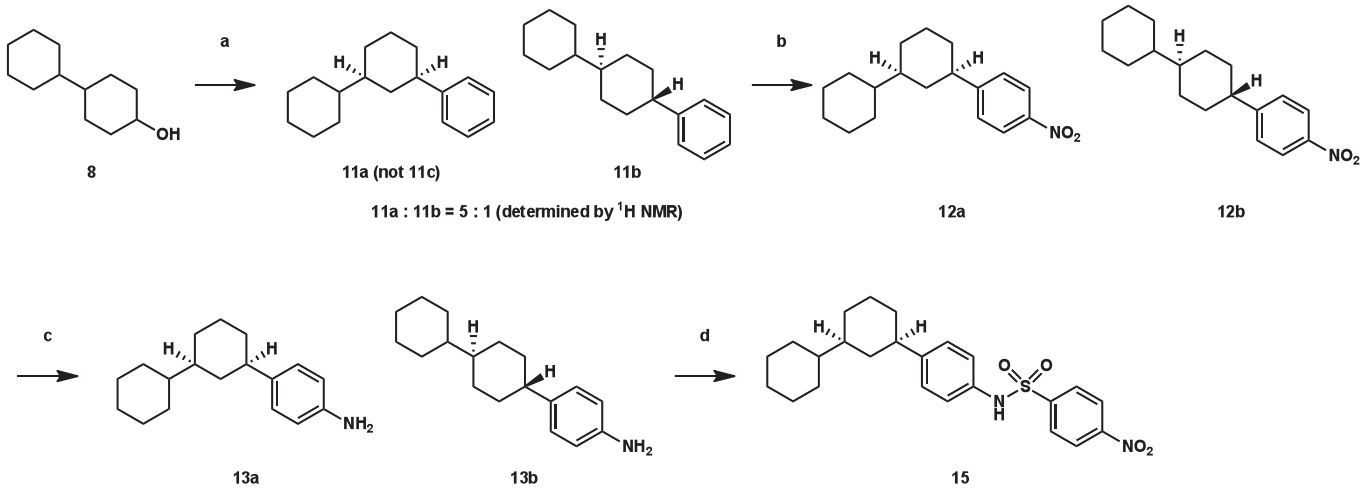


Figure 4. X-Ray crystal structure of compound **14**

Next we examined the synthesis of *p*-nitrobenzenesulfonamide derivative **15** of the major product **11a** in the Friedel-Crafts reaction. Nitration of the mixture of **11a** and **11b** followed by hydrogenation of the resulting nitro derivatives **12a** and **12b** afforded the corresponding anilines **13a** and **13b**, which were separated in this stage. The major aniline **13a** was converted to **15** by the usual manner (Scheme 5). The structure of **15** was determined by the X-ray analysis shown in Figure 5.



Scheme 5. *Reagents and conditions*: (a) $AlCl_3$, PhH; (b) Ac_2O, HNO_3, $CHCl_3$ (47%, 2steps); (c) H_2, Pd/C, EtOAc(**13a** : 61%, **13b** : 18%); (d) $ArSO_2Cl$, pyridine (quant.)

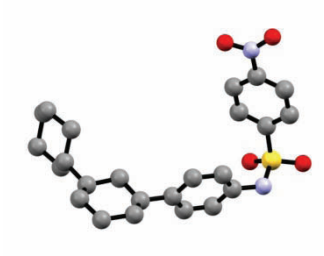


Figure 5. X-Ray crystal structure of compound **15**

The PM6 calculations for the intermediary cations in the Friedel-Crafts reaction revealed that the rearranged cation **A** is more stable than the cation **B**, initially produced by the action of the alcohol **8** with $AlCl_3$ (Figure 6). According to these results, we concluded the major product of the Friedel-Crafts reaction was 1,3-*cis*-substituted derivative **11a**.

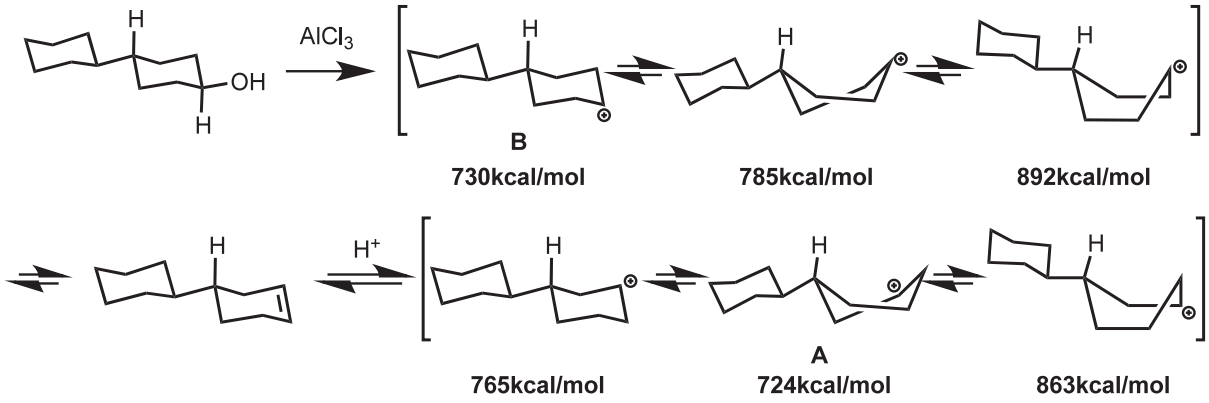
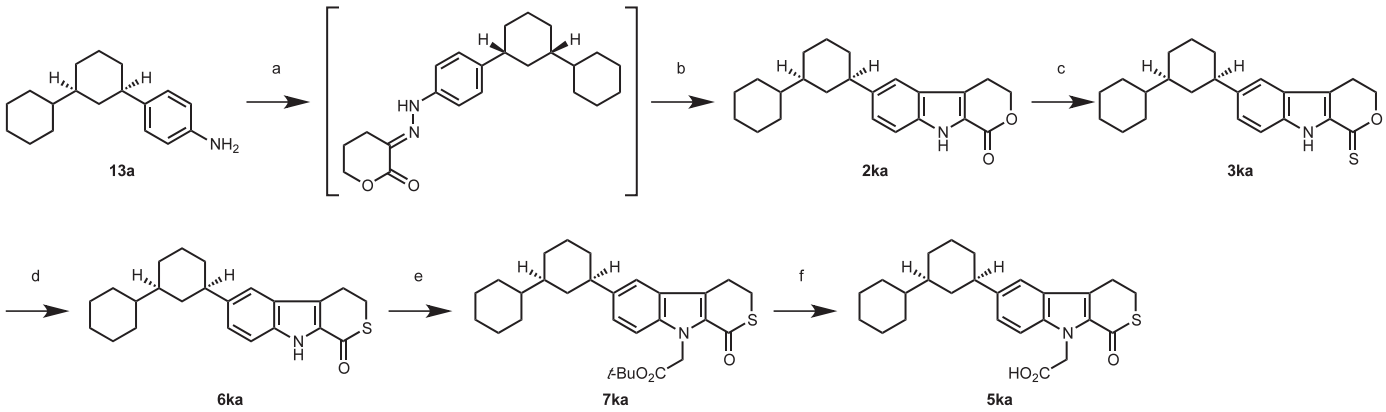


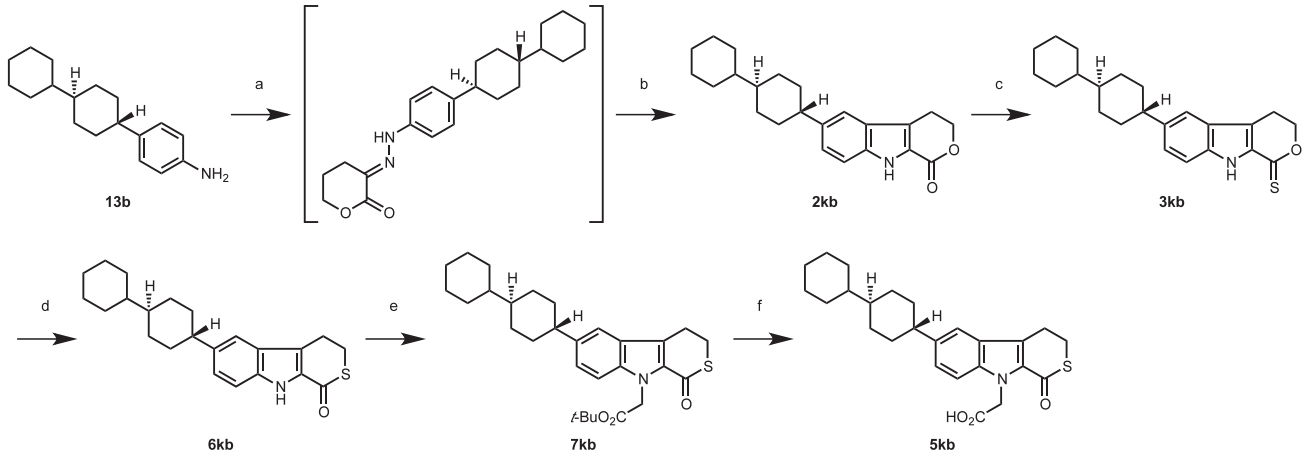
Figure 6. PM6 Calculations of the intermedially carbocations on the Friedel-Crafts reaction

The pure 1,3-*cis*-substituted aniline **13a** and 1,4-*trans*-substituted aniline **13b** in hand, we tried to synthesize the pure carboxylic acids **5ka** and **5kb** for the evaluations of the effect of these stereoisomers in the secretion of GLP-1. The aniline **13a** was converted to the lactone **2ka**, which was transformed into **5ka** via thiolactone **3ka**, S-lactone **6ka**, and ester **7ka** using the same procedure as shown in Scheme 2 (Scheme 6).



Scheme 6. *Reagents and conditions*: (a) HNO_2, then enol lactone, AcONa, THF/H_2O; (b) HCl, AcOH, reflux (69%, 2steps); (c) Lawesson's reagent, PhMe, reflux (quant.); (d) TMSI, $ClCH_2CH_2Cl$, reflux (92%); (e) NaH, then $BrCH_2CO_2t$-Bu (95%); (f) TMSI, $ClCH_2CH_2Cl$, reflux (89%)

The regioisomer **5kb** was also synthesized as shown in Scheme 7.



Scheme 7. *Reagents and conditions*: (a) HNO_2, then enol lactone, AcONa, THF/H_2O; (b) HCl, AcOH, reflux (67%, 2steps); (c) Lawesson's reagent, PhMe, reflux (81%); (d) TMSI, $ClCH_2CH_2Cl$, reflux (80%); (e) NaH, then $BrCH_2CO_2t$-Bu (97%); (f) TMSI, $ClCH_2CH_2Cl$, reflux (83%)

PHARMACOLOGICAL EFFECTS OF 5Ka AND 5Kb ON SECRETION OF GLP-1

Then, we evaluated the effect of these regioisomers (**5ka** and **5kb**) on GLP-1 secretion by NCI-H716. Intriguingly, as shown in Figure 7, **5ka** showed strong activity of GLP-1 secretion, while **5kb** had no effect. Therefore, the active compound is 1,3-*cis* form (**5ka**).

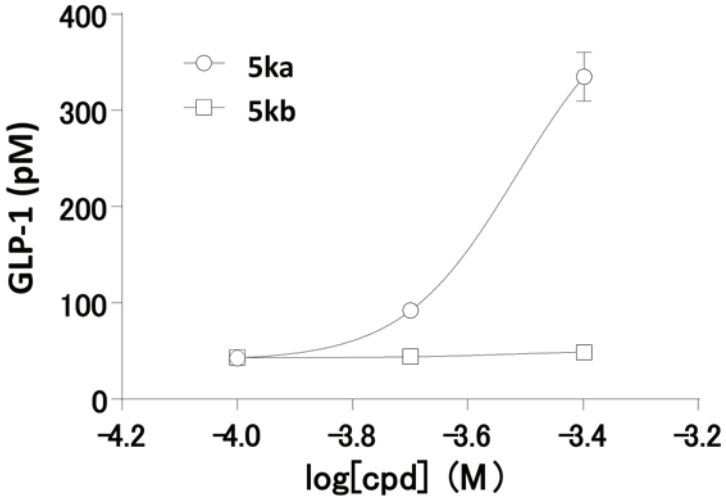


Figure 7. Comparison of the effect of regioisomers of **5ka** and **5kb** on GLP-1 secretion in NCI-H716 cells NCI-H716 cells ($1x10^5$/well) were incubated with **5ka** or **5kb** for 1 h, after which the GLP-1 level in the culture medium was measured. Mean ± SEM., n=3.

CONCLUSION

We prepared 14 tricyclic compounds **5a-5kb** having the different substituent on the 6-position. Evaluations of these compounds for the direct effect on GLP-1 secretion by intestinal L cells using a human intestinal L cell line (NCI-H716) revealed that the compound **5k** exhibited strong activity to induce GLP-1 secretion. In addition, **5k** showed the anti-diabetic effect in diabetic KKAy mice. We also identified the active compound for the above induction of GLP-1 and anti-diabetic effect was **5ka**, which has the 1,3-*cis*-substituted cyclohexylcyclohexyl substituent on the 6-position of tricyclic nuclei. This compound was unexpected product, and was produced in the Friedel-Crafts reaction by the interesting stereoselective rearrangement on this reaction process.

EXPERIMENTAL

General information

Melting points are uncorrected. Flash chromatography was performed on Kanto Kagaku silica gel 60N. NMR spectra were recorded on a JEOL a-GX 400 and JEOL JNX-ECX500 spectrometer using $CDCl_3$ as the solvent. Chemical shifts (δ) are given in ppm downfield from TMS and referenced to $CHCl_3$ (7.26

ppm) as an internal standard. Peak multiplicities are designated by the following abbreviations: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; br, broad and coupling constants in (*J*) Hz. High-resolution mass spectral data were obtained on a JEOL MStation JMS-700. All commercial reagents were used as received unless otherwise noted.

Synthesis of lactone 2b

According to the literature procedure,[25] lactone **2b** was synthesized.

6-Cyclohexyl-4,9-dihydro-3*H*-pyrano[3,4-*b*]indol-1-one (2b). Yield: 28%; mp 175-176 °C; IR (KBr): 3269, 2920, 2849, 1735 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 9.03 (1H, br), 7.43-7.38 (2H, m), 7.30-7.26 (1H, m), 4.70 (2H, t, *J* = 6.2 Hz), 3.15 (2H, t, *J* = 6.2 Hz), 2.60-2.57 (1H, m), 1.94-1.26 (10H, m); ^{13}C NMR (125 MHz, $CDCl_3$): δ 161.74, 140.82, 137.15, 126.94, 124.43, 122.91, 122.14, 117.43, 112.72, 69.48, 44.50, 34.87, 26.93, 26.13, 21.42; MS (EI) *m/z* 269 (M+); HRMS (EI) calcd for $C_{17}H_{19}NO_2$: 269.1416 (M+), found: 269.1414.

Synthesis of thiolactones 3a and 3b

To a stirred solution of lactone (1 mmol) in toluene (5 mL) was added Lawesson's reagent (0.55 eq), and the resulting mixture was refluxed for 20–24 h. After cooling, the solvent was removed, and the residue was chromatographed on SiO_2 (hexane–acetone = 20:1) to give the corresponding thiolactone.

6-*tert*-Butyl-4,9-dihydro-3*H*-pyrano[3,4-*b*]indole-1-thione (3a). Yield: 95%; mp 40-42 °C; IR (KBr): 2957, 1541, 1231 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ 8.80 (1H, br), 7.56 (1H, d, *J* = 1.7 Hz), 7.51 (1H, dd, *J* = 1.7, 9.0 Hz), 7.35 (1H, d, *J* = 9.0 Hz), 4.74 (2H, t, *J* = 6.4 Hz), 3.18 (2H, t, *J* = 6.4 Hz), 1.38 (9H, s); ^{13}C NMR (75 MHz, DMSO-d_6): δ 197.90, 142.94, 137.94, 132.31, 125.85, 123.49, 116.72, 116.39, 112.53, 71.26, 34.42, 31.39, 20.60; MS (EI) *m/z* 259 (M+); HRMS (EI) calcd for $C_{15}H_{17}NOS$: 259.1031 (M+), found: 259.1022.

6-Cyclohexyl-4,9-dihydro-3*H*-pyrano[3,4-*b*]indole-1-thione (3b). Yield: 95%; mp 40-42 °C; IR (KBr): 3348, 2922, 2848, 1541, 1226 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 8.75 (1H, br), 7.40 (1H, s), 7.31 (1H, d, *J* = 8.7 Hz), 7.27 (1H, dd, *J* = 1.4, 8.7 Hz), 4.72 (2H, t, *J* = 6.4 Hz), 3.15 (2H, t, *J* = 6.4 Hz), 2.57 (1H, tt, *J* = 3.2, 11.4 Hz), 1.92-1.84 (4H, m), 1.78-1.74 (1H, m), 1.50-1.23 (5H, m); ^{13}C NMR (75 MHz, DMSO-d_6): δ 197.93, 139.96, 138.34, 132.26, 127.18, 123.81, 118.34, 116.06, 112.68, 71.26, 43.82, 34.36, 26.49, 25.70, 20.57; MS (EI) *m/z* 285 (M+); HRMS (EI) calcd for $C_{17}H_{19}NOS$: 285.1187 (M+), found: 285.1191.

Synthesis of esters 4a and 4b

To a stirred solution of thiolactone (1 mmol) in DMF (5 mL) was added NaH (60%, 1.20 equiv) at 0 °C, and the reaction mixture was stirred at 0 °C for 30 min. To the mixture was added $BrCH_2CO_2t$-Bu at 0 °C,

and the resulting mixture was stirred at room temperature for 20–24 h. The reaction was quenched with H_2O (10 mL), and the aqueous mixture was extracted with Et_2O (10 mL × 3). The organic extracts were combined, dried over $MgSO_4$, and evaporated. The residue was chromatographed on SiO_2 (hexane–acetone = 20:1) to give the corresponding ester.

(6-*tert*-Butyl-1-thioxo-3,4-dihydro-1*H*-pyrano[3,4-*b*]indol-9-yl)acetic acid *tert*-butyl ester (4a). Yield: quant.; mp 144-145 °C; IR (KBr): 2967, 1748, 1527, 1228 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 7.56 (1H, d, *J* = 1.8 Hz), 7.53 (1H, dd, *J* = 1.8, 8.7 Hz), 7.19 (1H, d, *J* = 8.7 Hz), 5.47 (2H, s), 4.66 (2H, t, *J* = 6.4 Hz), 3.17 (2H, t, *J* = 6.4 Hz), 1.45 (9H, s), 1.36 (9H, s); ^{13}C NMR (75 MHz, $CDCl_3$): δ 196.28, 167.28, 144.19, 139.29, 131.38, 126.46, 122.04, 118.60, 116.69, 109.65, 81.82, 69.93, 46.55, 34.46, 31.36, 27.90, 21.26; MS (EI) *m/z* 373 (M+); HRMS (EI) calcd for $C_{21}H_{27}NO_3S$: 373.1712 (M+), found: 373.1732.

(6-Cyclohexyl-1-thioxo-3,4-dihydro-1*H*-pyrano[3,4-*b*]indol-9-yl)acetic acid *tert*-butyl ester (4b). Yield: quant.; mp 123-125 °C; IR (KBr): 2925, 2851, 1740, 1525, 1228 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ 7.44 (1H, s), 7.34 (1H, dd, *J* = 1.7, 9.0 Hz), 7.19 (1H, d, *J* = 9.0 Hz), 5.49 (2H, s), 4.67 (2H, t, *J* = 6.4 Hz), 3.17 (2H, t, *J* = 6.4 Hz), 2.61-2.57 (1H, m), 1.92-1.85 (4H, m), 1.78-1.76 (1H, m), 1.51-1.24 (5H, m), 1.46 (9H, s); ^{13}C NMR (75 MHz, $CDCl_3$): δ 196.63, 167.62, 141.62, 140.05, 131.72, 128.19, 122.71, 118.58, 118.50, 110.04, 82.28, 70.19, 46.92, 44.51, 34.87, 28.17, 27.01, 26.21, 21.59; MS (EI) *m/z* 399 (M+); HRMS (EI) calcd for $C_{23}H_{29}NO_3S$: 399.1868 (M+), found: 399.1858.

Synthesis of carboxylic acids 5a and 5b

To a stirred suspension of NaI (4.00 equiv) in $CHCl_3$ (3 mL) was added TMSCl (4.00 equiv), and the resulting mixture was stirred at room temperature for 15 min. To a solution of *t*-butyl ester (1 mmol) in $CHCl_3$ (5 mL) was transferred a solution of TMSI, prepared above, via a cannula, and then the resulting mixture was refluxed for 1 days. After cooling, the reaction was quenched with 10% HCl aq, and the aqueous mixture was extracted with $CHCl_3$ (10 mL × 3). The organic extracts were combined, dried over $MgSO_4$, and evaporated. The residue was chromatographed repeatedly on SiO_2 (hexane–acetone = 2:1) to give the carboxylic acid.

(6-*tert*-Butyl-1-oxo-3,4-dihydro-1*H*-2-thia-9-azafluoren-9-yl)acetic acid (5a). Yield: 66%; mp 237-239 °C; IR (KBr): 2964, 1727, 1623 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6): δ 7.67 (1H, s), 7.52 (2H, s), 5.19 (2H, s), 3.47 (2H, t, *J* = 6.1 Hz), 3.31 (2H, t, *J* = 6.1 Hz), 1.35 (9H, s); ^{13}C NMR (75 MHz, DMSO-d_6): δ 182.80, 169.96, 143.03, 136.61, 127.11, 126.46, 125.75, 123.68, 116.14, 110.41, 46.31, 34.42, 31.44, 30.84, 21.70; MS (EI) *m/z* 317 (M+); HRMS (EI) calcd for $C_{17}H_{19}NO_3S$: 317.1086 (M+), found: 317.1091.

(6-Cyclohexyl-1-oxo-3,4-dihydro-1*H*-2-thia-9-azafluoren-9-yl)acetic acid (5b). Yield: 64%; mp 245-247 °C; IR (KBr): 2924, 2850, 1717, 1619, 1251 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ 7.45 (1H, s),

7.33 (1H, d, *J* = 8.5 Hz), 7.21 (1H, d, *J* = 8.5 Hz), 5.26 (2H, s), 3.43 (2H, t, *J* = 6.2 Hz), 3.32 (2H, t, *J* = 6.2 Hz), 2.62-2.58 (1H, m), 1.92-1.85 (4H, m), 1.78-1.76 (1H, m), 1.50-1.38 (4H, m), 1.32-1.26 (1H, m); ^{13}C NMR (75 MHz, DMSO-d_6): δ 182.82, 169.96, 140.06, 137.00, 127.08, 127.02, 126.14, 124.04, 117.81, 110.58, 46.31, 43.77, 34.39, 30.83, 26.49, 25.67, 21.69; MS (EI) *m/z* 343 (M+); HRMS (EI) calcd for $C_{19}H_{21}NO_3S$: 343.1242 (M+), found: 343.1280.

Synthesis of lactones 2c-m

According to the literature procedure,[25] known lactones (**2c**, **2d**, **2i**, **2j**, **2m**) and the following lactones were prepared.

6-Ethyl-4,9-dihydro-3*H*-pyrano[3,4-*b*]indol-1-one (2e). Yield: 37%; mp 158-160 °C; IR (KBr): 3297, 1675 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.80 (1H, br), 7.42 (1H, s), 7.33 (1H, d, *J* = 8.7 Hz), 7.27 (1H, d, *J* = 8.7 Hz), 4.74 (2H, t, *J* = 6.5 Hz), 3.16 (2H, t, *J* = 6.5 Hz), 2.74 (2H, q, *J* = 7.6 Hz), 1.29 (3H, t, *J* = 7.6 Hz); ^{13}C NMR (75 MHz, $CDCl_3$): δ 161.70, 136.95, 136.31, 127.38, 124.13, 122.53, 121.75, 118.24, 112.66, 69.36, 28.72, 21.23, 16.02; MS (EI) *m/z* 215 (M+); HRMS (EI) calcd for $C_{13}H_{13}NO_2$: 215.0946 (M+), found: 215.0945.

6-*sec*-Butyl-4,9-dihydro-3*H*-pyrano[3,4-*b*]indol-1-one (2f). Yield: 43%; mp 155-157 °C; IR (KBr): 3280, 2958, 2919, 2871, 1733 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ 9.44 (1H, br), 7.45-7.40 (2H, m), 7.26-7.23 (1H, m), 4.71 (2H, t, *J* = 6.3 Hz), 3.16 (2H, t, *J* = 6.3 Hz), 2.70 (1H, sext, *J* = 6.9 Hz), 1.65 (2H, quint, *J* = 7.4 Hz), 1.29 (3H, d, *J* = 7.1 Hz), 0.84 (3H, t, *J* = 7.4 Hz); ^{13}C NMR (75 MHz, $CDCl_3$): δ 161.64, 140.12, 137.11, 126.64, 124.34, 122.77, 122.06, 117.95, 112.72, 69.51, 41.73, 31.46, 22.36, 21.57, 12.40; MS (EI) *m/z* 243 (M+); HRMS (EI) calcd for $C_{15}H_{17}NO_2$: 243.1259 (M+), found: 243.1255.

6-Cyclopentyl-4,9-dihydro-3*H*-pyrano[3,4-*b*]indol-1-one (2g). Yield: 34%; mp 144-146 °C; IR (KBr): 3296, 2950, 2865, 1707 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ 9.31 (1H, br), 7.46 (1H, s), 7.42 (1H, d, *J* = 8.7 Hz), 7.31 (1H, d, *J* = 8.7 Hz), 4.70 (2H, t, *J* = 6.3 Hz), 3.18-3.04 (3H, m), 2.18-1.57 (8H, m); ^{13}C NMR (75 MHz, $CDCl_3$): δ 161.47, 138.94, 136.91, 126.98, 124.36, 122.76, 122.13, 117.79, 112.61, 69.49, 46.02, 34.97, 25.58, 21.57; MS (EI) *m/z* 255 (M+); HRMS (EI) calcd for $C_{16}H_{17}NO_2$: 255.1259 (M+), found: 255.1259.

6-Cycloheptyl-4,9-dihydro-3*H*-pyrano[3,4-*b*]indol-1-one (2h). Yield: 53%; mp 168-170 °C; IR (KBr): 3277, 2921, 2851, 1735 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ 9.36 (1H, br), 7.41 (1H, s), 7.41 (1H, d, *J* = 8.1 Hz), 7.26 (1H, d, *J* = 8.1 Hz), 4.70 (2H, t, *J* = 6.2 Hz), 3.15 (2H, t, *J* = 6.2 Hz), 2.77 (1H, quint, *J* = 5.1 Hz), 1.98-1.92 (2H, m), 1.84-1.48 (10H, m); ^{13}C NMR (75 MHz, $CDCl_3$): δ 161.57, 142.55, 136.85, 126.67, 124.23, 122.76, 121.98, 117.11, 112.71, 69.45, 47.04, 37.23, 27.90, 27.23, 21.49; MS (EI) *m/z* 283 (M+); HRMS (EI) calcd for $C_{18}H_{21}NO_2$: 283.1572 (M+), found: 283.1574.

6-Bicyclohex-4-yl-4,9-dihydro-3*H*-pyrano[3,4-*b*]indol-1-one (2k). About 5:1 mixture of **2ka** and **2kb**;

Yield: 26%; IR (KBr): 3289, 2923, 2849, 1699 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 8.72 (1H, br), 7.43 (1H, s), 7.37 (1H, d, *J* = 8.5 Hz), 7.30 (1H, s), 4.69 (2H, t, *J* = 6.2 Hz), 3.15 (2H, t, *J* = 6.2 Hz), 2.61-2.44 (1H, m), 2.06-0.86 (20H, m).

6-Phenyl-4,9-dihydro-3*H*-pyrano[3,4-*b*]indol-1-one (2l). To a stirred solution of **2m** (266 mg, 1.00 mmol) in 1,4-dioxane (15 mL) were added $PhB(OH)_2$ (134 mg, 1.10 mmol), KBr (131 mg, 1.10 mmol), $K_3PO_4 \cdot 3H_2O$ (399 mg, 1.50 mmol) and $Pd(PPh_3)_4$ (116 mg, 0.10 mmol), and the resulting mixture was refluxed for 13 h. The reaction mixture was diluted with CH_2Cl_2, and the organic layer was washed with sat. $NaHCO_3$ aq. The organic layer was dried over $MgSO_4$, and evaporated. The residue was chromatographed on SiO_2 (hexane–acetone = 12:1) to give **2l** (77.4 mg, 29%). mp 247-250 °C; IR (KBr): 3278, 1692 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 8.86 (1H, br), 7.80 (1H, s), 7.64 (1H, d, *J* = 8.7 Hz), 7.61 (2H, d, *J* = 7.3 Hz), 7.51 (1H, d, *J* = 8.7 Hz), 7.44 (2H, t, *J* = 7.3 Hz), 7.33 (1H, t, *J* = 7.3 Hz), 4.71 (2H, t, *J* = 6.2 Hz), 3.19 (2H, t, *J* = 6.2 Hz); ^{13}C NMR (125 MHz, $CDCl_3$): δ 161.18, 141.50, 137.56, 134.63, 128.80, 127.28, 126.90, 125.07, 123.38, 122.94, 120.97, 119.07, 112.99, 69.47, 21.47; MS (EI) *m/z* 263 (M+); HRMS (EI) calcd for $C_{17}H_{13}NO_2$: 263.0946 (M+), found: 263.0949.

Synthesis of thiolactones 3c-l

To a stirred solution of lactone (1 mmol) in toluene (5 mL) was added Lawesson's reagent (0.55 eq), and the resulting mixture was refluxed for 20–24 h. After cooling, the solvent was removed, and the residue was chromatographed on SiO_2 (hexane–acetone = 20:1) to give the corresponding thiolactone.

4,9-Dihydro-3*H*-pyrano[3,4-*b*]indole-1-thione (3c). Yield: 93%; mp 115-116 °C; IR (KBr): 3347, 1537, 1208 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6): δ 11.67 (1H, br), 7.70 (1H, d, *J* = 8.2 Hz), 7.47 (1H, d, *J* = 8.3 Hz), 7.36 (1H, ddd, *J* = 1.4, 6.9, 8.2 Hz), 7.11 (1H, ddd, *J* = 1.1, 6.9, 8.3 Hz), 4.71 (2H, t, *J* = 6.5 Hz), 3.16 (2H, t, *J* = 6.5 Hz); ^{13}C NMR (75 MHz, DMSO-d_6): δ 198.04, 139.42, 132.08, 126.87, 123.73, 121.85, 120.49, 116.21, 112.84, 71.29, 20.52; MS (EI) *m/z* 203 (M+); HRMS (EI) calcd for $C_{11}H_9NOS$: 203.0405 (M+), found: 203.0439.

6-Methyl-4,9-dihydro-3*H*-pyrano[3,4-*b*]indole-1-thione (3d). Yield: 78%; mp 148-149 °C; IR (KBr): 3349, 1538, 1232 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 8.76 (1H, br), 7.40 (1H, s), 7.30 (1H, d, *J* = 8.5 Hz), 7.24 (1H, dd, *J* =1.2, 8.8 Hz), 4.74 (2H, t, *J* = 6.5 Hz), 3.15 (2H, t, *J* = 6.5 Hz), 2.44 (3H, s); ^{13}C NMR (75 MHz, DMSO-d_6): δ 197.95, 138.02, 132.18, 129.30, 128.97, 123.91, 120.75, 115.64, 112.60, 71.24, 21.09, 20.52; MS (EI) *m/z* 217 (M+); HRMS (EI) calcd for $C_{12}H_{11}NOS$: 217.0561 (M+), found: 217.0542.

6-Ethyl-4,9-dihydro-3*H*-pyrano[3,4-*b*]indole-1-thione (3e). Yield: 86%; mp 122-123 °C; IR (KBr): 3349, 2949, 1531, 1231 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 8.80 (1H, br), 7.39 (1H, s), 7.30 (1H, d, *J* = 8.5 Hz), 7.26-7.23 (1H, m), 4.71 (2H, t, *J* = 6.6 Hz), 3.13 (2H, t, *J* = 6.6 Hz), 2.72 (2H, q, *J* = 7.5 Hz),

1.27 (3H, t, *J* = 7.5 Hz); ^{13}C NMR (75 MHz, $CDCl_3$): δ 198.06, 137.59, 137.35, 132.19, 128.73, 124.83, 119.60, 115.52, 112.11, 71.18, 28.91, 21.29, 16.12; MS (EI) *m/z* 231 (M+); HRMS (EI) calcd for $C_{13}H_{13}NOS$: 231.0718 (M+), found: 231.0716.

6-*sec*-Butyl-4,9-dihydro-3*H*-pyrano[3,4-*b*]indole-1-thione (3f). Yield: 94%; orange oil; IR (neat): 3357, 2957, 2925, 1541, 1229 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.82 (1H, br), 7.39-7.24 (3H, m), 4.73 (2H, t, *J* = 6.5 Hz), 3.16 (2H, t, *J* = 6.5 Hz), 2.69 (1H, sext, *J* = 7.0 Hz), 1.64 (2H, quint, *J* = 7.3 Hz), 1.29 (3H, d, *J* = 7.1 Hz), 0.84 (3H, t, *J* = 7.4 Hz); ^{13}C NMR (75 MHz, $CDCl_3$): δ 197.94, 140.60, 137.72, 132.09, 127.59, 124.60, 118.92, 115.60, 112.11, 71.09, 41.58, 31.26, 22.18, 21.21, 12.30; MS (EI) *m/z* 259 (M+); HRMS (EI) calcd for $C_{15}H_{17}NOS$: 259.1031 (M+), found: 259.1034.

6-Cyclopentyl-4,9-dihydro-3*H*-pyrano[3,4-*b*]indole-1-thione (3g).

Yield: 94%; yellow oil; IR (neat): 3348, 2955, 1533, 1214 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.73 (1H, br), 7.38 (1H, s), 7.26 (2H, s), 4.67 (2H, t, *J* = 6.4 Hz), 3.11-2.99 (3H, m), 2.05-1.49 (8H, m); ^{13}C NMR (75 MHz, $CDCl_3$): δ 198.07, 139.58, 137.69, 132.25, 128.06, 124.75, 118.81, 115.60, 112.09, 71.19, 45.98, 34.90, 25.57, 21.38; MS (EI) *m/z* 271 (M+); HRMS (EI) calcd for $C_{16}H_{17}NOS$: 271.1031 (M+), found: 271.1028.

6-Cycloheptyl-4,9-dihydro-3*H*-pyrano[3,4-*b*]indole-1-thione (3h). Yield: 92%; yellow oil; IR (neat): 3338, 2919, 2851, 1541, 1229 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.78 (1H, br), 7.40 (1H, s), 7.32 (1H, d, *J* = 8.0 Hz), 7.27 (1H, d, *J* = 8.0 Hz), 4.73 (2H, t, *J* = 6.5 Hz), 3.16 (2H, t, *J* = 6.5 Hz), 2.75 (1H, quint, *J* = 5.1 Hz), 1.97-1.91 (2H, m), 1.83-1.57 (10H, m); ^{13}C NMR (75 MHz, $CDCl_3$): δ 197.99, 143.22, 137.56, 132.19, 127.82, 124.68, 118.24, 115.69, 112.14, 71.16, 47.02, 37.17, 27.91, 27.75, 21.31; MS (EI) *m/z* 299 (M+); HRMS (EI) calcd for $C_{18}H_{21}NOS$: 299.1344 (M+), found: 299.1344.

6-Chloro-4,9-dihydro-3*H*-pyrano[3,4-*b*]indole-1-thione (3i). Yield: quant.; mp: 155-157 °C; IR (KBr): 3219, 1537, 1228 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.88 (1H, br), 7.61 (1H, s), 7.35 (2H, s), 4.76 (2H, t, *J* = 6.4 Hz), 3.15 (2H, t, *J* = 6.4 Hz); ^{13}C NMR (75 MHz, DMSO-d_6): δ 198.06, 137.61, 132.99, 126.84, 124.86, 124.70, 120.95, 115.50, 114.51, 71.42, 20.38; MS (EI) *m/z* 237 (M+); HRMS (EI) calcd for $C_{11}H_8NOSCl$: 237.0015 (M+), found: 237.0001.

6-Methoxy-4,9-dihydro-3*H*-pyrano[3,4-*b*]indole-1-thione (3j). Yield: 93%; mp 153-155 °C; IR (KBr): 3326, 1534, 1219 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.79 (1H, br), 7.31 (1H, d, *J* = 9.1 Hz), 7.08 (1H, dd, *J* = 2.1, 9.1 Hz), 6.95 (1H, d, *J* = 2.1 Hz), 4.74 (2H, t, *J* = 6.5 Hz), 3.86 (3H, s), 3.15 (2H, t, *J* = 6.5 Hz); ^{13}C NMR (75 MHz, $CDCl_3$): δ 197.97, 154.94, 134.50, 132.58, 124.97, 119.60, 115.27, 113.32, 101.21, 71.21, 55.74, 21.39; MS (EI) *m/z* 233 (M+); HRMS (EI) calcd for $C_{12}H_{11}NO_2S$: 233.0510 (M+), found: 233.0512.

6-Bicyclohex-4-yl-4,9-dihydro-3*H*-pyrano[3,4-b]indole-1-thione (3k). About 5:1 mixture of **3ka** and **3kb**; Yield: 82%; IR (KBr): 2919, 2849, 1542, 1230 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.78 (1H, br),

7.42 (1H, s), 7.34-7.27 (2H, m), 4.73 (2H, t, *J* = 6.5 Hz), 3.16 (2H, dt, *J* = 2.7, 6.5 Hz), 2.63-2.50 (1H, m), 1.98-0.86 (20H, m).

6-Phenyl-4,9-dihydro-3*H*-pyrano[3,4-b]indole-1-thione (3l). Yield: 76%; yellow oil; IR (neat): 3364, 1542, 1232 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 8.90 (1H, br), 7.81 (1H, s), 7.67 (1H, d, *J* = 9.9 Hz), 7.63 (2H, d, *J* = 7.5 Hz), 7.48 (1H, d, *J* = 9.9 Hz), 7.46 (2H, t, *J* = 7.5 Hz), 7.35 (1H, t, *J* = 7.5 Hz), 4.79 (2H, t, *J* = 6.5 Hz), 3.23 (2H, t, *J* = 6.5 Hz); ^{13}C NMR (75 MHz, $CDCl_3$): δ 198.01, 141.17, 138.34, 134.97, 132.66, 128.73, 127.67, 127.11, 126.93, 125.31, 119.88, 112.58, 71.24, 31.02, 21.39; MS (EI) *m/z* 279 (M+); HRMS (EI) calcd for $C_{17}H_{13}NOS$: 279.0718 (M+), found: 279.0718.

Synthesis of S-lactones 6c-l

To a stirred solution of NaI (4.00 equiv) in $ClCH_2CH_2Cl$ (3 mL) was added TMSCl (4.00 equiv), and the resulting mixture was stirred at room temperature for 15 min. To a solution of thiolactone (1.00 mmol) in $ClCH_2CH_2Cl$ (5 mL) was transferred a solution of TMSI, prepared above, via a cannula, and then the resulting mixture was refluxed for 3-6 days. After cooling, the reaction was quenched with 10% HCl aq, and the aqueous mixture was extracted with $CHCl_3$ (10 mL × 3). The organic extracts were combined, dried over $MgSO_4$, and evaporated. The residue was chromatographed on SiO_2 (hexane–acetone = 20:1) to give the corresponding S-lactone including the known compound (**6c**).[26]

6-Methyl-4,9-dihydro-3*H*-2-thia-9-azafluoren-1-one (6d). Yield: 81%; mp 198-200 °C; IR (KBr): 3306, 1599 cm^{-1};^1H NMR (400 MHz, $CDCl_3$): δ 8.96 (1H, br), 7.41 (1H, s), 7.32 (1H, d, *J* = 8.4 Hz), 7.22 (1H, dd, *J* = 1.5, 8.4 Hz), 3.48 (2H, t, *J* = 6.3 Hz), 3.27 (2H, t, *J* = 6.3 Hz), 2.46 (3H, s); ^{13}C NMR (100 MHz, $CDCl_3$): δ 183.67, 135.02, 130.21, 129.26, 128.67, 125.99, 124.84, 120.15, 112.14, 31.52, 21.73, 21.43; MS (EI) *m/z* 217 (M+); HRMS (EI) calcd for $C_{12}H_{11}NOS$: 217.0561 (M+), found: 217.0563.

6-Ethyl-4,9-dihydro-3*H*-2-thia-9-azafluoren-1-one (6e). Yield: 56%; mp 120-123 °C; IR (KBr): 3314, 2962, 1609 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 8.96 (1H, br), 7.43 (1H, s), 7.35 (1H, d, *J* = 8.5 Hz), 7.27-7.24 (1H, m), 3.49 (2H, t, *J* = 6.3 Hz), 3.28 (2H, t, *J* = 6.3 Hz), 2.75 (2H, q, *J* = 7.4 Hz), 1.29 (3H, t, *J* = 7.4 Hz); ^{13}C NMR (75 MHz, $CDCl_3$): δ 183.83, 136.59, 135.34, 128.55, 128.16, 125.72, 124.99, 118.61, 112.43, 31.58, 28.96, 21.84, 16.21; MS (EI) *m/z* 231 (M+); HRMS (EI) calcd for $C_{13}H_{13}NOS$: 231.0718 (M+), found: 231.0716.

6-*sec*-Butyl-4,9-dihydro-3*H*-2-thia-9-azafluoren-1-one (6f). Yield: 71%; brown oil; IR (neat): 3297, 2958, 1609 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 9.23(1H, br), 7.40-7.37 (2H, d, *J* = 9.3 Hz), 7.23 (1H, d, *J* = 1.4 Hz), 3.49 (2H, t, *J* = 6.2 Hz), 3.30 (2H, t, *J* = 6.2 Hz), 2.70 (1H, sext, *J* = 6.9 Hz), 1.65 (2H, quint, *J* = 7.4 Hz), 1.30 (3H, d, *J* = 6.9 Hz), 0.85 (3H, t, *J* = 7.4 Hz); ^{13}C NMR (75 MHz, $CDCl_3$): δ 183.74, 140.05, 135.47, 128.58, 127.17, 125.68, 125.10, 118.11, 112.39, 41.76, 31.62, 31.46, 22.36, 21.91, 12.43; MS (EI) *m/z* 259 (M+); HRMS (EI) calcd for $C_{15}H_{17}NOS$: 259.1031 (M+), found: 259.1034.

6-Cyclopentyl-4,9-dihydro-3*H*-2-thia-9-azafluoren-1-one (6g). Yield: 30%; mp 168-170 °C; IR (KBr): 3311, 2948, 1607 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 8.74 (1H, br), 7.47 (1H, s), 7.36-7.29 (2H, m), 3.49 (2H, t, *J* = 6.4 Hz), 3.29 (2H, t, *J* = 6.4 Hz), 3.10 (1H, quint, *J* = 8.7 Hz), 2.18-2.10 (2H, m), 1.87-1.64 (6H, m); ^{13}C NMR (75 MHz, $CDCl_3$): δ 183.40, 138.92, 135.18, 128.61, 127.75, 125.50, 125.02, 117.97, 112.19, 46.05, 34.98, 31.63, 25.60, 21.91; MS (EI) *m/z* 271 (M+); HRMS (EI) calcd for $C_{16}H_{17}NOS$: 271.1031 (M+), found: 271.1026.

6-Cycloheptyl-4,9-dihydro-3*H*-2-thia-9-azafluoren-1-one (6h). Yield: 86%; mp 155-157 °C; IR (KBr): 3282, 2918, 2850, 1606 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 9.05 (1H, br), 7.41 (1H, s), 7.34 (1H, d, *J* = 8.8 Hz), 7.25 (1H, d, *J* = 8.8 Hz), 3.47 (2H, t, *J* = 6.3 Hz), 3.27 (2H, t, *J* = 6.3 Hz), 2.82-2.73 (1H, m), 2.02-1.22 (12H, m); ^{13}C NMR (75 MHz, $CDCl_3$): δ 183.70, 142.53, 135.26, 128.56, 127.25, 125.62, 125.13, 117.32, 112.42, 47.10, 37.26, 31.60, 27.96, 27.30, 21.88; MS (EI) *m/z* 299 (M+); HRMS (EI) calcd for $C_{18}H_{21}NOS$: 299.1344 (M+), found: 299.1344.

6-Chloro-4,9-dihydro-3*H*-2-thia-9-azafluoren-1-one (6i).Yield: 75% (brsm); mp 245-247 °C; IR (KBr): 3316, 1607 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 8.85 (1H, br), 7.62 (1H, s), 7.38-7.35 (2H, m), 3.52 (2H, t, *J* = 6.4 Hz), 3.28 (2H, t, *J* = 6.4 Hz); ^{13}C NMR (125 MHz, Acetone-d_6): δ 183.27, 136.25, 130.69, 127.62, 127.54, 126.35, 125.18, 121.07, 115.12, 31.95, 22.18; MS (EI) *m/z* 237 (M+); HRMS (EI) calcd for $C_{11}H_8NOSCl$: 237.0015 (M+), found: 237.0017.

6-Methoxy-4,9-dihydro-3*H*-2-thia-9-azafluoren-1-one (6j). Yield: 44%; mp 186-187 °C; IR (KBr): 3294, 1611 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 8.73 (1H, br), 7.32 (1H, d, *J* = 8.8 Hz), 7.06 (1H, dd, *J* = 2.5, 8.8 Hz), 6.98 (1H, d, *J* = 2.5 Hz), 3.87 (3H, s), 3.50 (2H, t, *J* = 6.4 Hz), 3.27 (2H, t, *J* = 6.4 Hz) ^{13}C NMR (125 MHz, $CDCl_3$): δ 183.60, 154.71, 132.04, 129.07, 126.01, 124.67, 119.00, 113.52, 100.71, 55.71, 31.51, 21.82; MS (EI) *m/z* 233 (M+); HRMS (EI) calcd for $C_{12}H_{11}NO_2S$: 233.0510 (M+), found: 237.0509.

6-Bicyclohex-4-yl-4,9-dihydro-3*H*-2-thia-9-azafluoren-1-one (6k). About 5:1 mixture of **6ka** and **6kb**; Yield: 87%; brown oil; IR (neat): 3306, 2921, 2849, 1616 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 8.98 (1H, br), 7.44 (1H, s), 7.35 (1H, d, *J* = 8.1 Hz), 7.27 (1H, d, *J* = 8.1 Hz), 3.49 (2H, t, *J* = 6.2 Hz), 3.29 (2H, t, *J* = 6.2 Hz), 2.65-2.51 (1H, m), 1.99-0.99 (20H, m).

6-Phenyl-4,9-dihydro-3*H*-2-thia-9-azafluoren-1-one (6l). Yield: 43% (brsm); mp 228-230 °C; IR (KBr): 3292, 1609 cm^{-1}; ^1H NMR (300MHz, $CDCl_3$): δ 8.84 (1H, br), 7.83 (1H, s), 7.65 (1H, d, *J* = 8.8 Hz), 7.63 (2H, d, *J* = 7.3 Hz), 7.49 (1H, d, *J* = 8.8 Hz), 7.46 (2H, t, *J* = 7.3 Hz), 7.35 (1H, t, *J* = 7.3 Hz), 3.53 (2H, t, *J* = 6.4 Hz), 3.35 (2H, t, *J* = 6.4 Hz); ^{13}C NMR (75 MHz, $CDCl_3$): δ 183.40, 141.41, 135.86, 134.41, 129.11, 128.71, 127.21, 127.17, 126.82, 126.28, 125.46, 119.08, 112.64, 31.60, 21.88; MS (EI) *m/z* 279 (M+); HRMS (EI) calcd for $C_{17}H_{13}NOS$: 279.0718 (M+), found: 279.0716.

Synthesis of esters 7c-l

To a stirred solution of S-lactone (1 mmol) in DMF (5 mL) was added NaH (60%, 1.2 equiv) at 0 °C, and the reaction mixture was stirred at 0 °C for 30 min. To the mixture was added $BrCH_2CO_2t$-Bu (1.2 equiv) at 0 °C, and the resulting mixture was stirred at room temperature for 20–24 h. The reaction was quenched with H_2O (10 mL), and the aqueous mixture was extracted with Et_2O (10 mL × 3). The organic extracts were combined, dried over $MgSO_4$, and evaporated. The residue was chromatographed on SiO_2 (hexane–acetone = 20:1) to give the corresponding ester.

(1-Oxo-3,4-dihydro-1*H*-2-thia-9-azafluoren-9-yl)acetic acid *tert*-butyl ester (7c). Yield: 75%; mp 122-123 °C; IR (KBr): 1745, 1617 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ 7.66 (1H, d, *J* = 8.0 Hz), 7.42 (1H, t, *J* = 8.2 Hz), 7.26 (1H, t, *J* = 8.8 Hz), 7.19 (1H, t, *J* = 7.5 Hz), 5.17 (2H, s), 3.44 (2H, t, *J* = 6.3 Hz), 3.34 (2H, t, *J* = 6.3 Hz), 1.45 (9H, s); ^{13}C NMR (75 MHz, DMSO-d_6): δ 185.83, 169.73, 137.60, 132.27, 125.53, 125.36, 120.54, 120.48, 120.25, 110.67, 49.37, 46.17, 29.66, 29.52, 6.17; MS (EI) *m/z* 317 (M+); HRMS (EI) calcd for $C_{17}H_{19}NO_3S$: 317.1086 (M+), found: 317.1085.

(6-Methyl-1-oxo-3,4-dihydro-1*H*-2-thia-9-azafluoren-9-yl)acetic acid *tert*-butyl ester (7d). Yield: 20%; mp: 174-176 °C; IR (KBr): 1743, 1618 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ 7.42 (1H, s), 7.25 (1H, d, *J* = 8.4 Hz), 7.15 (1H, d, *J* = 8.4 Hz), 5.14 (2H, s), 3.43 (2H, t, *J* = 6.5 Hz), 3.30 (2H, t, *J* = 6.5 Hz), 2.45 (3H, s), 1.44 (9H, s); ^{13}C NMR (125 MHz, $CDCl_3$): δ 183.95, 167.87, 137.07, 130.35, 129.31, 127.95, 125.93, 125.00, 120.41, 109.61, 82.26, 47.17, 31.28, 28.02, 22.36, 21.36; MS (EI) *m/z* 331 (M+); HRMS (EI) calcd for $C_{18}H_{21}NO_3S$: 331.1242 (M+), found: 331.1247.

(6-Ethyl-1-oxo-3,4-dihydro-1*H*-2-thia-9-azafluoren-9-yl)acetic acid *tert*-butyl ester (7e). Yield: 95%; mp 153-155 °C; IR (KBr): 2975, 2930, 1742, 1620 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 7.43 (1H, s), 7.28 (1H, d, *J* = 9.6 Hz), 7.17 (1H, d, *J* = 9.6 Hz), 5.14 (2H, s), 3.43 (2H, t, *J* = 6.3 Hz), 3.31 (2H, t, *J* = 6.3 Hz), 2.75 (2H, q, *J* = 7.6 Hz), 1.46 (9H, s), 1.28 (3H, t, *J* = 7.6 Hz); ^{13}C NMR (75 MHz, $CDCl_3$): δ 183.64, 167.64, 137.01, 136.77, 128.19, 127.76, 125.94, 124.78, 119.00, 109.59, 82.13, 47.12, 31.28, 28.87, 28.04, 22.39, 16.20; MS (EI) *m/z* 345 (M+); HRMS (EI) calcd for $C_{19}H_{23}NO_3S$: 345.1399 (M+), found: 345.1402.

(6-*sec*-Butyl-1-oxo-3,4-dihydro-1*H*-2-thia-9-azafluoren-9-yl)acetic acid *tert*-butyl ester (7f). Yield: quant.; mp 103-105 °C; IR (KBr): 2953, 1743, 1635 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ 7.40 (1H, brs), 7.27 (1H, dd, *J* = 1.5, 8.8 Hz), 7.18 (1H, d, *J* = 8.8 Hz), 5.14 (2H, s), 3.43 (2H, t, *J* = 6.3 Hz), 3.32 (2H, t, *J* = 6.3 Hz), 2.69 (1H, sext, *J* = 7.0 Hz), 1.64 (2H, quint, *J* = 7.2 Hz), 1.46 (9H, s), 1.28 (3H, d, *J* = 6.9 Hz), 0.83 (3H, t, *J* = 7.2 Hz); ^{13}C NMR (75 MHz, $CDCl_3$): δ 183.66, 167.72, 140.21, 137.20, 127.77, 127.19, 126.07, 124.71, 118.47, 109.60, 82.18, 47.15, 41.70, 31.44, 31.31, 28.09, 22.46, 22.36, 12.41; MS (EI) *m/z* 373 (M+); HRMS (EI) calcd for $C_{21}H_{27}NO_3S$: 373.1712 (M+), found: 373.1709.

(6-Cyclopentyl-1-oxo-3,4-dihydro-1*H*-2-thia-9-azafluoren-9-yl)acetic acid *tert*-butyl ester (7g).

Yield: 63%; mp 103-106 °C; IR (KBr): 2952, 1753, 1618 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 7.47 (1H, s), 7.33 (1H, d, *J* = 8.8 Hz), 7.18 (1H, d, *J* = 8.8 Hz), 5.14 (2H, s), 3.43 (2H, t, *J* = 6.5 Hz), 3.31 (2H, t, *J* = 6.5 Hz), 3.09 (1H, quint, *J* = 8.5 Hz), 2.11-2.09 (2H, m), 1.84-1.21 (15H, m); ^{13}C NMR (75 MHz, $CDCl_3$): δ 183.70, 167.72, 139.02, 137.17, 127.85, 127.56, 126.11, 124.71, 118.26, 109.62, 82.24, 47.21, 46.00, 34.98, 31.37, 28.14, 25.60, 22.52; MS (EI) *m/z* 385 (M+); HRMS (EI) calcd for $C_{22}H_{27}NO_3S$: 385.1712 (M+), found: 385.1711.

(6-Cycloheptyl-1-oxo-3,4-dihydro-1*H*-2-thia-9-azafluoren-9-yl)acetic acid *tert*-butyl ester (7h). Yield: 92% (brsm); mp 158-161 °C; IR (KBr): 2923, 2850, 1739, 1632 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 7.41 (1H, s), 7.27 (1H, d, *J* = 8.8 Hz), 7.15 (1H, d, *J* = 8.8 Hz), 5.12 (2H, s), 3.41 (2H, t, *J* = 6.0 Hz), 3.30 (2H, t, *J* = 6.0 Hz), 1.96-1.53 (12H, m), 1.45 (9H, s); ^{13}C NMR (75 MHz, $CDCl_3$): δ 183.61, 167.69, 142.67, 136.98, 127.76, 127.29, 126.11, 124.67, 117.71, 109.59, 82.15, 47.10, 47.04, 37.25, 31.31, 28.07, 27.94, 27.28, 22.43; MS (EI) *m/z* 413 (M+); HRMS (EI) calcd for $C_{24}H_{31}NO_3S$: 413.2025 (M+), found: 413.2024.

(6-Chloro-1-oxo-3,4-dihydro-1*H*-2-thia-9-azafluoren-9-yl)acetic acid *tert*-butyl ester (7i). Yield: quant.; mp 218-220 °C; IR (KBr): 1739, 1623 cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6): δ 7.90 (1H, d, *J* = 2.1 Hz), 7.64 (1H, d, *J* = 9.0 Hz), 7.34 (1H, dd, *J* = 2.1, 9.0 Hz), 5.09 (2H, s), 3.66 (2H, t, *J* = 7.7 Hz), 3.43 (2H, t, *J* = 7.7 Hz), 1.56 (9H, s); ^{13}C NMR (125 MHz, $CDCl_3$): δ 180.70, 167.59, 136.83, 128.89, 127.73, 126.76, 125.77, 125.51, 120.52, 111.29, 82.72, 47.37, 31.21, 28.11, 22.30; MS (EI) *m/z* 351 (M+); HRMS (EI) calcd for $C_{17}H_{18}NO_3SCl$: 351.0696 (M+), found: 351.0693.

(6-Methoxy-1-oxo-3,4-dihydro-1*H*-2-thia-9-azafluoren-9-yl)acetic acid *tert*-butyl ester (7j). Yield: quant.; mp 134-135 °C; IR (KBr): 1741, 1610 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 7.16 (1H, d, *J* = 9.1 Hz), 7.09 (1H, dd, *J* = 2.4, 9.1 Hz), 6.99 (1H, d, *J* = 2.4 Hz), 5.13 (2H, s), 3.86 (3H, s), 3.42 (2H, t, *J* = 6.5 Hz), 3.29 (2H, t, *J* = 6.5 Hz), 1.45 (9H, s); ^{13}C NMR (125 MHz, $CDCl_3$): δ 183.84, 167.81, 154.83, 134.04, 128.20, 125.59, 124.95, 118.97, 110.95, 101.00, 82.30, 55.77, 47.23, 31.22, 28.00, 22.42; MS (EI) *m/z* 347 (M+); HRMS (EI) calcd for $C_{18}H_{21}NO_4S$: 347.1191 (M+), found: 347.1191.

(6-Bicyclohex-4-yl-1-oxo-3,4-dihydro-1*H*-2-thia-9-azafluoren-9-yl)acetic acid *tert*-butyl ester (7k). About 5:1 mixture of **7ka** and **7kb**; Yield: 93%; yellow oil; IR (neat): 2923, 2849, 1748, 1634 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 7.44 (1H, s), 7.30 (1H, d, *J* = 8.8 Hz), 7.17 (1H, d, *J* = 8.8 Hz), 5.14 (2H, s), 3.43 (2H, t, *J* = 5.3 Hz), 3.32 (2H, t, *J* = 5.3 Hz), 2.61-2.51 (1H, m), 1.97-0.89 (20H, m), 1.46 (9H, s).

(1-Oxo-6-phenyl-3,4-dihydro-1*H*-2-thia-9-azafluoren-9-yl)acetic acid *tert*-butyl ester (7l). Yield: 98% (brsm); mp 128-130 °C; IR (KBr): 2979, 1741, 1624 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 7.83 (1H, d, *J* = 1.7 Hz), 7.67 (1H, dd, *J* = 1.7, 8.7 Hz), 7.63 (2H, d, *J* = 7.4 Hz), 7.46 (2H, t, *J* = 7.4 Hz), 7.35 (1H, t, *J* = 7.4 Hz), 7.33 (1H, d, *J* = 8.7 Hz), 5.20 (2H, s), 3.47 (2H, t, *J* = 5.7 Hz), 3.38 (2H, t, *J* = 5.7 Hz), 1.47 (9H, s); ^{13}C NMR (75 MHz, $CDCl_3$): δ 183.77, 167.57, 141.35, 137.84, 134.47, 128.68, 128.34, 127.21,

127.16, 126.77, 126.57, 125.18, 119.26, 110.15, 82.42, 47.29, 31.31, 28.12, 22.46; MS (EI) *m/z* 393 (M+); HRMS (EI) calcd for $C_{23}H_{23}NO_3S$: 393.1399 (M+), found: 393.1402.

Synthesis of carboxylic acids 5c-l

To a stirred solution of NaI (4 equiv) in $ClCH_2CH_2Cl$ (3 mL) was added TMSCl (4 equiv), and the resulting mixture was stirred at room temperature for 15 min. To a solution of *t*-butyl ester (1 mmol) in $ClCH_2CH_2Cl$ (5 mL) was transferred a solution of TMSI, prepared above, via a cannula, and then the resulting mixture was refluxed for 2 days. After cooling, the reaction was quenched with 10% HCl aq, and the aqueous mixture was extracted with $CHCl_3$ (10 mL × 3). The organic extracts were combined, dried over $MgSO_4$, and evaporated. The residue was chromatographed on SiO_2 (hexane–acetone = 2:1) to give the corresponding carboxylic acid.

(1-Oxo-3,4-dihydro-1*H*-2-thia-9-azafluoren-9-yl)acetic acid (5c). Yield: 73%; mp 207-209 °C; IR (KBr): 1720, 1612 cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6): δ 7.78 (1H, d, *J* = 8.1 Hz), 7.61 (1H, d, *J* = 8.5 Hz), 7.42 (1H, t, *J* = 8.3 Hz), 7.19 (1H, t, *J* = 7.5 Hz), 5.22 (2H, s), 3.49 (2H, t, *J* = 6.4 Hz), 3.34-3.32 (2H, m); ^{13}C NMR (75 MHz, DMSO-d_6): δ 182.96, 169.91, 138.10, 127.05, 126.93, 126.32, 124.01, 121.16, 120.51, 110.75, 46.33, 30.81, 21.67; MS (EI) *m/z* 261 (M+); HRMS (EI) calcd for $C_{13}H_{11}NO_3S$: 261.0460 (M+), found: 261.0454.

(6-Methyl-1-oxo-3,4-dihydro-1*H*-2-thia-9-azafluoren-9-yl)acetic acid (5d). Yield: 52%; mp 215-217 °C; IR (KBr): 1715, 1625 cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6): δ 7.54 (1H, s), 7.50 (1H, d, *J* = 8.5 Hz), 7.25 (1H, d, *J* = 8.5 Hz), 5.18 (2H, s), 3.47 (2H, t, *J* = 6.2 Hz), 3.27 (2H, t, *J* = 6.2 Hz), 2.41 (3H, s); ^{13}C NMR (75 MHz, $CDCl_3$): δ 182.82, 169.92, 136.72, 129.39, 128.88, 127.08, 125.72, 124.15, 120.16, 110.51, 46.33, 30.81, 21.67, 20.98; MS (EI) *m/z* 275 (M+); HRMS (EI) calcd for $C_{14}H_{13}NO_3S$: 275.0616 (M+), found: 275.0600.

(6-Ethyl-1-oxo-3,4-dihydro-1*H*-2-thia-9-azafluoren-9-yl)acetic acid (5e). Yield: 76%; mp 231-233 °C; IR (KBr): 2963, 1717, 1616 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 7.44 (1H, s), 7.31 (1H, d, *J* = 8.7 Hz), 7.21 (1H, d, *J* = 8.7 Hz), 5.27 (2H, s), 3.43 (2H, t, *J* = 6.3 Hz), 3.31 (2H, t, *J* = 6.3 Hz), 2.75 (2H, q, *J* = 7.7 Hz), 1.28 (3H, t, *J* = 7.7 Hz); ^{13}C NMR (125 MHz, DMSO-d_6): δ 183.13, 170.21, 137.10, 136.27, 128.10, 127.28, 126.12, 124.29, 119.13, 110.77, 46.36, 30.83, 28.18, 21.68, 16.23; MS (EI) *m/z* 289 (M+); HRMS (EI) calcd for $C_{15}H_{15}NO_3S$: 289.0773 (M+), found: 289.0776.

(6-*sec*-Butyl-1-oxo-3,4-dihydro-1*H*-2-thia-9-azafluoren-9-yl)acetic acid (5f). Yield: 68%; mp 184-185 °C; IR (KBr): 2960, 1718, 1624 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 7.41 (1H, s), 7.31 (1H, d, *J* = 8.8 Hz), 7.22 (1H, d, *J* = 8.8 Hz), 5.26 (2H, s), 3.44 (2H, t, *J* = 5.8 Hz), 3.32 (2H, t, *J* = 5.8 Hz), 2.70 (1H, d, *J* = 7.1 Hz), 1.64 (2H, quint, *J* = 7.4 Hz), 1.28 (3H, d, *J* = 6.9 Hz), 0.83 (3H, t, *J* = 7.4 Hz); ^{13}C NMR (75 MHz, $CDCl_3$): δ 184.23, 174.29, 140.62, 137.12, 127.64, 127.58, 126.59, 124.73, 118.57,

109.55, 46.28, 41.68, 31.42, 31.21, 22.36, 22.33, 12.41; MS (EI) *m/z* 317 (M+); HRMS (EI) calcd for $C_{17}H_{19}NO_3S$: 317.1086 (M+), found: 317.1086.

(6-Cyclopentyl-1-oxo-3,4-dihydro-1*H*-2-thia-9-azafluoren-9-yl)acetic acid (5g). Yield: 86%; mp 232-234 °C; IR (KBr): 2952, 1718, 1617 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 7.48 (1H, d, *J* = 0.82 Hz), 7.37 (1H, d, *J* = 8.7 Hz), 7.22 (1H, d, *J* = 8.7 Hz), 5.26 (2H, s), 3.43 (2H, t, *J* = 5.7 Hz), 3.32 (2H, t, *J* = 5.7 Hz), 3.10 (1H, quint, *J* = 9.0 Hz), 2.11-2.09 (2H, m), 1.84-1.63 (6H, m); ^{13}C NMR (125 MHz, $CDCl_3$): δ 184.59, 173.34, 139.60, 137.26, 128.14, 127.78, 126.80, 124.81, 118.46, 109.67, 46.33, 45.91, 34.89, 31.15, 25.49, 22.32; MS (EI) *m/z* 329 (M+); HRMS (EI) calcd for $C_{18}H_{19}NO_3S$: 329.1086 (M+), found: 329.1083.

(6-Cycloheptyl-1-oxo-3,4-dihydro-1*H*-2-thia-9-azafluoren-9-yl)acetic acid (5h). Yield: 89%; mp 213-215 °C; IR (KBr): 2923, 2849, 1725, 1627 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 7.43 (1H, s), 7.32 (1H, d, *J* = 9.0 Hz), 7.21 (1H, d, *J* = 9.0 Hz), 5.24 (2H, s), 3.43 (2H, t, *J* = 6.0 Hz), 3.31 (2H, t, *J* = 6.0 Hz), 2.77 (1H, m), 2.09-0.86 (12H, m); ^{13}C NMR (75 MHz, DMSO-d_6): δ 183.08, 170.23, 142.13, 137.03, 127.26, 127.03, 126.29, 124.16, 117.73, 110.80, 46.33, 46.29, 36.73, 30.84, 27.38, 26.66, 21.68; MS (EI) *m/z* 357 (M+); HRMS (EI) calcd for $C_{20}H_{23}NO_3S$: 357.1399 (M+), found: 357.1396.

(6-Chloro-1-oxo-3,4-dihydro-1*H*-2-thia-9-azafluoren-9-yl)acetic acid (5i). Yield: 50%; mp 221-222 °C; IR (KBr): 1727, 1624 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6): δ 7.90 (1H, d, *J* = 1.9 Hz), 7.69 (1H, d, *J* = 9.1 Hz), 7.41 (1H, dd, *J* = 1.9, 9.1 Hz), 5.22 (2H, s), 3.49 (2H, t, *J* = 6.4 Hz), 3.30 (2H, t, *J* = 6.4 Hz); ^{13}C NMR (75 MHz, DMSO-d_6): δ 183.19, 169.73, 136.48, 128.07, 126.84, 125.58, 125.09, 124.93, 120.35, 112.74, 46.59, 30.73, 21.49; MS (EI) *m/z* 295 (M+); HRMS (EI) calcd for $C_{13}H_{10}O_3NSCl$: 295.0070 (M+), found: 295.0042.

(6-Methoxy-1-oxo-3,4-dihydro-1*H*-2-thia-9-azafluoren-9-yl)acetic acid (5j). Yield: 34%; mp 205-207 °C; IR (KBr): 1715, 1623 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6): δ 7.53 (1H, d, *J* = 9.1 Hz), 7.21 (1H, d, *J* = 2.5 Hz), 7.06 (1H, d, *J* = 2.5, 9.1 Hz), 5.18 (2H, s), 3.81 (3H, s), 3.47 (2H, t, *J* = 6.2 Hz), 3.28 (2H, t, *J* = 6.2 Hz); ^{13}C NMR (75 MHz, DMSO-d_6): δ 182.75, 169.96, 154.10, 133.66, 127.29, 125.56, 124.22, 118.57, 111.89, 100.97, 55.44, 46.44, 30.84, 21.78; MS (EI) *m/z* 291 (M+); HRMS (EI) calcd for $C_{14}H_{13}NO_4S$: 291.0565 (M+), found: 291.0570.

(6-Bicyclohex-4-yl-1-oxo-3,4-dihydro-1*H*-2-thia-9-azafluoren-9-yl)acetic acid (5k). About 5:1 mixture of **5ka** and **5kb**; Yield: 86%; IR (KBr): 2921, 2849, 1722, 1632 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 7.44 (1H, s), 7.33 (1H, d, *J* = 9.5 Hz), 7.20 (1H, d, *J* = 9.5 Hz), 5.26 (2H, s), 3.42 (2H, t, *J* = 5.7 Hz), 3.32 (2H, t, *J* = 5.7 Hz), 2.64-2.55 (1H, m), 1.98-0.89 (20H, m).

(1-Oxo-6-phenyl-3,4-dihydro-1*H*-2-thia-9-azafluoren-9-yl)acetic acid (5l). Yield: 55%; mp 220-223 °C; IR (KBr): 2923, 1716, 1619 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 7.84 (1H, s), 7.71 (1H, d, *J* = 7.4 Hz), 7.62 (2H, d, *J* = 6.9 Hz), 7.46 (2H, t, *J* = 6.9 Hz), 7.39 (1H, d, *J* = 7.4 Hz), 7.37 (1H, t, *J* = 6.9

Hz), 5.33 (2H, s), 3.46 (2H, t, *J* = 6.5 Hz), 3.41 (2H, t, *J* = 6.5 Hz); ^{13}C NMR (125 MHz, DMSO-d_6): δ 183.29, 170.17, 140.59, 137.91, 133.15, 128.90, 127.86, 126.96, 126.88, 126.81, 126.55, 124.75, 119.13, 111.50, 46.58, 30.83, 21.66; MS (EI) *m/z* 337 (M+); HRMS (EI) calcd for $C_{19}H_{15}NO_3S$: 337.0773 (M+), found: 337.0775.

Bicyclohexyl-4-one (9)

To a suspension of 4-cyclohexylcyclohexanol **8** (7.17 g, 39.3 mmol) and Celite (20 g) in CH_2Cl_2 (80 mL) was added PCC (12.7 g, 59.0 mmol) at 0 °C, and the resulting mixture was stirred at the room temperature for 14 h. The catalyst was removed by filtration and the filtrate was concentrated in vacuo. The residue was chromatographed on SiO_2 (hexane : EtOAc = 20 : 1) to give **9** (6.78 g, 96%).[27]

4-Phenyl-bicyclohexyl-4-ol (10)

To a freshly made solution of PhMgBr (30 mmol) in THF (30 mL) was added **9** (2.16 g, 12.0 mmol) in THF (12.5 mL) at 0 °C, and the resulting mixture was stirred at the same temperature for 3 h. The reaction was quenched by the addition of sat. NH_4Cl aq, diluted with EtOAc. And then, the organic phase was separated, the aqueous mixture was extracted with EtOAc. The organic extracts were combined, dried over $MgSO_4$, and evaporated. The residue was chromatographed on SiO_2 (hexane : EtOAc = 30 : 1) to give **10** (2.88 g, 93%).

^{1}H NMR (500 MHz, $CDCl_3$): 7.55-7.50 (2H, m), 7.38-7.33 (2H, m), 7.29-7.22 (1H, m), 1.88-0.83 (20H, m).

***trans*-4-Phenyl-1,1'-bi(cyclohexane) (11b) and *cis*-4-Phenyl-1,1'-bi-(cyclohexane) (11c)**

To a solution of **10** (2.88 g, 11.1 mmol) in CH_2Cl_2 was added slowly Et_3SiH (3.60 mL, 22.2 mmol) , $BF_3 \cdot OEt_2$ (1.39 mL, 11.1 mmol) at -40 °C, and the resulting mixture was stirred at the same temperature for 4 h. The reaction was diluted with CH_2Cl_2 and neutralized with sat. $NaHCO_3$ aq. The organic phase was separated, dried over Na_2SO_4 and concentrated in vacuo. The residue was chromatographed on SiO_2 (hexane) to give mixture of **11b** and **11c (**the ratio of compound **11b** : **11c** = ca. 3 : 1, 2.47 g, 92%).

^{1}H NMR (500 MHz, $CDCl_3$): 8.16-8.13 (2H, m), 7.40 (1H, d, *J* = 8.6 Hz), 7.34 (1H, d, *J* = 8.5 Hz), 2.79-2.74 (**11c**; 1H, m), 2.56 (**11b**; 1H, tt, *J* = 3.1, 12.0 Hz), 1.94-0.99 (20H, m).

***trans*-4-(4-Nitrophenyl)-1,1'-bi(cyclohexane) (12b) and *cis*-4-(4-Nitrophenyl)-1,1'-bi(cyclohexane) (12c)**

To a solution of mixture of **11b** and **11c** (2.47 g) in $CHCl_3$ (10 mL) was added Ac_2O (2.9 mL) at 0 °C. At the same time HNO_3 (6.1 mL) was slowly added to Ac_2O (10 mL) at 0 °C, and this mixture was added

dropwise to the solution, prepared above, at 0 °C. The resulting mixture was stirred at room temperature for 13 h. The reaction was quenched by the addition of NaOH (10% aqueous solution), and the organic layer was separated, dried over Na_2SO_4 and concentrated in vacuo. The residue was chromatographed on SiO_2 (hexane) to give mixture of **12b** and **12c** (1.53 g, 52%).

^{1}H NMR (500 MHz, $CDCl_3$): 8.16-8.13 (2H, m), 7.40 (1H, d, *J* = 8.6 Hz), 7.34 (1H, d, *J* = 8.5 Hz), 2.79-2.74 (**12c**; 1H, m), 2.56 (**12b**; 1H, tt, *J* = 3.1, 12.0 Hz), 1.94-0.99 (20H, m).

4-(*trans*-[1,1'-Bi(cyclohexane)]-4-yl)aniline (13b) and 4-(*cis*-[1,1'-Bi(cyclohexane)]-4-yl)aniline (13c)

To a solution of mixture of **12b** and **12c** (1.53 g, 5.33 mmol) in EtOAc (23 mL) was added 10% Pd/C (60 mg), and the resulting suspension was stirred under a hydrogen atmosphere at 1 atm for 3 days. The catalyst was removed by filtration and the filtrate was concentrated in vacuo. The residue was chromatographed on SiO_2 (hexane : EtOAc = 15 : 1) to give **13b** (795 mg, 58%), **13c** (273 mg, 20%).

4-(*trans*-[1,1'-Bi(cyclohexane)]-4-yl)aniline (13b). mp 118-120 °C; IR (KBr): 3382, 3312, 2920, 2849, 1516 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.00 (2H, d, *J* = 8.3 Hz), 6.64 (2H, d, *J* = 8.3 Hz), 3.53 (2H, br), 2.34 (1H, tt, *J* = 3.5, 12.4 Hz), 1.89-0.96 (20H, m); ^{13}C NMR (125 MHz, $CDCl_3$): δ 144.08, 138.12, 127.41, 115.09, 43.68, 43.27, 42.92, 34.80, 30.26, 30.21, 26.81; MS (EI) *m/z* 257 (M+); HRMS (EI) calcd for $C_{18}H_{27}N$: 257.2143 (M+), found: 257.2141.

4-(*cis*-[1,1'-Bi(cyclohexane)]-4-yl)aniline (13c). mp 67-69 °C; IR (KBr): 3464, 3371, 2927, 2846, 1520 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$): 7.04 (2H, dd, *J* = 1.4, 6.3 Hz), 6.64 (2H, dd, *J* = 2.0, 6.3 Hz), 3.53 (2H, br), 2.57-2.51 (1H, m), 1.85-0.78 (20H, m); ^{13}C NMR (125 MHz, $CDCl_3$): δ 143.96, 137.68, 127.69, 115.12, 41.99, 39.19, 36.67, 30.95, 29.54, 27.41, 26.65; MS (EI) *m/z* 257 (M+); HRMS (EI) calcd for $C_{18}H_{27}N$: 257.2143 (M+), found: 257.2144.

***cis*-3-(4-Nitrophenyl)-1,1'-bi(cyclohexane) (12a) and *trans*-4-(4-nitrophenyl)-1,1'-bi(cyclohexane) (12b)**

To a solution of 4-cyclohexylcyclohexanol **8** (2.42 g, 13.3 mmol, about 1 : 1.3 mixture of *cis*- and *trans*-isomers) in dry benzene (60 mL) was slowly added $AlCl_3$ (1.77 g, 13.3 mmol) at room temperature. The resulting mixture was stirred at the same temperature for 17 h. The reaction was quenched by the addition of ice water, and then the organic phase was separated, washed with Na_2CO_3 (saturated aqueous solution), dried over Na_2SO_4 and concentrated in vacuo to yield mixture of *cis*-3-phenyl-1,1'-bi(cyclohexane) **11a** and *trans*-4-phenyl-1,1'-bi(cyclohexane) **11b** (the ratio of compound **11a** : **11b** = ca. 5 : 1). The product was used for the next reaction without further purification. To a solution of crude (3.22 g) in $CHCl_3$ (13 mL) was added Ac_2O (2.7 mL) at 0 °C. At the same time HNO_3 (1.3 mL) was slowly added to Ac_2O (2.7 mL) at 0 °C, and this mixture was added dropwise to the

solution, prepared above, at 0 °C. The resulting mixture was stirred at room temperature for 14 h. The reaction was quenched by the addition of NaOH (10% aqueous solution), and the organic layer was separated, dried over Na_2SO_4 and concentrated in vacuo. The residue was chromatographed on SiO_2 (hexane : EtOAc = 70 : 1) to give mixture of **12a** and **12b** (1.78 g, 47% in two steps).

^{1}H NMR (500 MHz, $CDCl_3$): δ 8.14 (2H, dd, *J* = 2.0, 7.9 Hz), 7.35 (2H, dd, *J* = 2.0 Hz, 7.9 Hz), 2.65-2.53 (1H, m), 1.94-0.96 (20H, m)

4-(*cis*-[1,1'-Bi(cyclohexane)]-3-yl)aniline (13a) and 4-(*trans*-[1,1'-Bi(cyclohexane)]-4-yl)aniline (13b)

To a solution of **12a** and **12b** (3.30 g, 11.5 mmol) in EtOAc (15 mL) was added Pd/C (59 mg), and the resulting suspension was stirred under a hydrogen atmosphere at 1 atm for 5 days. The catalyst was removed by filtration and the filtrate was concentrated in vacuo. The residue was chromatographed on SiO_2 (hexane : EtOAc = 12 : 1) to give **13a** (1.80g, 61%), **13b** (531mg, 18%).

4-(*cis*-[1,1'-Bi(cyclohexane)]-3-yl)aniline (13a). mp 58-60 °C; IR (KBr): 3423, 3348, 2920, 2846, 1517 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.01 (2H, d, *J* = 8.3 Hz), 6.64 (2H, d, *J* = 8.3 Hz), 3.54 (2H, br), 2.39 (1H, tt, *J* = 3.3, 11.9 Hz), 1.89-0.94 (20H, m); ^{13}C NMR (125 MHz, $CDCl_3$): δ 144.09, 138.37, 127.41, 115.10, 43.76, 43.67, 43.44, 38.35, 34.55, 30.16, 30.04, 29.53, 26.87, 26.80; MS (EI) *m/z* 257 (M+); HRMS (EI) calcd for $C_{18}H_{27}N$: 257.2143 (M+), found: 257.2147.

1',3'-*cis*-6-Bicyclohex-3-yl-4,9-dihydro-3*H*-pyrano[3,4-*b*]indol-1-one (2ka)

To a suspension of **13a** (105 mg, 0.41 mmol) and concentrated HCl (0.1 mL) in THF (0.63 mL) and H_2O (0.42 mL) was slowly added a solution of $NaNO_2$ (31 mg, 0.44 mmol) in H_2O (0.15 mL) at 0 °C. The reaction mixture was stirred at 0 °C for 30 min. At the same time, to a solution of enol lactone (98 mg, 0.49 mmol) and sodium acetate (134 mg, 1.63 mmol) in THF (0.21 mL) and H_2O (0.11 mL) was slowly added the diazonium salt, prepared above, at 0 °C. The resulting mixture was stirred at 0 °C for 2 h. The reaction was diluted with CH_2Cl_2 and the organic phase was separated, dried over Na_2SO_4 and concentrated in vacuo to yield hydrazone, which was used for the next reaction without further purification. A solution of the above hydrazone (138 mg) in AcOH (1.2 mL) and HCl (1.2 mL, 1.0 M in AcOH solution) was refluxed for 4h, and cooled to room temperature. The resulting mixture was diluted with CH_2Cl_2 and the organic phase was separated, washed with H_2O, neutralized with $NaHCO_3$ (saturated aqueous solution), dried over Na_2SO_4 and concentrated in vacuo. The residue was chromatographed on SiO_2 (hexane : EtOAc = 15 : 1) to give **2ka** (99 mg, 69% in two steps).

brown oil, IR (neat): 3291, 2922, 2850, 1700 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.76 (1H, br), 7.43 (1H, brs), 7.38 (1H, d, *J* = 8.6 Hz), 7.28 (1H, dd, *J* = 1.7, 8.6 Hz), 4.69 (2H, t, *J* = 6.3 Hz), 3.15 (2H, t, *J* = 6.3 Hz), 2.63-2.59 (1H, m), 1.92-0.96 (20H, m); ^{13}C NMR (125 MHz, $CDCl_3$): δ 161.54, 140.96, 137.02,

127.02, 124.55, 122.89, 122.23, 117.55, 112.59, 69.46, 44.73, 43.77, 43.47, 38.57, 34.81, 30.21, 30.10, 29.57, 26.90, 26.80, 21.47; MS (EI) *m/z* 351 (M+); HRMS (EI) calcd for $C_{23}H_{29}NO_2$: 351.2198 (M+), found: 351.2199.

1',3'-*cis*-6-Bicyclohex-3-yl-4,9-dihydro-3*H*-pyrano[3,4-*b*]indole-1-thione (3ka)

To a stirred solution of **2ka** (43 mg, 0.12 mmol) in toluene (3 mL) was added Lawesson's reagent (27 mg, 0.067 mmol), and the resulting mixture was refluxed for 18 h. After cooling, the solvent was removed, and the residue was chromatographed on SiO_2 (hexane–acetone = 20:1) to give **3ka** (50 mg, quant.).

yellow oil, IR (neat): 3356, 2921, 2850, 1540, 1231 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ 8.76 (1H, br), 7.42 (1H, brs), 7.32 (1H, d, *J* = 8.6 Hz), 7.29 (1H, dd, *J* = 1.5, 8.6 Hz), 4.74 (2H, t, *J* = 6.6 Hz), 3.17 (2H, t, *J* = 6.6 Hz), 2.59 (1H, tt, *J* = 3.2, 11.8 Hz), 1.92-0.96 (20H, m); ^{13}C NMR (125 MHz, $CDCl_3$): δ 198.23, 141.48, 137.83, 132.30, 128.07, 124.85, 118.53, 115.74, 112.12, 71.15, 44.67, 43.69, 43.41, 38.43, 34.68, 30.16, 30.07, 29.50, 26.84, 26.77, 21.22; MS (EI) *m/z* 367 (M+); HRMS (EI) calcd for $C_{23}H_{29}NOS$: 367.1970 (M+), found: 367.1970.

1',3'-*cis*-6-Bicyclohex-3-yl-4,9-dihydro-3*H*-2-thia-9-azafluoren-1-one (6ka)

To a stirred solution of NaI (85 mg, 0.57 mmol) in $ClCH_2CH_2Cl$ (10 mL) was added TMSCl (72 μL, 0.57 mmol), and the resulting mixture was stirred at room temperature for 15 min. To a solution of **3ka** (52mg, 0.14 mmol) in $ClCH_2CH_2Cl$ (5 mL) was transferred a solution of TMSI, prepared above, via a cannula, and then the resulting mixture was refluxed for 15 days. After cooling, the reaction was quenched with 10% HCl aq, and the aqueous mixture was extracted with $CHCl_3$. The organic extracts were combined, dried over Na_2SO_4, and evaporated. The residue was chromatographed on SiO_2 (hexane–acetone = 30 : 1) to give **6ka** (48 mg, 92%).

mp 147-149 °C; IR (KBr): 3306, 2909, 2848, 1606 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ 8.74 (1H, br), 7.44 (1H, brs), 7.33 (1H, d, *J* = 8.6 Hz), 7.27 (1H, dd, *J* = 1.7 Hz, 8.6 Hz), 3.48 (2H, t, *J* = 6.2 Hz), 3.29 (2H, t, *J* = 6.2 Hz), 2.60 (1H, tt, *J* = 3.1 Hz, 11.8 Hz), 1.93-0.96 (20H, m); ^{13}C NMR (125 MHz, $CDCl_3$): δ 183.75, 140.86, 135.45, 128.69, 127.54, 125.86, 125.21, 117.67, 112.31, 44.75, 43.77, 43.48, 38.56, 34.80, 31.54, 30.22, 30.11, 29.58, 26.91, 26.82, 21.80; MS (EI) *m/z* 367 (M+); HRMS (EI) calcd for $C_{23}H_{29}NOS$: 367.1970 (M+), found: 367.1970.

1',3'-*cis*-(6-Bicyclohex-3-yl-1-oxo-3,4-dihydro-1*H*-2-thia-9-azafluoren-9-yl)acetic acid *tert*-butyl ester (7ka)

To a stirred solution of **6ka** (104 mg, 0.28 mmol) in DMF (3 mL) was added NaH (60%, 17mg, 0.42 mmol) at 0 °C, and the reaction mixture was stirred at 0 °C for 30 min. To the mixture was added

$BrCH_2CO_2t$-Bu (63 μL, 0.42 mmol) at 0 °C, and the resulting mixture was stirred at room temperature for 17 h. The reaction was quenched with H_2O, and the aqueous mixture was extracted with Et_2O. The organic extracts were combined, dried over Na_2SO_4, and evaporated. The residue was chromatographed on SiO_2 (hexane–acetone = 30:1) to give **7ka** (130 mg, 95%).

mp 163-165 °C; IR (KBr): 2922, 2851, 1742, 1627 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ 7.44 (1H, brs), 7.30 (1H, dd, *J* = 1.4, 8.6 Hz), 7.17 (1H, d, *J* = 8.6 Hz), 5.13 (2H, s), 3.43 (2H, t, *J* = 6.2 Hz), 3.32 (2H, t, *J* = 6.2 Hz), 2.60 (1H, tt, *J* = 3.1, 11.6 Hz), 1.91-0.98 (20H, m), 1.45 (9H, s); ^{13}C NMR (125 MHz, $CDCl_3$): δ 183.89, 167.88, 140.98, 137.30, 127.91, 127.59, 126.25, 124.85, 118.02, 109.62, 82.21, 47.11, 44.71, 43.78, 43.49, 38.51, 34.83, 31.27, 30.23, 30.13, 29.58, 28.01, 26.92, 26.83, 22.40; MS (EI) *m/z* 481 (M+); HRMS (EI) calcd for $C_{23}H_{39}NO_3S$: 481.2651 (M+), found: 481.2652.

1',3'-*cis*-(6-Bicyclohex-3-yl-1-oxo-3,4-dihydro-1*H*-2-thia-9-azafluoren-9-yl)acetic acid (5ka)

To a stirred solution of NaI (162 mg, 1.08 mmol) in $ClCH_2CH_2Cl$ (5 mL) was added TMSCl (0.14 mL, 1.08 mmol), and the resulting mixture was stirred at room temperature for 15 min. To a solution of **7ka** (130mg, 0.27 mmol) in $ClCH_2CH_2Cl$ (5 mL) was transferred a solution of TMSI, prepared above, via a cannula, and then the resulting mixture was refluxed for 19 h. After cooling, the reaction was quenched with 10% HCl aq, and the aqueous mixture was extracted with EtOAc. The organic extracts were combined, dried over Na_2SO_4, and evaporated. The residue was chromatographed on SiO_2 (hexane–acetone = 3 : 1) to give **5ka** (102 mg, 89%).

mp 219-221 °C; IR (KBr): 2925, 2850, 1723, 1631 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ 7.45 (1H, brs), 7.33 (1H, d, *J* = 8.6 Hz), 7.21 (1H, dd, *J* = 1.5, 8.6 Hz), 5.26 (2H, s), 3.42 (2H, t, *J* = 6.0 Hz), 3.32 (2H, t, *J* = 6.0 Hz), 2.61 (1H, tt, *J* = 3.3, 11.7 Hz), 1.90-0.96 (20H, m), ^{13}C NMR (125 MHz, $CDCl_3$): δ 184.46, 174.08, 141.39, 137.26, 128.03, 127.73, 126.79, 124.87, 118.11, 109.60, 46.28, 44.68, 43.77, 43.48, 38.52, 34.76, 31.15, 30.22, 30.12, 29.57, 26.90, 26.82, 22.31; MS (EI) *m/z* 425 (M+); HRMS (EI) calcd for $C_{25}H_{31}NO_3S$: 425.2025 (M+), found: 425.2029.

1',4'-*trans*-6-Bicyclohex-4-yl-4,9-dihydro-3*H*-pyrano[3,4-*b*]indol-1-one (2kb)

To a suspension of **13b** (120 mg, 0.47 mmol) and concentrated HCl (0.12 mL) in THF (0.72 mL) and H_2O (0.24 mL) was slowly added a solution of $NaNO_2$ (35 mg, 0.50 mmol) in H_2O (0.16 mL) at 0 °C. The reaction mixture was stirred at 0 °C for 30 min. At the same time, to a solution of enol lactone (112 mg, 0.56 mmol) and sodium acetate (153 mg, 1.86 mmol) in THF (0.48 mL) and H_2O (0.12 mL) was slowly added the diazonium salt, prepared above, at 0 °C. The resulting mixture was stirred at 0 °C for 2 h. The reaction was diluted with CH_2Cl_2 and the organic phase was separated, dried over Na_2SO_4 and concentrated in vacuo to yield hydrazone. The product was used for the next reaction without further

purification. A solution of crude (153 mg) in AcOH (1.3 mL) and HCl (1.3 mL, 1.0 M in AcOH solution) was refluxed for 4 h, and cooled to room temperature. The resulting mixture was diluted with CH_2Cl_2 and the organic phase was separated, washed with H_2O, neutralized with $NaHCO_3$ (saturated aqueous solution), dried over Na_2SO_4 and concentrated in vacuo. The residue was chromatographed on SiO_2 (hexane : EtOAc = 8 : 1) to give **2kb** (110 mg, 67% in two steps).

mp 272-273 °C; IR (KBr): 3265, 2919, 2850, 1734 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ 8.67 (1H, br), 7.43 (1H, brs), 7.37 (1H, d, *J* = 8.6 Hz), 7.28 (1H, dd, *J* = 1.4, 8.6 Hz), 4.69 (2H, t, *J* = 6.2 Hz), 3.14 (2H, t, *J* = 6.2 Hz), 2.55 (1H, tt, *J* = 3.3, 12.2 Hz), 1.98-0.97 (20H, m); ^{13}C NMR (125 MHz, $CDCl_3$): δ 161.51, 140.75, 137.00, 127.00, 124.56, 122.90, 122.24, 117.55, 112.56, 69.45, 44.64, 43.29, 43.00, 35.05, 30.32, 30.27, 26.84, 21.46; MS (EI) *m/z* 351 (M+); HRMS (EI) calcd for $C_{23}H_{29}NO_2$: 351.2198 (M+), found: 351.2201.

1',4'-*trans*-6-Bicyclohex-4-yl-4,9-dihydro-3*H*-pyrano[3,4-*b*]indole-1-thione (3kb)

To a stirred solution of **2kb** (150 mg, 0.43 mmol) in toluene (6 mL) was added Lawesson's reagent (96 mg, 0.24 mmol), and the resulting mixture was refluxed for 17 h. After cooling, the solvent was removed, and the residue was chromatographed on SiO_2 (hexane–acetone = 40:1) to give **3kb** (126 mg, 81%).

IR (neat): 3363, 2922, 2850, 1540, 1231 cm^{-1}; ^1H NMR (500MHz, $CDCl_3$): δ 8.76 (1H, br), 7.42 (1H, brs), 7.32 (1H, d, *J* = 8.6 Hz), 7.29 (1H, dd, *J* = 1.4 Hz, 8.6 Hz), 4.74 (2H, t, *J* = 6.5 Hz), 3.16 (2H, t, *J* = 6.5 Hz), 2.53 (1H, tt, *J* = 3.1 Hz, 12.0 Hz), 1.97-0.97 (20H, m); ^{13}C NMR (125 MHz, $CDCl_3$): δ 198.30, 141.32, 137.87, 132.36, 128.09, 124.92, 118.56, 115.75, 112.11, 71.18, 44.62, 43.28, 42.98, 34.96, 30.28, 26.84, 21.27; MS (EI) *m/z* 367 (M+); HRMS (EI) calcd for $C_{23}H_{29}NOS$: 367.1970 (M+), found: 367.1968.

1',4'-*trans*-6-Bicyclohex-4-yl-4,9-dihydro-3*H*-2-thia-9-azafluoren-1-one (6kb)

To a stirred solution of NaI (206 mg, 1.37 mmol) in $ClCH_2CH_2Cl$ (10 mL) was added TMSCl (0.18 mL, 1.37 mmol), and the resulting mixture was stirred at room temperature for 15 min. To a solution of **3kb** (126mg, 0.34 mmol) in $ClCH_2CH_2Cl$ (10 mL) was transferred a solution of TMSI, prepared above, via a cannula, and then the resulting mixture was refluxed for 4 days. After cooling, the reaction was quenched with 10% HCl aq, and the aqueous mixture was extracted with $CHCl_3$. The organic extracts were combined, dried over Na_2SO_4, and evaporated. The residue was chromatographed on SiO_2 (hexane–acetone = 35 : 1) to give **6kb** (101 mg, 80%).

mp 275-276 °C; IR (KBr): 3288, 2915, 2848, 1628 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ 8.74 (1H, br), 7.44 (1H, brs), 7.33 (1H, d, *J* = 8.6 Hz), 7.27 (1H, dd, *J* = 1.4, 8.6 Hz), 3.48 (2H, t, *J* = 6.5 Hz), 3.28 (2H, t, *J* = 6.5 Hz), 2.55 (1H, tt, *J* = 3.4, 12.2 Hz), 1.98-0.98 (20H, m); ^{13}C NMR (125 MHz, $CDCl_3$): δ 183.61,

140.68, 135.35, 128.70, 127.52, 125.90, 125.18, 117.70, 112.18, 44.67, 43.30, 43.01, 35.05, 31.54, 30.33, 30.29, 26.85, 21.79; MS (EI) *m/z* 367 (M+); HRMS (EI) calcd for $C_{23}H_{29}NOS$: 367.1970 (M+), found: 367.1971.

1',4'-*trans*-(6-Bicyclohex-4-yl-1-oxo-3,4-dihydro-1*H*-2-thia-9-azafluoren-9-yl)acetic acid *tert*-butyl ester (7kb)

To a stirred solution of **6kb** (101 mg, 0.28 mmol) in DMF (3 mL) was added NaH (60%, 17 mg, 0.41 mmol) at 0 °C, and the reaction mixture was stirred at 0 °C for 30 min. To the mixture was added $BrCH_2CO_2t$-Bu (61 μL, 0.41 mmol) at 0 °C, and the resulting mixture was stirred at room temperature for 15 h. The reaction was quenched with H_2O, and the aqueous mixture was extracted with Et_2O. The organic extracts were combined, dried over Na_2SO_4, and evaporated. The residue was chromatographed on SiO_2 (hexane–acetone = 30:1) to give **7kb** (128 mg, 97%).

mp 178-180 °C; IR (KBr): 2921, 2850, 1740, 1641 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ 7.44 (1H, brs), 7.30 (1H, d, *J* = 8.7 Hz), 7.17 (1H, d, *J* = 8.7 Hz), 5.13 (2H, s), 3.42 (2H, t, *J* = 6.4 Hz), 3.31 (2H, t, *J* = 6.4 Hz), 2.54 (1H, tt, *J* = 3.3, 12.2 Hz), 1.97-0.98 (20H, m), 1.45 (9H, s); ^{13}C NMR (125 MHz, $CDCl_3$): δ 183.87, 167.90, 140.73, 137.31, 127.90, 127.54, 126.24, 124.84, 118.00, 109.60, 82.21, 47.11, 44.61, 43.30, 43.00, 35.02, 31.26, 30.31, 30.27, 28.01, 26.84, 22.37; MS (EI) *m/z* 481 (M+); HRMS (EI) calcd for $C_{29}H_{39}NO_3S$: 481.2651 (M+), found: 481.2655.

1',4'-*trans*-(6-Bicyclohex-4-yl-1-oxo-3,4-dihydro-1*H*-2-thia-9-azafluoren-9-yl)acetic acid (5kb)

To a stirred solution of NaI (138 mg, 0.92 mmol) in $ClCH_2CH_2Cl$ (5 mL) was added TMSCl (0.12 mL, 0.92 mmol), and the resulting mixture was stirred at room temperature for 15 min. To a solution of **7kb** (111 mg, 0.23 mmol) in $ClCH_2CH_2Cl$ (5 mL) was transferred a solution of TMSI, prepared above, via a cannula, and then the resulting mixture was refluxed for 19 h. After cooling, the reaction was quenched with 10% HCl aq, and the aqueous mixture was extracted with EtOAc. The organic extracts were combined, dried over Na_2SO_4, and evaporated. The residue was chromatographed on SiO_2 (hexane–acetone = 3 : 1) to give **5kb** (81 mg, 83%).

mp 234-236 °C; IR (KBr): 2908, 2850, 1723, 1632 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ 7.44 (1H, brs), 7.33 (1H, dd, *J* = 1.5, 8.6 Hz), 7.21 (1H, d, *J* = 8.6 Hz), 5.25 (2H, s), 3.42 (2H, t, *J* = 6.3 Hz), 3.31 (2H, t, *J* = 6.3 Hz), 2.55 (1H, tt, *J* = 3.3, 12.0 Hz), 1.96-0.97 (20H, m); ^{13}C NMR (125 MHz, $CDCl_3$): δ 184.59, 172.37, 141.15, 137.36, 128.02, 127.81, 126.82, 124.87, 118.11, 109.67, 46.42, 44.60, 43.30, 43.01, 35.01, 31.16, 30.30, 29.68, 26.86, 22.32; MS (EI) *m/z* 425 (M+); HRMS (EI) calcd for $C_{25}H_{31}NO_3S$: 425.2025 (M+), found: 425.2024.

4-(*trans*-[1,1'-Bi(cyclohexane)]-4-yl)aniline *p*-toluenesulfonate (14)

To a stirred solution of **13b** (63 mg, 0.25 mmol) in MeOH (3 mL) was added *p*-toluenesulfonic acid (46.6 mg, 0.25 mmol), and the resulting mixture was stirred at room temperature for 2.5 h. The solvent was concentrated in vacuo to give **14** (107 mg, quant.)

mp 250-251 °C; IR (KBr): 2920, 2849, 1516 cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6): δ 9.88 (1H, br), 7.48 (2H, d, J = 8.0 Hz), 7.33 (2H, d, J = 8.0 Hz), 7.25 (2H, d, J = 8.0 Hz), 7.11 (2H, d, J = 8.0 Hz), 2.47 (1H, tt, J = 3.2, 12.0 Hz), 2.28 (3H, s), 1.81-0.94 (20H, m); ^{13}C NMR (125 MHz, DMSO-d_6): δ 147.65, 145.35, 137.84, 129.11, 128.13, 127.98, 125.48, 123.08, 43.35, 42.72, 42.21, 33.98, 29.72, 29.60, 26.34, 26.31, 20.79.

***N*-(*cis*-4-Bicyclohex-3-yl-phenyl)-4-nitro-benzenesulfonamide (15)**

To a stirred solution of **13a** (108 mg, 0.37 mmol) in pyridine (2 mL) was added 4-nitrobenzenesulfonyl chloride (90 mg, 0.41 mmol), and the resulting mixture was stirred at room temperature for 23 h. The mixture was diluted with CH_2Cl_2 and washed with 10% HCl aq. The organic layer was dried over Na_2SO_4, and evaporated. The residue was chromatographed on SiO_2 (hexane–CH_2Cl_2 = 1 : 2) to give 1**5** (186 mg, quant.).

mp 174-175 °C; IR (KBr): 3220, 2926, 2920, 1535, 1349 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ 8.28 (2H, d, J = 9.2 Hz), 7.90 (2H, d, J = 9.2 Hz), 7.11 (2H, d, J = 8.3 Hz), 6.96 (2H, d, J = 8.3 Hz), 6.44 (1H, br), 2.45 (1H, tt, J = 3.2, 12.0 Hz) 1.89-0.95 (20H, m); ^{13}C NMR (125 MHz, $CDCl_3$): δ 150.15, 146.78, 144.78, 132.69, 128.53, 127.96, 124.21, 122.87, 44.10, 43.59, 43.40, 37.93, 34.28, 30.16, 30.13, 29.39, 26.79, 26.70; MS (EI) *m/z* 442 (M+); HRMS (EI) calcd for $C_{24}H_{30}N_2O_4S$: 442.1926 (M+), found: 442.1928.

GLP-1 secretion studies *in vitro*

Human enteroendocrine NCI-H716 cells were obtained from the American Type Culture Collection (Manassas, VA, U.S.A.), and were maintained in suspension culture as instructed by the supplier. Two days before each experiment, the cells were seeded in 96-well plates pre-coated with poly-L-lysine ($1x10^5$ cells /well). On the day of the experiment, the culture medium was replaced by assay buffer (146mM NaCl, 5mM KCl, 1.5mM $CaCl_2$, 1mM $MgSO_4$, 20 mM *N*-(2-hydroxyethyl)-piperazine-*N*-(2-ethanesulfonic acid) (HEPES), 5.6 mM glucose, 2 mg/mL bovine serum albumin, and 10 mM sitagliptin, pH 7.4) with or without test agents, and the cells were incubated for 1 h at 37 °C. Then the GLP-1 level in the assay buffer was measured by ELISA (LINCO, Billerica, MA, U.S.A.).

Acute and long-term effect of 5k on blood glucose *in vivo*

This study was approved by the Animal Care and Use Committee of Ajinomoto. Male C57BL/6J and KKAy mice were purchased from Clea Japan (Tokyo, Japan) at 5 weeks of age, and each mouse was housed in a polycarbonate cage with wood chip bedding. Water and commercial chow were provided ad libitum. The animal room was kept on a 12-h light/dark cycle (7:00 AM to 7:00 PM, dark; 7:00 PM to 7:00 AM, light), with a temperature range of 22 °C ± 1 °C and a relative humidity of 55% ± 5% throughout the experimental period. The animals were acclimatized to the laboratory condition for 4 weeks.

For evaluation of acute hypoglycemic effect of **5k**, C57BL/6J mice were fasted overnight, and were administered either the vehicle (0.5% methylcellulose), or 100 mg/kg of **5k** by oral gavage with or without subcutaneous injection of GLP-1 antagonist exendin (9-39) (24 nmol/kg). Then, 2 g/kg of glucose was given orally immediately after **5k** administration. Blood samples were collected from the tail vein to measure the blood glucose levels.

For evaluation of long-term effect of **5k** on blood glucose control, KKAy mice were divided into 4 groups, and either the vehicle, **5k** (100 mg/kg), sitagliptin (10 mg/kg), or pioglitazone (10 mg/kg) was administered orally twice a day for 3 weeks. At the end of the study, blood samples were collected from the tail vein and the blood glucose, HbA1c, plasma insulin, and plasma glucagon levels were measured. Blood glucose was measured with an autoanalyzer (Fuji Dri-Chem 5500; Fujifilm, Tokyo, Japan). Plasma insulin was measured by ELISA (Morinaga, Tokyo, Japan). Plasma glucagon was also measured by ELISA (Yanaihara Institute Inc., Shizuoka, Japan). HbA1c was measured by HPLC (TOSOH, Tokyo, Japan).

Statistical analysis

Results are expressed the mean±SEM. Statistical analysis was performed with StatView software (version 5.0, SAS institute, Cary, NC, USA). Differences were evaluated by one-way analysis of variance (ANOVA), followed by Dunnett's test. Statistical significance was accepted at $p<0.05$.

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