Journal of Medicinal Chemistry

Article

Discovery and Optimization of Chromeno[2,3-c]pyrrol-9(2H)-ones as Novel Selective and Orally Bioavailable Phosphodiesterase 5 Inhibitors for the Treatment of Pulmonary Arterial Hypertension

Deyan Wu, Tianhua Zhang, Yiping Chen, Yadan Huang, Haiju Geng, Yanfa Yu, Chen Zhang, Zengwei Lai, Yinuo Wu, Xiaolei Guo, Jianwen Chen, and Hai-Bin Luo

J. Med. Chem., **Just Accepted Manuscript** • DOI: 10.1021/acs.jmedchem.7b00523 • Publication Date (Web): 07 Jul 2017

Discovery and Optimization of Chromeno[2,3-c]pyrrol-9(2H)-ones as Novel Selective and Orally Bioavailable Phosphodiesterase 5 Inhibitors for the Treatment of Pulmonary Arterial Hypertension

Deyan Wu[a,#], *Tianhua Zhang*[a,#], *Yiping Chen*[a], *Yadan Huang*[a], *Haiju Geng*[a], *Yanfa Yu*[a], *Chen Zhang*[a], *Zengwei Lai*[a], *Yinuo Wu*[a], *Xiaolei Guo*[c], *Jianwen Chen*[a,*], *Hai-Bin Luo*[a, b,*]

[a] School of Pharmaceutical Sciences, Sun Yat-Sen University, Guangzhou 510006, P. R. China

[b] Collaborative Innovation Center of High Performance Computing, National University of Defense Technology, Changsha 410073, China

[c] Infinitus (China) Co. Ltd, Guangzhou 510663, China

ABSTRACT

Phosphodiesterase 5 (PDE5) inhibitors have been used as clinical agents to treat erectile dysfunction and pulmonary arterial hypertension (PAH). Herein, we detail the discovery of a novel series of chromeno[2,3-c]pyrrol-9(2H)-one derivatives as selective and orally bioavailable inhibitors against phosphodiesterase 5. Medicinal chemistry optimization resulted in **2**, which exhibits a desirable inhibitory potency of 5.6 nM with remarkable selectivity as well as excellent pharmacokinetic properties and an oral bioavailability of 63.4%. In addition, oral administration of **2** at a dose of 5.0 mg/kg caused better pharmacodynamics effects on both mPAP (mean pulmonary artery pressure) and RVHI (index of right ventricle hypertrophy) than sildenafil citrate at a dose of 10.0 mg/kg. These activities along with its reasonable drug-like properties, such as human liver microsomal stability, cytochrome inhibition, hERG inhibition, and pharmacological safety, indicate that **2** is a potential candidate for the treatment of PAH.

INTRODUCTION

Pulmonary arterial hypertension (PAH) is a syndrome resulting from the restricted flow of blood through the pulmonary arterial circulatory system, which leads to pathological increases in pulmonary vascular resistance (PVR) and ultimately right-sided heart failure.[1, 2] In addition, PAH is a progressive and deadly disease with a poor prognosis and approximately 15% mortality within one year.[3, 4] Recently, multiple pathogenic pathways have been implicated in the development of PAH, and three of these pathways are important because they have been targeted by drugs (i.e., prostacyclin derivatives (epoprostenol, treprostinil, and iloprost), endothelin receptor antagonists (bosentan, bitaxsentan, and ambrisentan), and phosphodiesterase 5 (PDE5) inhibitors (sildenafil and tadalafil)).[5, 6]

PDE5 is a cGMP-specific enzyme that is primarily distributed in smooth muscle. PDE5 was initially discovered in bovine lung and rat platelets[7, 8] and later found in several other tissues (i.e., corpus cavernosum, heart, lung, liver, brain, platelets, prostate, urethra, bladder, and stomach).[9-13] PDE5 plays an important role in vascular relaxation mediated by the NO/cGMP pathway in vascular smooth muscle cells.[14, 15] Therefore, PDE5 is a prime target for the development of inhibitors to treat the diseases associated with low cGMP levels.[16] Currently, several PDE5 inhibitors have been approved to treat several diseases, such as erectile dysfunction (sildenafil, vardenafil, tadalafil, avanafil, udenafil, and mirodenafil)[17-21] and PAH (sildenafil and tadalafil).[12-13, 16, 22-28] Sildenafil was approved by the FDA in 1998 as the first oral drug for the treatment of erectile dysfunction. In addition, this drug was approved for the treatment of PAH in 2005.[13, 16, 24-27] However, extensive studies have revealed several side effects of sildenafil, such as vision disturbance[29] and hearing loss[30]. Therefore, the discovery of novel PDE5 inhibitors with new scaffolds continues to attract much attention in both academics and industry.

We previously discovered a series of 1-aryl chromeno[2,3-*c*]pyrrol-9(2*H*)-ones as potent PDE5 inhibitors,[31] and the hit 3-(4-hydroxybenzyl)-1-(thiophen-2-yl)chromeno[2,3-*c*]pyrrol-9(2*H*)-one (**1**,

Figure 1) exhibited considerable inhibitory affinity (IC_{50} = 17 nM) against PDE5. However, **1** has relatively weak pharmacokinetic properties with an oral bioavailability of 4.9 %. Herein, we report the medicinal chemistry optimization (Figure 2) of **1** to improve its binding affinity and oral bioavailability. This optimization led to the discovery of **2** with higher inhibitory potency and significantly improved pharmacokinetic properties with an oral bioavailability of 63.4%. The best compound **2** (Figure 1) has an IC_{50} of 5.6 nM against PDE5 with remarkable selectivity across the PDE families, excellent pharmacokinetic properties, and a marked pharmacodynamic profile against PAH *in vivo*. These activities along with reasonable drug-like properties, such as human/rat liver microsomal stability, hERG inhibition, cytochrome inhibition, pharmacological safety, indicate that **2** is a potential candidate for the treatment of PAH.

CHEMISTRY

The targeted compounds were prepared by the synthetic routes reported in Schemes 1-5. Our initial efforts focused on the syntheses of dihydrochromeno [2,3-*c*]pyrroles-1-carboxylates and (1-carbonyl)chromeno[2,3-*c*] pyrrol-9(2*H*)-ones (Schemes 1). Ethyl 4-(2-hydroxyphenyl)-2,4-dioxobutanoate (**4**) was synthesized by the reaction of 2'-hydroxyacetophenone and diethyl oxalate in the presence of sodium hydride.[32] The key intermediate (3-(4-(*tert*-butoxy)benzyl)-9-oxo-2,9-dihydrochromeno[2,3-*c*]pyrrole-1-carboxylate (**5**)) was synthesized according to our previously reported procedure.[31, 33] Compound **9** was the deprotected product, and compound **6** was the hydrolysate product. Compound **8** was obtained using the transesterification reaction followed by deprotection from **5**. In addition, compounds **10a-10c** were synthesized by the amidation reaction of **6** and amines,[34] and compounds **11a-11c** were the deprotected products from **10a-c**, respectively.

(1-Aryl)chromeno[2,3-*c*]pyrrol-9(2*H*)-ones **17a-17j** and **21a-21c** were also synthesized according to our previously reported procedures (Schemes 2 and 3).[31, 33] The key step of each route was the synthesis of propane-1,3-diones **15a-15j** or **19a-19b**. As shown in Schemes 2, all the propane-1,3-diones **15a-15j** were synthesized by the reported procedure with a Baker-Venkataraman rearrangement. However, the propane-1,3-diones **19a-19b** were synthesized using a more effective procedure that was similar to the synthesis of compound **4** (Schemes 3). To synthesize compound **23**, carboxylic acid **6** was used as the starting material. This material was treated with acethydrazide and HATU in the presence of DIPEA to obtain intermediate **22** followed by treatment with $POCl_3$ to afford 1,3,4-oxadiazole **23** (Scheme 4).[35] The syntheses of 1-(thiazol-2-yl)chromeno[2,3-*c*]pyrrol-9(2*H*)-ones **2** and **30** began with a published route to obtain the racemic amino acid chloride salts **28a-b**,[36] and then, the Fmoc group was introduced with fluorenylmethoxycarbonyl chloride (Fmoc-Cl) in dioxane/aqueous Na_2CO_3 (Scheme 5).[37-39] The resulting Fmoc-protected amino acids were subsequently treated with **15j** to obtain product **2** or **30** using the same procedure as that employed in the synthesis of **17**.

RESULTS AND DISCUSSION

Rational Design of Novel PDE5 inhibitors to Improve Binding Affinities and Pharmacokinetic Properties.

We previously discovered a series of (1-aryl)chromeno[2,3-*c*]pyrrol-9(2*H*)-ones as novel PDE5 inhibitors, and the hit (**1**) had an IC_{50} of 17 nM against PDE5.[31] However, **1** has relatively weak pharmacokinetic properties with an oral bioavailability of less than 5%. To improve its inhibitory potency and metabolic stability, structural optimization of compound **1** was necessary. Using structure-based design (Figure 2, molecular docking and molecular dynamics simulations), we replaced the thiophene ring with a thiazole ring with the hope that it can form stronger bidentate H-bond interactions

between the inhibitors and crucial residue Q817, which was the key site for improving the binding affinities. In addition, the replacement of the 4-hydroxybenzyl group with other aromatic groups or heterocycle groups may help to overcome its metabolic instability.

To avoid interference of false positive compounds with our subsequent study, PAINS screening of the designed compounds was performed using an online program (i.e., "PAINS-Remover", http://www.cbligand.org/PAINS/),[40] and all the compounds passed the filter.

Structure-activity Relationships (SARs) of Substituent Groups at the C1 Position of Chromeno[2,3-*c*]pyrrol-1-carboxylates.

As shown in the previous study[31], the C1 position must retain a H-bond receptor to form the H-bond interactions between the inhibitors and residue Q817. Therefore, our initial investigations of the SARs of chromeno[2,3-*c*]pyrrol-9(2*H*)-one derivatives were carried out by substituting the C1 position with carboxylates or amides. The inhibitory activities of these substituted compounds (**8** with a carboxylic methyl ether and **11a-11c** with an amide group) against PDE5 are shown in Table 1. However, all the compounds exhibited weaker PDE5 inhibitory activities than compound **1**, indicating that carboxylate substitution at this position is unfavorable for the formation of H-bond interactions and the subpocket is more favorable for holding an aromatic ring than an alkyl substituent.

SARs of Substituent Groups at the C1 Position of (1-Aryl)chromeno[2,3-*c*]pyrrol-9(2*H*)-ones.

Based on these results, we decided to change the aromatic rings at the C1 position, and the results of these substituted compounds against PDE5 are summarized in Table 2. Compounds **17a-17i** and **21a-21c** have a six-membered aromatic ring, and compounds **17j** and **23** bear a five-membered aromatic ring at position C1. The introduction of six-membered aromatic rings at position C1 resulted in weaker

inhibitions than those with five-membered aromatic rings (**1** and **17j**). The derivatives with a six-membered aromatic ring (**17a** and **17i**) have better IC_{50} values of 20.5 nM and 16.4 nM, respectively. Finally, compound **17j** bearing a thiazol-2-yl group enhanced the inhibition with an IC_{50} of 5.4 nM, suggesting that a thiazol-2-yl group at this position was favorable for the formation of H-bond interactions with residue Q817 in the substrate binding pocket. Therefore, the thiazol-2-yl group was selected as the best substituent group at position C1 because it may increase the inhibitory affinities and improve the solubility.

SARs of Substituent Groups at the C3 Position.

Among the studied compounds, **17j** exhibited the best potency with an IC_{50} of 5.4 nM against the PDE5A catalytic domain. However, this compound has a 4-hydroxybenzyl group at the C3 position, which could be easily oxidized. Enhancement of the stability of functional groups is an efficient approach for increasing the metabolic stability and improving the pharmacokinetic properties of a molecule. Further investigation (Table 3) revealed that the compound with a benzo[*d*][1,3]dioxol-5-ylmethyl group at the C3 position (**2**) has a similar inhibitory potency (IC_{50} = 5.6 nM) against PDE5. However, other modifications, such as replacement of a 4-hydroxybenzyl group with a 4-(tert-butoxy)benzyl group (**16j**) or a 4-(trifluoromethyl)benzyl group (**30**), failed to improve their binding affinities. Finally, compounds **17j** and **2** were selected as the best inhibitors for subsequent study.

Significant Improvement of the Pharmacokinetic Properties of Compound 2 over 1

Due to the relatively higher inhibitory affinities of the two compounds (**17j** and **2**), both compounds were subjected to preliminary pharmacokinetic assessment *in vivo* to choose the best one for screening of the selectivity across PDE families and the pharmacodynamics evaluation. As a result, compound **2**

exhibited excellent pharmacokinetic properties, and its pharmacokinetic data are summarized in Table 4. In addition, the results for **17j** (its oral bioavailability < 10%) are summarized in Table S4. After oral administration of a 5 mg/kg dose of **2** to rats, pharmacokinetic analysis revealed that **2** had a C_{max} of 368 ng/mL, $t_{1/2}$ of 5.17 h, and oral bioavailability of 63.4% (Table 4). Its oral bioavailability was remarkably higher than that of sildenafil (23%)[41] or sildenafil citrate (41%)[42], which demonstrated that **2** is suitable for use in subsequent pharmacodynamic tests.

Binding of 2 to the PDE5 Catalytic Domain

The binding pattern of PDE5 in complex with **2** after 20 ns molecular dynamics (MD) simulations provided insight into the activity data (Figure 3). Compound **2** formed two H-bond interactions (2.7 Å and 3.1 Å) with invariant residue Q817 and aromatic π–π stacking interactions against residue F820, which are two characteristic interactions of inhibitors with various PDE families. Surprisingly, this compound possessed an additional H-bond of 2.8 Å with residue Y79 in the active site of PDE5, which was not observed in the binding pattern between sildenafil and PDE5. Therefore, the new scaffold and different binding pattern of compound **2** provide a good example of the rational design of PDE5 inhibitors.

Remarkable Selectivity of Compound 2 across PDE Families

The selectivity of compound **2** across PDE families was also measured (Table 5). Its inhibitions towards PDE1B, PDE3A, PDE7A1, and PDE9A2 were very weak (IC_{50} > 10000 nM). Its IC_{50} values against PDE8A1, PDE4D2, PDE2A, PDE10A, and PDE6A were 1111-fold, 494-fold, 65-fold, 27-fold, and 10-fold higher, respectively, than that against PDE5A1, which demonstrated that **2** exhibited remarkable selectivity over other PDEs *in vitro*. For the sildenafil reference compound, its IC_{50} value

against PDE6A was 5-fold higher than that against PDE5A1, which is comparable to the literature values [18].

Reasonable drug-like properties of compound 2.

Based on its pharmacokinetic profile with an oral bioavailability of 63.4% and $t_{1/2}$ of 5.17 h after oral administration, compound **2** was further subjected to preliminary drug-like evaluations, such as human/rat liver microsomal stability, cytochrome inhibition, hERG inhibition, and pharmacological safety.

Human/rat liver microsomal stability. Human and rat liver microsomes are extensively used in the pharmaceutical industry for *in vitro* drug metabolism assays and evaluating the ADME properties of drugs in development. Herein, we examined compound **2** using a standard microsomal stability assay with comparison to the midazolam control compound (Sigma Aldrich). The results indicate that **2** was stable in the human and rat liver microsomes based on a $t_{1/2}$ of 56.0 and 24.7 min, respectively, and E_h (hepatic extraction ratio) of 54% and 65% (Table 6), respectively, which is significantly better than those for the positive control (midazolam, $t_{1/2}$ of approximately 2.7 and 2.4 min, respectively, and E_h of approximately 96% and 95%, respectively).

Cytochrome inhibition. Cytochrome P450s (CYPs) are the major enzymes involved in the metabolism of various xenobiotics. Among the various CYP isoenzymes, several human hepatic CYP enzymes play a dominant role in the metabolism of drugs and other xenobiotics.[43, 44] In this study, the inhibitory activities of compound **2** against seven human hepatic CYP enzymes (Table 7) were tested. Therefore, **2** has an IC_{50} of 7.6 μM against CYP1A2, and its IC_{50} values for the other six CYPs (CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, and CYP3A4) were uniformly more than 20 μM. The results suggest that **2** exhibited a very weak inhibitory effect on these CYP isoenzymes except CYP1A2. Therefore,

compound **2** is unlikely to exhibit significant pharmacokinetic interactions with drugs that are metabolized by the seven major CYP isoforms.

hERG inhibition. hERG (human ether-a-go-go related gene) forms the major portion of one of the ion channel proteins that conducts potassium ions out of the muscle cells of the heart, and this current is critical in correctly timing the return to the resting state of the cell membrane during the cardiac action potential, which has made hERG inhibition an important antitarget that must be avoided during drug development.[45] In our study, compound **2** inhibited hERG with an IC_{50} of more than 10 μM using an automated patch clamp electrophysiology measurement in CHO-hERG cells. The results suggest that **2** exhibited a weak inhibitory effect on hERG, which indicates that further development of compound **2** is appropriate.

Pharmacological Safety. The favorable pharmacokinetic profile of **2** along with its highly desirable inhibitory potency against PDE5 and remarkable selectivity across PDEs warranted its use in *in vitro* safety studies. First, the maximum tolerated dose of **2** was determined for acute toxicity in mice. Twenty-four mice were randomly divided into three groups and given single oral doses of 0 mg/kg, 1000 mg/kg, or 1500 mg/kg **2** on the first day. The animals treated with **2** did not exhibit any poisoning symptoms or mortality immediately or during the post-treatment period of two weeks. In addition, no abnormal behaviors or significant changes in the water/food consumption and body weight were observed during the period of the experiments. Therefore, inhibitor **2** was well tolerated up to a dose of 1500 mg/kg with no acute toxicity. Second, the maximum tolerated dose of **2** for short-term (2-week) toxicity in rats was also determined. Twenty-four SD rats were randomly divided into four groups and given daily oral doses of 0 mg/kg, 30 mg/kg, 100 mg/kg or 300 mg/kg **2**. Our results demonstrated that the compound did not cause any adverse effect on the body weight or any other signs of overt toxicity at daily doses up to 300 mg/kg for two weeks.

Pharmacodynamics Profile against PAH in Rats.

Effects on mPAP (mean pulmonary artery pressure) and RVHI (index of right ventricle hypertrophy). The pharmacodynamics effects of compound **2** against PAH *in vivo* are shown in Figure 4. A significant increase of mPAP was detected in the model group compared to that in the control group ($p<0.01$), and the RVHI of the model group was significantly higher than that of the control group ($p<0.01$), which suggests that it successfully induced the rats with PAH 3 weeks after monocrotaline (MCT) injection successfully. Therefore, the mPAPs (18.65 mmHg and 22.13 mmHg) of the groups treated with compound **2** at a dose of 5.0 mg/kg and sildenafil citrate at a dose of 10.0 mg/kg significantly decreased ($p<0.01$) compared to that of the model group (32.74 mmHg). For the RVHI, similar trends were also observed. At a dose of 5.0 mg/kg, compound **2** exhibited better effects on both mPAP and RVHI than sildenafil citrate at a dose of 10.0 mg/kg.

Effects on the thickness of the small pulmonary arteries in rats with PAH. The effects of compound **2** on the thickness of the small pulmonary arteries are shown in Figure 5. The model group had significantly thicker small pulmonary arteries than the other groups ($p<0.05$, $p<0.01$). The wall thickness percentage of the external diameter (WT %) of the model group increased significantly ($p<0.05$, $p<0.01$). As shown in Figure 5, compound **2** and the reference compound (sildenafil citrate) exhibited a marked reduction compared to that of the model group and performed well.

CONCLUSION

In summary, a series of chromeno[2,3-c]pyrrol-9(2H)-one derivatives as novel PDE5 inhibitors starting from compound **1** were successfully designed and synthesized using a structure-based discovery strategy. In total, twenty-one derivatives of chromeno[2,3-c]pyrrol-9(2H)-ones were discovered, resulting in ten compounds with IC_{50} values ranging from 1 to 100 nM and two compounds with IC_{50} <

10 nM. Compound **2** exhibited an IC_{50} of 5.6 nM with remarkable selectivity over other PDEs. After oral administration of a 5 mg/kg dose of **2** to rats, the pharmacokinetic analysis revealed that **2** had a C_{max} of 368 ng/mL, $t_{1/2}$ of 5.17 h, and oral bioavailability of 63.4%. Furthermore, its IC_{50} value(s) against CYP1A2 and six other CYPs (CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, and CYP3A4) were 7.6 μM and uniformly > 20 μM, respectively. In addition, **2** was essentially inactive at hERG with an IC_{50} of >10 μM. In addition, **2** was well tolerated up to a dose of 1500 mg/kg with no acute toxicity and up to a daily dose of 300 mg/kg. Moreover, 2 did not exhibit short-term (2-week) toxicity. These activities led to the selection of **2** as a potential candidate for treatment of PAH.

EXPERIMENTAL SECTION

Syntheses. All starting materials and reagents were purchased from commercial suppliers (Sigma-Aldrich, Adamas, Energy, Bide, ShuYa, J&K, and Meryer) and used directly without further purification. Chemical HG/T2354-92 silica gel (200-300 mesh, Haiyang®) was used for chromatography, and silica gel plates with fluorescence F254 (0.25 mm, Huanghai®) were used for thin-layer chromatography (TLC) analysis. Reactions that required anhydrous conditions were performed under argon or a calcium chloride tube. The ^{1}H NMR and ^{13}C NMR spectra were recorded at room temperature on a Bruker AVANCE III 400 instrument with tetramethylsilane (TMS) as an internal standard. The following abbreviations are used: s (singlet), d (doublet), dd (two doublets), ddd (three doublets), t (triplet), q (quartet), br s (broad singlet) and m (multiplet). The coupling constants are reported in Hz. The low- and high-resolution mass spectra (LRMS and HRMS) were recorded on a MAT-95 spectrometer. The purity of the compounds was determined by reverse-phase high-performance liquid chromatography (HPLC) analysis and confirmed to be more than 95%. HPLC instrument: SHIMADZU LC-20AT (column: Hypersil BDS C_{18}, 5.0 μm, 4.6 × 150 mm (Elite); Detector: SPD-20A UV/VIS

detector, UV detection at 254 nm; Elution, MeOH in water (80%, v/v); T = 25°C; and flow rate = 1.0 mL/min.

*Ethyl 4-(2-hydroxyphenyl)-2,4-dioxobutanoate (**4**)*. To a solution of 2'-hydroxyacetophenone (**3**) (2.72 g, 20.0 mmol) in toluene (80 mL) at 0°C was added sodium hydride (4.0 g, 100 mmol). The mixture was stirred at room temperature for 15 min and diethyl oxalate (4.0 mL, 30.0 mmol) was added and the mixture was stirred at 60°C for 2 h. After the solution had cooled to room temperature it was poured into a mixture of ice and water and acidified by the addition of 2 N aqueous HCl. The resulting solution was extracted with portions of ethyl acetate (2×100 mL). The combined organic extracts were dried over anhydrous sodium sulfate, and concentrated to give a crude, which was purified by silica gel column chromatography (petroleum ether/EtOAc, 3:1) to get the product **4** (3.2 g) as a yellow solid. Yield: 68%; MS (ESI) *m/z* calcd $C_{12}H_{13}O_5^+$ $[M+H]^+$ 237.1, found 237.1.

*Ethyl 3-(4-(tert-butoxy)benzyl)-9-oxo-2,9-dihydrochromeno[2,3-c]pyrrole-1-carboxylate (**5**)*. To a solution of **4** (3.2 g, 13.6 mmol), Fmoc-O-tert-butyl-L-tyrosine (11.2 g, 24.4 mmol), 4-dimethylpyridine (663 mg, 5.4 mmol) in pyridine (50 mL) was added DCC (5.6 g, 27.2 mmol). The mixture was stirred at room temperature for 3 h until the start material disappeared as monitored by TLC. The reaction temperature was raised to 50°C for 6 h. After the reaction mixture was evaporated under vacuum, the residue was diluted with ethyl acetate (150 mL) and filtered to remove the side product DCU. The filtrate was evaporated and purified by silica gel column chromatography (petroleum ether/EtOAc, 5:1) to afford **5** (4.0 g) as a yellow solid. Yield: 70%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.97 (br s, 1H), 8.38 (dd, J = 8.0, 1.4 Hz, 1H), 7.69 – 7.59 (m, 1H), 7.41 (d, J = 8.3 Hz, 1H), 7.31 (t, J = 7.5 Hz, 1H), 7.14 (d, J = 8.4 Hz, 2H), 6.95 (d, J = 8.4 Hz, 2H), 4.45 (q, J = 7.1 Hz, 2H), 4.21 (s, 2H), 1.47 (t, J = 7.1 Hz, 3H), 1.33 (s, 9H).

*3-(4-(tert-Butoxy)benzyl)-9-oxo-2,9-dihydrochromeno[2,3-c]pyrrole-1-carboxylic acid (**6**).* To a solution of **5** (2.0 g, 4.8 mmol) in THF (40 mL) and water (160 mL) was added Potassium hydroxide (4.0 g, 71.6 mmol). The mixture was stirred at 60°C for 12 h. After the solution had cooled to room temperature it was evaporated to remove most of the solvent and acidified by the addition of 4 N aqueous HCl. The resulting solution was extracted with portions of ethyl acetate (3×100 mL). The combined organic extracts were dried over anhydrous sodium sulfate, and concentrated to afford the product **6** as a yellow solid, which was used directly in the next step without further purification. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.05 (br s, 1H), 8.32 (d, J = 7.7 Hz, 1H), 7.62 (t, J = 7.6 Hz, 1H), 7.39 (d, J = 8.3 Hz, 1H), 7.30 - 7.25 (m, 1H), 7.12 (d, J = 8.0 Hz, 2H), 6.92 (d, J = 7.9 Hz, 2H), 4.14 (s, 2H), 1.32 (s, 9H).

*Methyl 3-(4-(tert-butoxy)benzyl)-9-oxo-2,9-dihydrochromeno[2,3-c]pyrrole-1-carboxylate (**7**).* To a solution of **5** (210 mg, 0.5 mmol) in methanol (100mL) was added lithium hydroxide hydrate (63 mg, 1.5 mmol). The mixture was stirred at room temperature for 12 h. Then it was evaporated to remove most of the solvent and acidified by the addition of 2 N aqueous HCl. The resulting solution was extracted with portions of ethyl acetate (2×30 mL). The combined organic extracts were dried over anhydrous sodium sulfate, and concentrated to afford the product **7** (202 mg) as a yellow solid. Yield: 100%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 13.29 (br s, 1H), 8.16 (dd, J = 8.0, 1.6 Hz, 1H), 7.75 (ddd, J = 8.6, 7.1, 1.7 Hz, 1H), 7.53 (d, J = 7.9 Hz, 1H), 7.44 – 7.34 (m, 1H), 7.20 (d, J = 8.5 Hz, 2H), 6.91 (d, J = 8.5 Hz, 2H), 4.15 (s, 2H), 3.86 (s, 3H), 1.25 (s, 9H).

*Methyl 3-(4-hydroxybenzyl)-9-oxo-2,9-dihydrochromeno[2,3-c]pyrrole-1-carboxylate (**8**).* To a solution of **7** (101 mg, 0.25 mmol) in dichloromethane (3.0 mL) was added trifluoroacetic acid (1.0 mL). The mixture was stirred at room temperature for 2 h. Then it was diluted with dichloromethane (50 mL) and washed with saturated aqueous sodium bicarbonate and water. The organic layer was dried over anhydrous sodium sulfate, and purified by silica gel column chromatography (petroleum ether/EtOAc,

2:1) to afford the product **8** (77 mg) as a yellow solid. Yield: 88%; purity: 99%; ^{1}H NMR (400 MHz, DMSO – d_6) δ 13.27 (br, 1H), 9.25 (s, 1H), 8.16 (d, J = 7.9 Hz, 1H), 7.75 (t, J = 7.7 Hz, 1H), 7.54 (d, J = 8.3 Hz, 1H), 7.38 (t, J = 7.5 Hz, 1H), 7.10 (d, J = 8.3 Hz, 2H), 6.68 (d, J = 8.4 Hz, 2H), 4.06 (s, 2H), 3.86 (s, 3H); ^{13}C NMR (101 MHz, DMSO – d_6) δ 172.89, 160.44, 156.31, 156.05, 142.90, 134.71, 129.64×2, 129.24, 126.94, 123.88, 122.91, 120.54, 118.26, 115.72×2, 113.75, 112.50, 52.12, 28.99.

*Ethyl 3-(4-hydroxybenzyl)-9-oxo-2,9-dihydrochromeno[2,3-c]pyrrole-1-carboxylate (**9**).* To a solution of **5** (105 mg, 0.25 mmol) in dichloromethane (3.0 mL) was added trifluoroacetic acid (1.0 mL). The mixture was stirred at room temperature for 2 h. Then it was diluted with dichloromethane (50 mL) and washed with saturated aqueous sodium bicarbonate and water. The organic layer was dried over anhydrous sodium sulfate, and purified by silica gel column chromatography (petroleum ether/EtOAc, 2:1) to afford the product **9** (85 mg) as a yellow solid. Yield 92%; purity: 99%; ^{1}H NMR (400 MHz, DMSO – d_6) δ 13.20 (br, 1H), 9.26 (s, 1H), 8.16 (d, J = 7.9 Hz, 1H), 7.74 (t, J = 7.7 Hz, 1H), 7.53 (d, J = 8.4 Hz, 1H), 7.38 (t, J = 7.5 Hz, 1H), 7.10 (d, J = 8.0 Hz, 2H), 6.68 (d, J = 8.0 Hz, 2H), 4.33 (q, J = 7.0 Hz, 2H), 4.07 (s, 2H), 1.36 (t, J = 7.0 Hz, 3H); ^{13}C NMR (101 MHz, DMSO – d_6) δ 172.88, 160.04, 156.31, 156.05, 142.88, 134.66, 129.63×2, 129.28, 126.98, 123.85, 122.95, 120.32, 118.24, 115.72×2, 114.17, 112.44, 60.80, 28.97, 14.82; HRMS (ESI) m/z calcd $C_{21}H_{18}NO_5^+$ $[M+H]^+$ 364.1179, found 364.1184.

General Procedure for Synthesis of Compounds 10a-10c. To a solution of **6** (0.6 mmol) in dichloromethane (8.0 mL) was added amines (0.9 mmol), triethylamine (125 μL, 0.9 mmol) and HATU (459 mg, 1.2 mmol). The mixture was stirred at room temperature for 12 h. Then it was concentrated to give a crude, which was purified by silica gel column chromatography (petroleum ether/EtOAc, 5:1) to get the product as a white solid.

3-(4-(tert-Butoxy)benzyl)-1-(morpholine-4-carbonyl)chromeno[2,3-c]pyrrol-9(2H)-one ***(10a)****.* Yield: 41%; ^{1}H NMR (400 MHz, Acetone – d_6) δ 11.81 (br s, 1H), 8.24 (dd, J = 7.9, 1.5 Hz, 1H), 7.74 (ddd, J = 8.8, 7.1, 1.8 Hz, 1H), 7.51 (dd, J = 8.4, 0.7 Hz, 1H), 7.36 (ddd, J = 8.1, 7.1, 1.0 Hz, 1H), 7.23 (d, J = 8.6 Hz, 2H), 6.90 (d, J = 8.5 Hz, 2H), 4.21 (s, 2H), 3.70 (s, 8H), 1.28 (s, 9H).

3-(4-(tert-Butoxy)benzyl)-1-(4-isopropylpiperazine-1-carbonyl)chromeno[2,3-c]pyrrol-9(2H)-one ***(10b)****.* Yield: 39%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.88 (br s, 1H), 8.25 (dd, J = 8.0, 1.4 Hz, 1H), 7.65 (ddd, J = 8.6, 7.2, 1.7 Hz, 1H), 7.42 (d, J = 8.0 Hz, 1H), 7.33 – 7.28 (m, 1H), 7.09 (d, J = 8.4 Hz, 2H), 6.81 (d, J = 8.4 Hz, 2H), 4.10 (d, J = 9.8 Hz, 2H), 3.84 (s, 4H), 3.13 – 3.05 (m, 1H), 2.96 (s, 4H), 1.24 (s, 9H), 1.21 (d, J = 6.5 Hz, 6H).

3-(4-(tert-Butoxy)benzyl)-1-(4-cyclopropylpiperazine-1-carbonyl)chromeno[2,3-c]pyrrol-9(2H)-one ***(10c)****.* Yield: 44%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 12.00 (br s, 1H), 8.29 (dd, J = 8.0, 1.6 Hz, 1H), 7.62 (ddd, J = 8.6, 7.2, 1.7 Hz, 1H), 7.38 (d, J = 8.0 Hz, 1H), 7.33 – 7.27 (m, 1H), 6.97 (d, J = 8.4 Hz, 2H), 6.69 (d, J = 8.4 Hz, 2H), 3.99 (s, 2H), 3.76 (d, J = 26.1 Hz, 4H), 2.71 (d, J = 15.9 Hz, 4H), 1.78 (s, 1H), 1.21 (d, J = 28.6 Hz, 9H), 0.55 – 0.35 (m, 4H).

General Procedure for Synthesis of Compounds 11a-11c. To a solution of **10** (0.25 mmol) in dichloromethane (3.0 mL) was added trifluoroacetic acid (1.0 mL). The mixture was stirred at room temperature for 2 h. Then it was diluted with dichloromethane (50 mL) and washed with saturated aqueous sodium bicarbonate and water. The organic layer was dried over anhydrous sodium sulfate, and purified by silica gel column chromatography (petroleum ether/EtOAc, 2:1) to afford the product **11** as a yellow solid.

3-(4-Hydroxybenzyl)-1-(morpholine-4-carbonyl)chromeno[2,3-c]pyrrol-9(2H)-one ***(11a)****.* Yield: 87%; purity$>$99%; ^{1}H NMR (400 MHz, DMSO – d_6) δ 12.83 (br s, 1H), 9.26 (s, 1H), 8.15 (d, J = 7.6 Hz, 1H), 7.74 (t, J = 7.4 Hz, 1H), 7.53 (d, J = 8.3 Hz, 1H), 7.36 (t, J = 7.4 Hz, 1H), 7.10 (d, J = 8.3 Hz, 2H), 6.70

(d, J = 8.4 Hz, 2H), 4.01 (s, 2H), 3.67 (s, 4H), 3.58 (s, 2H), 3.48 (s, 2H); ^{13}C NMR (101 MHz, DMSO – d_6) δ 174.16, 161.52, 156.68, 156.29, 140.55, 134.78, 129.68×2, 129.65, 126.69, 123.52, 122.28, 118.51, 118.30, 116.13, 115.72×2, 109.38, 66.70, 66.54, 47.69, 42.85, 29.06; HRMS (ESI) m/z calcd $C_{23}H_{21}N_2O_5^+$ $[M+H]^+$ 405.1445, found 405.1437.

3-(4-Hydroxybenzyl)-1-(4-isopropylpiperazine-1-carbonyl)chromeno[2,3-c]pyrrol-9(2H)-one ***(11b)***. Yield: 91%; purity: 98%; ^{1}H NMR (400 MHz, DMSO – d_6) δ 12.74 (br s, 1H), 9.22 (s, 1H), 8.14 (dd, J = 7.9, 1.6 Hz, 1H), 7.74 (ddd, J = 8.7, 7.1, 1.8 Hz, 1H), 7.52 (d, J = 7.8 Hz, 1H), 7.40 – 7.30 (m, 1H), 7.10 (d, J = 8.5 Hz, 2H), 6.69 (d, J = 8.5 Hz, 2H), 4.00 (s, 2H), 3.65 (s, 2H), 3.43 (s, 2H), 3.18 (d, J = 5.2 Hz, 1H), 2.68 (s, 1H), 2.38 (s, 3H), 0.97 (d, J = 6.3 Hz, 6H); ^{13}C NMR (101 MHz, DMSO – d_6) δ 174.02, 161.21, 156.70, 156.27, 140.46, 134.68, 129.68, 129.64×2, 126.66, 123.46, 122.32, 119.08, 118.26, 115.71×3, 109.29, 54.23, 49.12, 48.95, 48.47, 47.44, 29.08, 18.51×2; HRMS (ESI) m/z calcd $C_{26}H_{28}N_3O_4^+$ $[M+H]^+$ 446.2074, found 446.2065.

1-(4-Cyclopropylpiperazine-1-carbonyl)-3-(4-hydroxybenzyl)chromeno[2,3-c]pyrrol-9(2H)-one ***(11c)***. Yield: 91%; purity: 99%; ^{1}H NMR (400 MHz, DMSO – d_6) δ 12.76 (br s, 1H), 9.22 (s, 1H), 8.13 (dd, J = 7.9, 1.6 Hz, 1H), 7.74 (ddd, J = 8.7, 7.1, 1.7 Hz, 1H), 7.53 (d, J = 8.4 Hz, 1H), 7.41 – 7.29 (m, 1H), 7.10 (d, J = 8.5 Hz, 2H), 6.69 (d, J = 8.5 Hz, 2H), 4.00 (s, 2H), 3.63 (s, 2H), 3.41 (s, 2H), 2.60 (s, 2H), 2.48 (s, 2H), 1.71 – 1.59 (m, 1H), 0.51 – 0.27 (m, 4H); ^{13}C NMR (101 MHz, DMSO – d_6) δ 174.05, 161.33, 156.72, 156.28, 140.49, 134.71, 129.68, 129.65×2, 126.67, 123.48, 122.34, 119.03, 118.27, 115.75, 115.72×2, 109.32, 53.40, 52.96, 47.05, 46.45, 38.39, 29.09, 6.22×2; HRMS (ESI) m/z calcd $C_{26}H_{26}N_3O_4^+$ $[M+H]^+$ 444.1918, found 444.1909.

General Procedure for Synthesis of Compounds 13a-13j. The acids **12a-j** (12.0 mmol) were dissolved in thionyl chloride (5.0 mL), respectively. The mixture was then heated at reflux for 12 h.

After cooling to room temperature, the solution was evaporated under vacuum to afford the carbonyl chlorides **13a-j** as a yellow oil, which was used directly in the next step without further purification.

General Procedure for Synthesis of Compounds 14a-b, 14e, 14g, and 14h. To a solution of 2'-hydroxyacetophenone (1.36 g, 10.0 mmol) and pyridine (4.0 mL, 50 mmol) in dichloromethane (30 mL) was added dropwise of **13a** (or **13b**, **13e**, **13g**, **13h**; from the previous step) in dichloromethane (10 mL) at 0°C, respectively. The mixture was stirred at room temperature for 2 h. Then the reaction mixture was diluted with dichloromethane (60 mL) and washed with 3 M aqueous HCl and water. The organic layer was dried over anhydrous sodium sulfate, and concentrated to give a crude, which was purified by silica gel column chromatography (petroleum ether/EtOAc, 8:1) to get the product as a yellow solid.

*2-Acetylphenyl picolinate (**14a**).* Yield: 64%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.87 (d, J = 4.0 Hz, 1H), 8.32 (d, J = 7.8 Hz, 1H), 8.00 – 7.87 (m, 2H), 7.64 – 7.57 (m, 2H), 7.41 (t, J = 7.6 Hz, 1H), 7.32 (d, J = 8.1 Hz, 1H), 2.60 (s, 3H).

*2-acetylphenyl pyrimidine-4-carboxylate (**14b**).* Yield: 58%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.53 (d, J = 1.4 Hz, 1H), 9.11 (d, J = 5.0 Hz, 1H), 8.21 (dd, J = 5.0, 1.4 Hz, 1H), 7.94 (dd, J = 7.8, 1.6 Hz, 1H), 7.66 (ddd, J = 8.1, 7.5, 1.7 Hz, 1H), 7.45 (td, J = 7.7, 1.2 Hz, 1H), 7.32 (dd, J = 8.1, 1.0 Hz, 1H), 2.60 (s, 3H).

*2-Acetylphenyl-4-chloropicolinate (**14e**).* Yield: 98%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.74 (d, J = 5.1 Hz, 1H), 8.29 (s, 1H), 7.90 (d, J = 7.8 Hz, 1H), 7.66 – 7.52 (m, 2H), 7.41 (t, J = 7.6 Hz, 1H), 7.27 (d, J = 5.6 Hz, 1H), 2.58 (s, 3H).

*2-acetylphenyl 2-chloronicotinate (**14g**).* Yield: 95%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.61 (dd, J = 4.8, 2.0 Hz, 1H), 8.54 (dd, J = 7.7, 2.0 Hz, 1H), 7.91 (dd, J = 7.8, 1.6 Hz, 1H), 7.64 (td, J = 7.8, 1.6 Hz, 1H), 7.48 – 7.40 (m, 2H), 7.30 (dd, J = 8.4, 1.3 Hz, 1H), 2.60 (s, 3H).

*2-Acetylphenyl-3-chloroisonicotinate (**14h**).* Yield: 51%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.82 (s, 1H), 8.72 (d, J = 4.8 Hz, 1H), 8.03 (d, J = 4.9 Hz, 1H), 7.93 (d, J = 7.8 Hz, 1H), 7.66 (s, 1H), 7.45 (s, 1H), 7.29 (s, 1H), 2.61 (s, 3H).

General Procedure for Synthesis of Compounds 14c-d, 14f, and 14i-j. To a solution of 2'-hydroxyacetophenone (1.63 g, 12.0 mmol) and pyridine (2.88 mL, 36 mmol) in dichloromethane (30 mL) was added dropwise of **13c** (or **13d**, **13f**, **13i-j**; from the previous step) in dichloromethane (10 mL) at 0°C, respectively. The mixture was then stirred at 0°C for 1h and stirred at room temperature for 2 h. Then the reaction mixture was diluted with dichloromethane (60 mL) and washed with 3 M aqueous HCl and water. The organic layer was dried over anhydrous sodium sulfate, and concentrated to get the product as a yellow solid, which was used directly in the next step without further purification.

General Procedure for Synthesis of Compounds 15a-15j. Procedure A. To a solution of **14** (6.0 mmol) in THF (50 mL) was added potassium *tert*-butoxide (875 mg, 7.8 mmol). The mixture was stirred at room temperature for 12 h under argon. Then it was quenched with water (50 mL) and acidified with 2 N aqueous HCl to pH = 6.0. The mixture was evaporated under vaccum to remove the THF. Then the solid was filtered and washed with hexane to get the product **15** as a yellow solid, which was used directly in the next step without further purification.

Procedure B. To a solution of **14** (3.0 mmol) in pyridine (20 mL) was added potassium hydroxide (202 mg, 3.6 mmol). The mixture was stirred at 60°C for 8 h under argon. Then it was diluted with water (200 mL) and acidified with 4 N aqueous HCl. The resulting solution was extracted with portions of ethyl acetate (3×40 mL). The combined organic extracts were dried over anhydrous sodium sulfate, and concentrated to give a crude, which was purified silica gel column chromatography (petroleum ether/EtOAc, 6:1 to 2:1) to get the product **15** as a yellow solid.

*1-(2-Hydroxyphenyl)-3-(pyridin-2-yl)propane-1,3-dione (**15a**).* **Procedure A.** Yield: 16%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 15.18 (br s, 1H), 12.14 (s, 1H), 8.73 (ddd, J = 4.7, 1.7, 0.9 Hz, 1H), 8.14 – 8.09 (m, 1H), 7.97 (dd, J = 8.1, 1.6 Hz, 1H), 7.89 (td, J = 7.8, 1.8 Hz, 1H), 7.62 (s, 1H), 7.54 – 7.49 (m, 1H), 7.49 – 7.43 (m, 1H), 7.03 (dd, J = 8.4, 1.0 Hz, 1H), 6.96 (ddd, J = 8.2, 7.2, 1.1 Hz, 1H).

*1-(2-Hydroxyphenyl)-3-(pyrimidin-4-yl)propane-1,3-dione (**15b**).* **Procedure A.** Yield: 51%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 14.70 (br s, 1H), 12.00 (s, 1H), 9.34 (s, 1H), 9.00 (d, J = 5.0 Hz, 1H), 8.01 (d, J = 5.1 Hz, 1H), 7.96 (d, J = 7.9 Hz, 1H), 7.70 (s, 1H), 7.55 (t, J = 7.8 Hz, 1H), 7.05 (d, J = 8.4 Hz, 1H), 6.99 (t, J = 7.6 Hz, 1H).

*1-(2-Hydroxyphenyl)-3-(pyridin-3-yl)propane-1,3-dione (**15c**).* **Procedure A.** Yield: 61%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 15.18 (br s, 1H), 12.14 (s, 1H), 8.73 (ddd, J = 4.7, 1.7, 0.9 Hz, 1H), 8.14 – 8.09 (m, 1H), 7.97 (dd, J = 8.1, 1.6 Hz, 1H), 7.89 (td, J = 7.8, 1.8 Hz, 1H), 7.62 (s, 1H), 7.54 – 7.49 (m, 1H), 7.49 – 7.43 (m, 1H), 7.03 (dd, J = 8.4, 1.0 Hz, 1H), 6.96 (ddd, J = 8.2, 7.2, 1.1 Hz, 1H).

*1-(2-Hydroxyphenyl)-3-(pyridin-4-yl)propane-1,3-dione (**15d**).* **Procedure B.** Yield: 74%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 15.14 (br s, 1H), 11.94 (s, 1H), 8.83 (s, 2H), 7.83 (s, 3H), 7.54 (t, J = 7.7 Hz, 1H), 7.06 (d, J = 8.4 Hz, 1H), 6.98 (t, J = 7.6 Hz, 1H), 6.94 (s, 1H).

*1-(4-Chloropyridin-2-yl)-3-(2-hydroxyphenyl)propane-1,3-dione (**15e**).* **Procedure A.** Yield: 14%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.67 (d, J = 5.1 Hz, 1H), 8.29 (d, J = 8.4 Hz, 1H), 8.13 (s, 1H), 7.77 (t, J = 7.4 Hz, 1H), 7.64 (d, J = 8.1 Hz, 1H), 7.50 – 7.46 (m, 3H).

*1-(5-Chloropyridin-2-yl)-3-(2-hydroxyphenyl)propane-1,3-dione (**15f**).* **Procedure B.** Yield: 14%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 15.13 (br s, 1H), 12.09 (s, 1H), 8.66 (s, 1H), 8.06 (d, J = 8.3 Hz, 1H), 7.94 (d, J = 8.0 Hz, 1H), 7.85 (d, J = 8.1 Hz, 1H), 7.57 (s, 1H), 7.51 (t, J = 7.4 Hz, 1H), 7.03 (d, J = 8.5 Hz, 1H), 6.97 (t, J = 7.4 Hz, 1H).

*1-(2-Chloropyridin-3-yl)-3-(2-hydroxyphenyl)propane-1,3-dione (**15g**).* **Procedure A.** Yield: 36%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 15.19 (br s, 1H), 11.91 (s, 1H), 8.54 (dd, J = 4.8, 2.0 Hz, 1H), 8.11 (dd, J = 7.7, 2.0 Hz, 1H), 7.74 (dd, J = 8.1, 1.6 Hz, 1H), 7.52 (ddd, J = 8.6, 7.3, 1.6 Hz, 1H), 7.42 (dd, J = 7.7, 4.8 Hz, 1H), 7.05 (dd, J = 8.4, 1.0 Hz, 1H), 6.99 – 6.91 (m, 2H).

*1-(3-Chloropyridin-4-yl)-3-(2-hydroxyphenyl)propane-1,3-dione (**15h**).* **Procedure B.** Yield: 76%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 15.04 (br s, 1H), 11.87 (s, 1H), 8.77 (s, 1H), 8.66 (s, 1H), 7.72 (d, J = 7.8 Hz, 1H), 7.65 (d, J = 4.6 Hz, 1H), 7.53 (t, J = 7.6 Hz, 1H), 7.05 (d, J = 8.4 Hz, 1H), 6.96 (t, J = 7.5 Hz, 1H), 6.92 (s, 1H).

*1-(2-Fluorophenyl)-3-(2-hydroxyphenyl)propane-1,3-dione (**15i**).* **Procedure A.** Yield: 23%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.07 (td, J = 7.6, 1.8 Hz, 1H), 7.89 (dtd, J = 19.1, 7.5, 1.8 Hz, 1H), 7.67 – 7.56 (m, 2H), 7.53 – 7.43 (m, 1H), 7.28 – 7.16 (m, 3H), 7.14 – 7.03 (m, 1H).

*1-(2-Hydroxyphenyl)-3-(thiazol-2-yl)propane-1,3-dione (**15j**).* **Procedure A.** Yield: 31%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 15.16 (br s, 1H), 11.89 (s, 1H), 8.05 (d, J = 3.0 Hz, 1H), 7.90 (dd, J = 8.1, 1.6 Hz, 1H), 7.69 (d, J = 3.1 Hz, 1H), 7.52 (ddd, J = 8.6, 7.3, 1.5 Hz, 1H), 7.37 (s, 1H), 7.03 (dt, J = 8.4, 1.5 Hz, 1H), 6.99 – 6.93 (m, 1H).

General Procedure for Synthesis of Compounds 16a-16j. To a solution of **15** (1.0 mmol), Fmoc-O-tert-butyl-L-tyrosine (827 mg, 1.8 mmol), 4-dimethylpyridine (49 mg, 0.4 mmol) in pyridine (10 mL) was added DCC (412 mg, 2.0 mmol). The mixture was stirred at room temperature for 3 h until the start material disappeared as monitored by TLC. The reaction temperature was raised to 50°C for 6 h, and a major yellow spot could be observed by TLC. After the reaction mixture was evaporated under vacuum, the residue was diluted with ethyl acetate (40 mL) and filtered to remove the side product DCU. The filtrate was evaporated and purified by silica gel column chromatography (petroleum ether/EtOAc, 5:1) to afford **16** as a yellow solid.

3-(4-(tert-Butoxy)benzyl)-1-(pyridin-2-yl)chromeno[2,3-c]pyrrol-9(2H)-one ***(16a)***. Yield: 30%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.42 (d, J = 7.8 Hz, 1H), 8.48 (d, J = 4.1 Hz, 1H), 8.41 (dd, J = 7.9, 1.6 Hz, 1H), 7.81 (td, J = 7.8, 1.8 Hz, 1H), 7.69 – 7.63 (m, 1H), 7.42 (d, J = 8.4 Hz, 1H), 7.34 (d, J = 8.0 Hz, 1H), 7.20 (d, J = 8.4 Hz, 2H), 7.19 – 7.15 (m, 1H), 6.98 (d, J = 8.5 Hz, 2H), 4.24 (s, 2H), 1.35 (s, 9H).

3-(4-(tert-Butoxy)benzyl)-1-(pyrimidin-4-yl)chromeno[2,3-c]pyrrol-9(2H)-one ***(16b)***. Yield: 54%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.36 (br s, 1H), 9.35 (dd, J = 5.4, 1.3 Hz, 1H), 9.04 (d, J = 1.2 Hz, 1H), 8.77 (d, J = 5.4 Hz, 1H), 8.40 (dd, J = 8.0, 1.7 Hz, 1H), 7.70 (t, J = 6.9 Hz, 1H), 7.46 (d, J = 8.5 Hz, 1H), 7.37 (t, J = 7.5 Hz, 1H), 7.21 (d, J = 8.4 Hz, 2H), 7.01 (d, J = 8.4 Hz, 2H), 4.27 (s, 2H), 1.37 (s, 9H).

3-(4-(tert-Butoxy)benzyl)-1-(pyridin-3-yl)chromeno[2,3-c]pyrrol-9(2H)-one ***(16c)***. Yield: 56%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.42 (br s, 1H), 8.90 (d, J = 1.7 Hz, 1H), 8.65 – 8.55 (m, 1H), 8.34 (dd, J = 8.0, 1.7 Hz, 1H), 8.28 (dd, J = 4.8, 1.6 Hz, 1H), 7.65 (ddd, J = 8.6, 7.1, 1.7 Hz, 1H), 7.41 (dd, J = 8.4, 0.7 Hz, 1H), 7.33 – 7.29 (m, 1H), 7.28 – 7.24 (m, 1H), 7.16 (d, J = 8.5 Hz, 2H), 6.94 (d, J = 8.5 Hz, 2H), 4.21 (s, 2H), 1.33 (s, 9H).

3-(4-(tert-Butoxy)benzyl)-1-(pyridin-4-yl)chromeno[2,3-c]pyrrol-9(2H)-one ***(16d)***. Yield: 55%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.65 (d, J = 4.9 Hz, 2H), 8.38 (d, J = 8.0 Hz, 1H), 7.93 (d, J = 4.7 Hz, 2H), 7.67 (d, J = 7.8 Hz, 1H), 7.44 (d, J = 8.5 Hz, 1H), 7.34 (d, J = 7.8 Hz, 1H), 7.20 (d, J = 7.7 Hz, 2H), 7.01 (d, J = 8.0 Hz, 2H), 4.27 (s, 2H), 1.37 (s, 9H).

3-(4-(tert-Butoxy)benzyl)-1-(4-chloropyridin-2-yl)chromeno[2,3-c]pyrrol-9(2H)-one ***(16e)***. Yield: 50%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.37 (br s, 1H), 9.57 (d, J = 1.8 Hz, 1H), 8.42 (dd, J = 8.0, 1.6 Hz, 1H), 8.32 (d, J = 5.3 Hz, 1H), 7.66 (ddd, J = 8.6, 7.1, 1.7 Hz, 1H), 7.42 (d, J = 7.7 Hz, 1H), 7.34 (ddd, J = 8.1, 7.2, 1.0 Hz, 1H), 7.23 – 7.10 (m, 3H), 6.96 (d, J = 8.5 Hz, 2H), 4.21 (s, 2H), 1.34 (s, 9H).

*3-(4-(tert-Butoxy)benzyl)-1-(5-chloropyridin-2-yl)chromeno[2,3-c]pyrrol-9(2H)-one (**16f**).* Yield: 45%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.15 (br s, 1H), 9.43 (d, J = 8.7 Hz, 1H), 8.41 (s, 1H), 8.38 (d, J = 7.8 Hz, 1H), 7.76 (d, J = 8.6 Hz, 1H), 7.66 (t, J = 7.7 Hz, 1H), 7.42 (d, J = 8.5 Hz, 1H), 7.33 (t, J = 7.7 Hz, 1H), 7.20 (d, J = 7.7 Hz, 2H), 6.98 (d, J = 7.9 Hz, 2H), 4.24 (s, 2H), 1.36 (s, 9H).

*3-(4-(tert-Butoxy)benzyl)-1-(2-chloropyridin-3-yl)chromeno[2,3-c]pyrrol-9(2H)-one (**16g**).* Yield: 48%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.43 (s, 1H), 8.37 (dd, J = 7.8, 1.9 Hz, 1H), 8.31 – 8.24 (m, 2H), 7.65 (ddd, J = 8.7, 7.1, 1.7 Hz, 1H), 7.43 (d, J = 8.4 Hz, 1H), 7.35 – 7.29 (m, 2H), 7.21 (d, J = 8.5 Hz, 2H), 6.99 (d, J = 8.5 Hz, 2H), 4.24 (s, 2H), 1.35 (s, 9H).

*3-(4-(tert-Butoxy)benzyl)-1-(3-chloropyridin-4-yl)chromeno[2,3-c]pyrrol-9(2H)-one (**16h**).* Yield: 85%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.29 (s, 1H), 8.60 (s, 1H), 8.56 (d, J = 4.2 Hz, 1H), 8.34 (d, J = 8.1 Hz, 1H), 8.20 (d, J = 4.8 Hz, 1H), 7.69 (t, J = 7.7 Hz, 1H), 7.46 (d, J = 8.3 Hz, 1H), 7.34 (t, J = 7.4 Hz, 1H), 7.23 (d, J = 7.8 Hz, 2H), 7.03 (d, J = 7.5 Hz, 2H), 4.27 (s, 2H), 1.37 (s, 9H).

*3-(4-(tert-Butoxy)benzyl)-1-(2-fluorophenyl)chromeno[2,3-c]pyrrol-9(2H)-one (**16i**).* Yield: 53%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.07 (s, 1H), 8.77 – 8.65 (m, 1H), 8.37 (dd, J = 8.0, 1.6 Hz, 1H), 7.68 – 7.62 (m, 1H), 7.47 – 7.39 (m, 2H), 7.34 – 7.31 (m, 2H), 7.21 (d, J = 8.6 Hz, 2H), 7.15 – 7.09 (m, 1H), 7.00 (d, J = 8.5 Hz, 2H), 4.24 (s, 2H), 1.37 (s, 9H).

*3-(4-(tert-Butoxy)benzyl)-1-(thiazol-2-yl)chromeno[2,3-c]pyrrol-9(2H)-one (**16j**).* Yield: 49%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.76 (br s, 1H), 8.40 (dd, J = 8.0, 1.7 Hz, 1H), 7.74 (d, J = 3.2 Hz, 1H), 7.66 (ddd, J = 8.6, 7.2, 1.7 Hz, 1H), 7.42 (d, J = 8.3 Hz, 1H), 7.37 (d, J = 3.2 Hz, 1H), 7.34 (t, J = 7.5 Hz, 1H), 6.99 (t, J = 12.4 Hz, 2H), 6.85 (d, J = 7.9 Hz, 2H), 4.11 (s, 2H), 1.25 (s, 9H).

General Procedure for Synthesis of Compounds 17a-17j. To a solution of **16** (0.25 mmol) in dichloromethane (3.0 mL) was added trifluoroacetic acid (1.0 mL). The mixture was stirred at room temperature for 2 h. Then it was diluted with dichloromethane (50 mL) and washed with saturated

aqueous sodium bicarbonate and water. The organic layer was dried over anhydrous sodium sulfate, and purified by silica gel column chromatography (petroleum ether/EtOAc, 3:1 to 1:1) to afford the product **17** as a yellow solid.

*3-(4-Hydroxybenzyl)-1-(pyridin-2-yl)chromeno[2,3-c]pyrrol-9(2H)-one (**17a**).* Yield: 63%; purity: 98%; ^{1}H NMR (400 MHz, Acetone – d_6) δ 11.66 (br s, 1H), 9.41 (d, J = 8.1 Hz, 1H), 8.60 – 8.44 (m, 1H), 8.32 (dd, J = 7.9, 1.5 Hz, 1H), 8.14 (br, 1H), 7.86 (td, J = 8.0, 1.8 Hz, 1H), 7.72 (ddd, J = 8.7, 7.1, 1.7 Hz, 1H), 7.49 (d, J = 8.4 Hz, 1H), 7.41 – 7.30 (m, 1H), 7.25 – 7.20 (m, 3H), 6.77 (d, J = 8.5 Hz, 2H), 4.26 (s, 2H); ^{13}C NMR (101 MHz, Acetone – d_6) δ 174.73, 156.58, 155.94, 148.81, 148.71, 142.88, 137.06, 133.87, 130.12, 129.41×3, 126.87, 122.75, 122.65, 122.30, 122.14, 117.47, 115.58, 115.28, 109.10, 99.99, 28.62. HRMS (ESI) m/z calcd $C_{23}H_{17}N_2O_3^+$ $[M+H]^+$ 369.1234, found 369.1241.

*3-(4-Hydroxybenzyl)-1-(pyrimidin-4-yl)chromeno[2,3-c]pyrrol-9(2H)-one (**17b**).* Yield: 84%; purity: 99%; ^{1}H NMR (400 MHz, DMSO – d_6) δ 13.26 (br s, 1H), 9.20 (s, 1H), 9.18 (d, J = 11.7 Hz, 1H), 8.83 (d, J = 5.3 Hz, 1H), 8.23 (d, J = 7.5 Hz, 1H), 7.77 (t, J = 7.3 Hz, 1H), 7.55 (d, J = 8.2 Hz, 1H), 7.39 (t, J = 7.4 Hz, 1H), 7.16 (d, J = 8.1 Hz, 2H), 6.69 (d, J = 8.2 Hz, 2H), 4.14 (s, 2H); ^{13}C NMR (101 MHz, DMSO – d_6) δ 174.33, 157.98×2, 155.77×2, 154.44, 143.04, 134.49, 129.20×2, 129.12, 126.52, 123.34, 121.90, 119.25, 117.70, 117.61, 115.20×2, 110.93, 99.49, 28.39. HRMS (ESI) m/z calcd $C_{22}H_{16}N_3O_3^+$ $[M+H]^+$ 370.1186, found 370.1190.

*3-(4-Hydroxybenzyl)-1-(pyridin-3-yl)chromeno[2,3-c]pyrrol-9(2H)-one (**17c**).* Yield: 93%; purity: 95%; ^{1}H NMR (400 MHz, DMSO – d_6) δ 12.53 (br s, 1H), 9.22 – 9.21 (m, 2H), 8.53 (dd, J = 4.8, 1.6 Hz, 1H), 8.46 (ddd, J = 8.1, 2.3, 1.7 Hz, 1H), 8.18 (dd, J = 7.9, 1.6 Hz, 1H), 7.72 (ddd, J = 8.6, 7.1, 1.8 Hz, 1H), 7.57 – 7.44 (m, 2H), 7.35 (t, J = 8.0 Hz, 1H), 7.13 (d, J = 8.5 Hz, 2H), 6.72 (d, J = 8.5 Hz, 2H), 4.08 (s, 2H); ^{13}C NMR (101 MHz, DMSO – d_6) δ 174.71, 156.56, 156.27, 148.85, 148.58, 142.24, 135.33,

134.59, 129.86, 129.53×2, 127.48, 126.87, 124.18, 123.58, 123.34, 122.31, 118.02, 115.75×2, 115.39, 108.55, 29.03. HRMS (ESI) *m/z* calcd $C_{23}H_{17}N_2O_3^+$ $[M+H]^+$ 369.1234, found 369.1244.

*3-(4-Hydroxybenzyl)-1-(pyridin-4-yl)chromeno[2,3-c]pyrrol-9(2H)-one (**17d**).* Yield: 92%; purity: 98%; ^{1}H NMR (400 MHz, DMSO – d_6) δ 12.73 (s, 1H), 9.23 (s, 1H), 8.63 (d, J = 4.7 Hz, 2H), 8.31 – 8.11 (m, 3H), 7.75 (t, J = 7.6 Hz, 1H), 7.52 (d, J = 8.3 Hz, 1H), 7.37 (t, J = 7.5 Hz, 1H), 7.11 (d, J = 7.7 Hz, 2H), 6.70 (d, J = 7.6 Hz, 2H), 4.11 (s, 2H); ^{13}C NMR (101 MHz, DMSO – d_6) δ 174.73, 156.27, 149.91×2, 142.92, 138.32, 134.80, 129.59, 129.54×3, 126.99, 123.87, 123.58, 122.36, 121.36, 118.06, 117.05, 115.75×3, 109.64, 28.99. HRMS (ESI) *m/z* calcd $C_{23}H_{17}N_2O_3^+$ $[M+H]^+$ 369.1234, found 369.1240.

*1-(4-Chloropyridin-2-yl)-3-(4-hydroxybenzyl)chromeno[2,3-c]pyrrol-9(2H)-one (**17e**).* Yield: 75%; purity＞99%; ^{1}H NMR (400 MHz, DMSO – d_6) δ 12.95 (br s, 1H), 9.45 (d, J = 1.6 Hz, 1H), 9.18 (s, 1H), 8.58 (d, J = 5.0, 1H), 8.24 (dd, J = 8.0, 1.6 Hz, 1H), 7.75 (ddd, J = 8.6, 7.1, 1.7 Hz, 1H), 7.52 (d, J = 7.8 Hz, 1H), 7.42 (dd, J = 5.3, 2.0 Hz, 1H), 7.38 (t, J = 7.0 Hz, 1H), 7.15 (d, J = 8.5 Hz, 2H), 6.69 (d, J = 8.6 Hz, 2H), 4.12 (s, 2H); ^{13}C NMR (101 MHz, DMSO – d_6) δ 174.52, 155.84, 155.70, 150.15, 150.10, 143.75, 142.29, 134.35, 129.45, 129.18×2, 126.58, 123.74, 123.13, 121.84, 121.41, 117.58, 117.17, 115.16×3, 109.07, 28.30. HRMS (ESI) *m/z* calcd $C_{23}H_{16}ClN_2O_3^+$ $[M+H]^+$ 403.0844, found 403.0850.

*1-(5-Chloropyridin-2-yl)-3-(4-hydroxybenzyl)chromeno[2,3-c]pyrrol-9(2H)-one (**17f**).* Yield: 74%; purity: 99%; ^{1}H NMR (400 MHz, DMSO – d_6) δ 12.88 (s, 1H), 9.26 (d, J = 8.6 Hz, 1H), 9.19 (s, 1H), 8.63 (s, 1H), 8.21 (d, J = 7.7 Hz, 1H), 8.03 (d, J = 8.7 Hz, 1H), 7.74 (t, J = 7.6 Hz, 1H), 7.52 (d, J = 8.4 Hz, 1H), 7.37 (t, J = 7.4 Hz, 1H), 7.14 (d, J = 8.0 Hz, 2H), 6.68 (d, J = 8.0 Hz, 2H), 4.11 (s, 2H); ^{13}C NMR (101 MHz, DMSO – d_6) δ 174.40, 155.87, 155.68, 147.23, 147.21, 142.17, 136.88, 134.29, 129.52, 129.16×2, 128.90, 126.50, 124.01, 123.14, 123.06, 121.88, 117.57, 116.74, 115.15×2, 108.81, 28.31. HRMS (ESI) *m/z* calcd $C_{23}H_{16}ClN_2O_3^+$ $[M+H]^+$ 403.0844, found 403.0851.

*1-(2-Chloropyridin-3-yl)-3-(4-hydroxybenzyl)chromeno[2,3-c]pyrrol-9(2H)-one (**17g**).* Yield: 76%; purity: 99%; ^{1}H NMR (400 MHz, DMSO – d_6) δ 12.48 (br s, 1H), 9.24 (s, 1H), 8.48 (dd, J = 4.8, 1.9 Hz, 1H), 8.08 (dd, J = 8.0, 1.6 Hz, 1H), 8.04 (dd, J = 7.6, 1.9 Hz, 1H), 7.73 (ddd, J = 8.7, 7.1, 1.8 Hz, 1H), 7.61 – 7.47 (m, 2H), 7.39 – 7.27 (m, 1H), 7.12 (d, J = 8.5 Hz, 2H), 6.71 (d, J = 8.5 Hz, 2H), 4.06 (s, 2H); ^{13}C NMR (101 MHz, DMSO – d_6) δ 174.31, 156.94, 156.27, 150.14, 149.64, 142.52, 141.18, 134.58, 129.82, 129.55×2, 127.83, 126.50, 123.35, 122.95, 122.30, 121.38, 118.24, 115.75×2, 114.61, 109.56, 29.13. HRMS (ESI) m/z calcd $C_{23}H_{14}ClN_2O_3^-$ $[M-H]^-$ 401.0698, found 401.0707.

*1-(3-Chloropyridin-4-yl)-3-(4-hydroxybenzyl)chromeno[2,3-c]pyrrol-9(2H)-one (**17h**).* Yield: 55%; purity: 99%; ^{1}H NMR (400 MHz, DMSO – d_6) δ 12.58 (br s, 1H), 9.23 (s, 1H), 8.75 (s, 1H), 8.59 (d, J = 5.0 Hz, 1H), 8.09 (dd, J = 7.9, 1.6 Hz, 1H), 7.79 – 7.70 (m, 1H), 7.63 (d, J = 5.0 Hz, 1H), 7.53 (d, J = 8.4 Hz, 1H), 7.35 (t, J = 7.5 Hz, 1H), 7.12 (d, J = 8.5 Hz, 2H), 6.71 (d, J = 8.5 Hz, 2H), 4.08 (s, 2H); ^{13}C NMR (101 MHz, DMSO – d_6) δ 174.25, 156.85, 156.29, 149.67, 147.87, 141.53, 138.20, 134.70, 130.81, 129.66, 129.57×2, 127.39, 126.56, 123.48, 122.31, 120.05, 118.28, 115.86, 115.77×2, 109.92, 29.16. HRMS (ESI) m/z calcd $C_{23}H_{14}ClN_2O_3^-$ $[M-H]^-$ 401.0698, found 401.0709.

*1-(2-Fluorophenyl)-3-(4-hydroxybenzyl)chromeno[2,3-c]pyrrol-9(2H)-one (**17i**).* Yield: 91%; purity: 98%; ^{1}H NMR (400 MHz, DMSO – d_6) δ 12.35 (br s, 1H), 9.22 (s, 1H), 8.10 (dd, J = 7.9, 1.6 Hz, 1H), 7.73 (ddd, J = 15.9, 8.2, 1.7 Hz, 2H), 7.58 – 7.39 (m, 2H), 7.35 – 7.28 (m, 3H), 7.12 (d, J = 8.5 Hz, 2H), 6.70 (d, J = 8.5 Hz, 2H), 4.06 (s, 2H); ^{13}C NMR (101 MHz, DMSO – d_6) δ 174.27, 156.78, 156.22, 141.42, 134.42, 132.80, 130.51, 130.03, 129.54×2, 126.61, 124.41, 124.38, 123.24, 122.45, 120.41, 118.13, 116.13, 115.91, 115.71×2, 114.55, 108.94, 29.10; HRMS (ESI) m/z calcd $C_{24}H_{17}FNO_3^+$ $[M+H]^+$ 386.1187, found 386.1195.

*3-(4-Hydroxybenzyl)-1-(thiazol-2-yl)chromeno[2,3-c]pyrrol-9(2H)-one (**17j**).* Yield: 91%; purity: 95%; ^{1}H NMR (400 MHz, DMSO – d_6) δ 13.05 (br s, 1H), 9.20 (s, 1H), 8.20 (dd, J = 7.9, 1.5 Hz, 1H), 7.93

(d, *J* = 3.2 Hz, 1H), 7.79 – 7.65 (m, 2H), 7.53 (d, *J* = 8.3 Hz, 1H), 7.38 (t, *J* = 7.5 Hz, 1H), 7.14 (d, *J* = 8.4 Hz, 2H), 6.69 (d, *J* = 8.5 Hz, 2H), 4.09 (s, 2H); ^{13}C NMR (101 MHz, DMSO – d_6) δ 174.19, 156.89, 156.29, 155.72, 142.30, 141.11, 134.42, 129.36, 129.15×2, 126.15, 123.19, 121.71, 120.40, 119.71, 117.80, 116.51, 115.17×2, 108.64, 28.38; HRMS (ESI) *m*/*z* calcd $C_{21}H_{15}N_2O_3S^+$ $[M+H]^+$ 375.0798, found 375.0804.

General Procedure for Synthesis of Compounds 19a-19b. To a solution of 2'-hydroxyacetophenone (**3**) (272 mg, 2.0 mmol) in toluene (10 mL) at 0°C was added sodium hydride (60% in mineral oil, 400 mg, 10.0 mmol). The mixture was stirred at room temperature for 15 min and **18** (2.0 mmol) was added and the mixture was stirred at 60°C for 2 h. After the solution had cooled to room temperature it was poured into a mixture of ice and water and acidified by the addition of 2 N aqueous HCl. The resulting solution was extracted with portions of ethyl acetate (2×50 mL). The combined organic extracts were dried over anhydrous sodium sulfate, and concentrated to give a crude, which was purified by silica gel column chromatography (petroleum ether/EtOAc, 6:1 to 2:1) to get the product **19** as a yellow solid.

*1-(2-Hydroxyphenyl)-3-(p-tolyl)propane-1,3-dione (**19a**).* Yield: 37%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 15.18 (br s, 1H), 12.14 (s, 1H), 8.73 (ddd, *J* = 4.7, 1.7, 0.9 Hz, 1H), 8.14 – 8.09 (m, 1H), 7.97 (dd, *J* = 8.1, 1.6 Hz, 1H), 7.89 (td, *J* = 7.8, 1.8 Hz, 1H), 7.62 (s, 1H), 7.54 – 7.49 (m, 1H), 7.49 – 7.43 (m, 1H), 7.03 (dd, *J* = 8.4, 1.0 Hz, 1H), 6.96 (ddd, *J* = 8.2, 7.2, 1.1 Hz, 1H).

*1-(2-Hydroxyphenyl)-3-(2-methoxypyridin-3-yl)propane-1,3-dione (**19b**).* Yield: 24%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 15.47 (br s, 1H), 12.12 (s, 1H), 8.34 (dd, *J* = 7.7, 1.9 Hz, 1H), 8.29 (dd, *J* = 4.8, 1.9 Hz, 1H), 7.75 (dd, *J* = 8.1, 1.3 Hz, 1H), 7.50 – 7.44 (m, 1H), 7.42 (s, 1H), 7.05 (dd, *J* = 7.6, 4.9 Hz, 1H), 7.00 (d, *J* = 8.1 Hz, 1H), 6.94 (t, *J* = 7.2 Hz, 1H), 4.14 (s, 3H).

General Procedure for Synthesis of Compounds 20a-20b. To a solution of **19** (1.0 mmol), Fmoc-O-tert-butyl-L-tyrosine (827 mg, 1.8 mmol), 4-dimethylpyridine (49 mg, 0.4 mmol) in pyridine (10 mL)

was added DCC (412 mg, 2.0 mmol). The mixture was stirred at room temperature for 3 h until the start material disappeared as monitored by TLC. The reaction temperature was raised to 50°C for 6 h, and a major yellow spot could be observed by TLC. After the reaction mixture was evaporated under vacuum, the residue was diluted with ethyl acetate (40 mL) and filtered to remove the side product DCU. The filtrate was evaporated and purified by silica gel column chromatography (petroleum ether/EtOAc, 5:1) to afford **20** as a yellow solid.

*3-(4-(tert-butoxy)benzyl)-1-(p-tolyl)chromeno[2,3-c]pyrrol-9(2H)-one (**20a**).* Yield: 16%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.49 (s, 1H), 8.35 (dd, J = 7.9, 1.7 Hz, 1H), 7.81 (d, J = 8.2 Hz, 2H), 7.63 (ddd, J = 8.7, 7.1, 1.7 Hz, 1H), 7.39 (d, J = 8.4 Hz, 1H), 7.33 – 7.29 (m, 1H), 7.24 (d, J = 8.0 Hz, 2H), 7.19 (d, J = 8.5 Hz, 2H), 6.98 (d, J = 8.5 Hz, 2H), 4.21 (s, 2H), 2.39 (s, 3H), 1.36 (s, 9H).

*3-(4-(tert-Butoxy)benzyl)-1-(2-methoxypyridin-3-yl)chromeno[2,3-c]pyrrol-9(2H)-one (**20b**).* Yield: 65%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.37 (br s, 1H), 9.59 (dd, J = 7.7, 1.8 Hz, 1H), 8.38 (dd, J = 8.0, 1.7 Hz, 1H), 8.08 (dd, J = 4.8, 1.8 Hz, 1H), 7.69 – 7.60 (m, 1H), 7.41 (d, J = 8.4 Hz, 1H), 7.32 (d, J = 7.2 Hz, 1H), 7.24 (d, J = 8.4 Hz, 2H), 7.11 (dd, J = 7.7, 4.8 Hz, 1H), 7.04 (d, J = 8.4 Hz, 2H), 4.23 (s, 2H), 3.97 (s, 3H), 1.38 (s, 9H).

General Procedure for Synthesis of Compounds 21a-21b. To a solution of **20** (0.25 mmol) in dichloromethane (3.0 mL) was added trifluoroacetic acid (1.0 mL). The mixture was stirred at room temperature for 2 h. Then it was diluted with dichloromethane (50 mL) and washed with saturated aqueous sodium bicarbonate and water. The organic layer was dried over anhydrous sodium sulfate, and purified by silica gel column chromatography (petroleum ether/EtOAc, 3:1 to 1:1) to afford the product **21a/21b** as a yellow solid.

*3-(4-hydroxybenzyl)-1-(p-tolyl)chromeno[2,3-c]pyrrol-9(2H)-one (**21a**).* Yield: 92%; purity: 98%; ^{1}H NMR (400 MHz, DMSO – d_6) δ 12.24 (br s, 1H), 9.20 (s, 1H), 8.17 (dd, J = 7.9, 1.6 Hz, 1H), 8.01 (d, J

= 8.2 Hz, 2H), 7.70 (ddd, *J* = 8.6, 7.2, 1.7 Hz, 1H), 7.47 (d, *J* = 7.9 Hz, 1H), 7.35 – 7.30 (m, 1H), 7.27 (d, *J* = 8.1 Hz, 2H), 7.11 (d, *J* = 8.5 Hz, 2H), 6.70 (d, *J* = 8.5 Hz, 2H), 4.06 (s, 2H), 2.36 (s, 3H); ^{13}C NMR (101 MHz, DMSO – d_6) δ 174.52, 156.47, 156.18, 141.91, 137.54, 134.35, 130.18, 129.48×2, 129.18×2, 128.70, 128.11×2, 128.08, 126.92, 123.15, 122.53, 117.88, 115.69×2, 113.79, 107.56, 28.94, 21.34; HRMS (ESI) *m*/*z* calcd $C_{25}H_{20}NO_3^+$ $[M+H]^+$ 382.1438, found 382.1448.

*3-(4-Hydroxybenzyl)-1-(2-methoxypyridin-3-yl)chromeno[2,3-c]pyrrol-9(2H)-one (**21b**).* Yield: 88%; purity: 99%; ^{1}H NMR (400 MHz, DMSO – d_6) δ 12.10 (br s, 1H), 9.23 (s, 1H), 8.19 (dd, *J* = 6.0, 4.3 Hz, 2H), 8.11 (d, *J* = 7.9 Hz, 1H), 7.71 (t, *J* = 7.8 Hz, 1H), 7.50 (d, *J* = 8.3 Hz, 1H), 7.32 (t, *J* = 7.5 Hz, 1H), 7.13 – 7.08 (m, 3H), 6.70 (d, *J* = 8.4 Hz, 2H), 4.06 (s, 2H), 3.90 (s, 3H); ^{13}C NMR (101 MHz, DMSO – d_6) δ 174.34, 160.52, 156.64, 156.20, 146.53, 141.37, 140.94, 134.43, 130.06, 129.56×2, 126.70, 123.24, 122.38, 121.96, 118.07, 117.12, 115.68×2, 114.72, 113.86, 108.70, 53.95, 28.98; HRMS (ESI) *m*/*z* calcd $C_{24}H_{18}N_2NaO_4^+$ $[M+Na]^+$ 421.1159, found 421.1150.

*3-(4-Hydroxybenzyl)-1-(2-hydroxypyridin-3-yl)chromeno[2,3-c]pyrrol-9(2H)-one (**21c**).* To a solution of **21b** (100 mg, 0.25 mmol) in dichloromethane (10 mL) at -20°C was added boron tribromide (72 μL, 0.75 mmol). The mixture was stirred at -20°C for 2h and quenched with ice water (10 mL). The solution was then extracted with portions of dichloromethane (3×20 mL), dried over anhydrous sodium sulfate, and evaporated to afford a residue, which was purified by silica gel column chromatography (petroleum ether/EtOAc, 1:3) to get the product **21c** (44 mg) as a yellow solid. Yield: 46%; ^{1}H NMR (400 MHz, DMSO – d_6) δ 13.27 (s, 1H), 12.32 (br s, 1H), 9.73 (dd, *J* = 7.4, 1.9 Hz, 1H), 9.24 (s, 1H), 8.22 (dd, *J* = 7.9, 1.6 Hz, 1H), 7.74 (ddd, *J* = 8.7, 7.1, 1.7 Hz, 1H), 7.59 – 7.46 (m, 2H), 7.43 – 7.30 (m, 1H), 7.11 (d, *J* = 8.5 Hz, 2H), 6.71 (d, *J* = 8.5 Hz, 2H), 6.55 (dd, *J* = 7.3, 6.3 Hz, 1H), 4.12 (s, 2H); ^{13}C NMR (101 MHz, DMSO – d_6) δ 173.87, 161.56, 155.86, 155.74, 141.65, 138.64, 134.00, 133.81, 129.25×2, 128.84,

126.57, 125.61, 122.89, 121.95, 118.68, 117.33, 115.38×2, 112.11, 106.68, 103.81, 28.45; HRMS (ESI) *m*/*z* calcd $C_{23}H_{16}N_2NaO_4^+$ $[M+Na]^+$ 407.1002, found 407.0991.

N'-acetyl-3-(4-(tert-butoxy)benzyl)-9-oxo-2,9-dihydrochromeno[2,3-c]pyrrole-1-carbohydrazide ***(22)***. To a solution of **6** (250 mg, 0.6 mmol) in dichloromethane (8.0 mL) was added acethydrazide (67 mg, 0.9 mmol), triethylamine (125 μL, 0.9 mmol) and HATU (459 mg, 1.2 mmol). The mixture was stirred at room temperature for 12 h. Then it was concentrated to give a crude, which was purified by silica gel column chromatography (petroleum ether/EtOAc, 5:1 to 3:1) to get the product **22** (169 mg) as a white solid. Yield: 63%; ^{1}H NMR (400 MHz, DMSO – d_6) δ 13.44 (br s, 1H), 12.25 (s, 1H), 10.32 (s, 1H), 8.25 (dd, J = 8.0, 1.4 Hz, 1H), 7.84 (t, J = 7.0 Hz, 1H), 7.61 (d, J = 8.4 Hz, 1H), 7.45 (t, J = 7.6 Hz, 1H), 7.21 (d, J = 8.4 Hz, 2H), 6.90 (d, J = 8.4 Hz, 2H), 4.17 (s, 2H), 1.96 (s, 3H), 1.25 (s, 9H).

3-(4-hydroxybenzyl)-1-(5-methyl-1,3,4-oxadiazol-2-yl)chromeno[2,3-c]pyrrol-9(2H)-one ***(23)***. Compound **22** (140mg, 0.31 mmol) was dissolved in phosphorus oxychloride (3.0 mL) and the mixture was stirred at 60°C for 30 min. After the reaction mixture had cooled to room temperature it was poured into a mixture of ice and water. The resulting solution was extracted with portions of ethyl acetate (3×20 mL). The combined organic extracts were dried over anhydrous sodium sulfate, and concentrated to give a crude, which was purified by silica gel column chromatography (petroleum ether/EtOAc, 3:1 to 1:1) to get the product **23** (67 mg) as a yellow solid. Yield: 58%; purity: 98%; ^{1}H NMR (400 MHz, DMSO – d_6) δ 13.49 (br s, 1H), 9.25 (s, 1H), 8.17 (d, J = 7.9 Hz, 1H), 7.77 (t, J = 7.0 Hz, 1H), 7.56 (d, J = 8.3 Hz, 1H), 7.39 (t, J = 7.5 Hz, 1H), 7.12 (d, J = 8.3 Hz, 2H), 6.69 (d, J = 8.4 Hz, 2H), 4.08 (s, 2H), 2.61 (s, 3H); ^{13}C NMR (101 MHz, DMSO – d_6) δ 173.40, 163.90, 158.98, 156.53, 156.34, 142.25, 134.97, 129.67×2, 129.37, 126.74, 123.88, 122.46, 119.66, 118.38, 115.75×2, 111.16, 108.08, 31.17, 11.17; HRMS (ESI) *m*/*z* calcd $C_{21}H_{15}N_3NaO_4^+$ $[M+H]^+$ 396.0955, found 396.0946.

*5-(Bromomethyl)benzo[d][1,3]dioxole (**26a**).* To a solution of piperitol (1.52 g, 10.0 mmol) in dichloromethane (40 mL) at 0°C was added phosphorus tribromide (2.8 mL, 30.0 mmol). The mixture was stirred at room temperature for 4h and quenched with ice water (100 mL). The solution was then extracted with portions of dichloromethane (3×50 mL), dried over anhydrous sodium sulfate, and evaporated to afford the product **26a** as a colorless oil, which was used directly without further purification. Yield: 87%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.92 – 6.86 (m, 2H), 6.77 (d, J = 7.9 Hz, 1H), 5.99 (s, 2H), 4.48 (s, 2H).

*1-(Bromomethyl)-4-(trifluoromethyl)benzene (**26b**).* To a solution of 1-methyl-4-(trifluoromethyl)benzene (800 mg, 5.0 mmol) in carbon tetrachloride (20 mL) was added N-bromosuccinimide (908 mg, 5.1 mmol) and benzoyl peroxide (60 mg, 0.25 mmol). The reaction mixture was heated at reflux for 6 h under argon. After cooling to room temperature, the mixture was filtered and the filtrate was evaporated under vacuum to afford a residue, which was purified by column chromatography to get the product **26b** (1027 mg) as a colorless oil, which was used directly without further purification. Yield: 86%.

General Procedure for Synthesis of Compounds 27a-27b. To a solution of **26** (10.0 mmol) in acetonitrile (10 mL) was added diethyl acetamidomalonate (2.4 g, 11.0 mmol), potassium carbonate (2.76 g, 20.0 mmol) and potassium iodide (1.66 g, 10.0 mmol). The reaction mixture was heated at reflux for 12h. After cooling to room temperature, the mixture was filtered and washed with ethyl acetate (30 mL). The filtrate was evaporated under vacuum to remove most of the solvent, diluted with ethyl acetate (150 mL) and washed with water (50 mL). The organic layer was dried over anhydrous sodium sulfate and evaporated under vacuum to afford a residue, which was purified by silica gel column chromatography (petroleum ether/EtOAc, 5:1 to 2:1) to get the product **27** as a colorless oil.

*Diethyl 2-acetamido-2-(benzo[d][1,3]dioxol-5-ylmethyl)malonate (**27a**).* Yield: 58%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.70 (dd, J = 8.3, 0.8 Hz, 1H), 6.59 (s, 1H), 6.49 – 6.46 (m, 2H), 5.93 (d, J = 1.1 Hz, 2H), 4.28 (qdd, J = 7.1, 4.2, 0.9 Hz, 4H), 3.57 (s, 2H), 2.05 (d, J = 0.9 Hz, 3H), 1.30 (td, J = 7.1, 0.9 Hz, 6H).

*Diethyl 2-acetamido-2-(4-(trifluoromethyl)benzyl)malonate (**27b**).* Yield: 65%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.52 (d, J = 8.0 Hz, 2H), 7.14 (d, J = 7.9 Hz, 2H), 6.57 (br s, 1H), 4.31 – 4.23 (m, 4H), 3.73 (s, 2H), 2.04 (s, 3H), 1.30 (ddd, J = 7.1, 5.4, 1.8 Hz, 6H).

General Procedure for Synthesis of Compounds 28a-28b. A suspension of compound **27a-b** (8.5 mmol) in 3 M HCl (60 mL) was heated to reflux for 16 h before cooling to room temperature. Water was evaporated under reduced pressure and the solid washed with ether (3×10 mL) and then dried under vacuum to afford compound **28a-b** as a gray solid, which was used directly without further purification.

*3-(Benzo[d][1,3]dioxol-5-ylmethyl)-1-(thiazol-2-yl)chromeno[2,3-c]pyrrol-9(2H)-one (**2**).* A solution of 9-fluorenylmethyl chloroformate (2.07 g, 8.0 mmol) in dioxane (20 mL) was added to a suspension of the **28a** (1.97 g, 8.0 mmol) in dioxane (20 mL) and 10% aqueous Na_2CO_3 (20 mL) at 0°C. The mixture was stirred for 1 h at 0 °C and then for 1 h at room temperature. The reaction mixture was poured into water and washed with ether (2×30 mL). The aqueous phase was acidified with concentrated aqueous HCl, and extracted with portions of ethyl acetate (3×50 mL). The combined organic phases were dried over anhydrous sodium sulfate, and evaporated under vacuum to afford the product **29a** as a white solid, which was used directly without further purification; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.77 (d, J = 7.4 Hz, 2H), 7.56 (t, J = 7.0 Hz, 2H), 7.40 (t, J = 7.4 Hz, 2H), 7.36 – 7.28 (m, 2H), 6.73 (d, J = 7.7 Hz, 1H), 6.64 (s, 1H), 6.58 (d, J = 7.7 Hz, 1H), 5.92 (s, 2H), 5.23 (d, J = 7.5 Hz, 1H), 4.65 (d, J = 6.6 Hz, 1H), 4.54 – 4.42 (m, 1H), 4.42 – 4.32 (m, 1H), 4.21 (t, J = 6.5 Hz, 1H), 3.08 (dd, J = 12.8, 5.1 Hz, 2H). To a solution of **15j** (247 mg, 1.0 mmol), **29a** (776 mg, 1.8 mmol), 4-dimethylpyridine (49 mg, 0.4 mmol) in

pyridine (10 mL) was added DCC (412 mg, 2.0 mmol). The mixture was stirred at room temperature for 3 h until the start material disappeared as monitored by TLC. The reaction temperature was raised to 50°C for 6 h, and a major yellow spot could be observed by TLC. After the reaction mixture was evaporated under vacuum, the residue was diluted with ethyl acetate (40 mL) and filtered to remove the side product DCU. The filtrate was evaporated and purified by silica gel column chromatography (petroleum ether/EtOAc, 5:1) to afford the product as a yellow solid. Yield: 53%; purity: 98%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 11.15 (br s, 1H), 8.38 (dd, J = 8.0, 1.6 Hz, 1H), 7.75 (d, J = 3.2 Hz, 1H), 7.65 (ddd, J = 8.7, 7.2, 1.7 Hz, 1H), 7.40 (d, J = 8.2 Hz, 1H), 7.38 (d, J = 3.2 Hz, 1H), 7.33 (t, J = 7.5 Hz, 1H), 6.58 (d, J = 7.9 Hz, 1H), 6.50 (s, 1H), 6.45 (d, J = 7.9 Hz, 1H), 5.74 (s, 2H), 4.01 (s, 2H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 175.57, 158.02, 157.03, 147.79, 146.28, 142.40, 141.60, 134.05, 131.54, 126.84, 123.08, 122.46, 121.00×2, 119.87, 117.65, 114.84, 109.63, 108.55, 108.30, 100.96, 29.60; HRMS (ESI) m/z calcd $C_{22}H_{15}N_2O_4S^+$ $[M+H]^+$ 403.0747, found 403.0752.

*1-(Thiazol-2-yl)-3-(4-(trifluoromethyl)benzyl)chromeno[2,3-c]pyrrol-9(2H)-one (**30**).* A solution of 9-fluorenylmethyl chloroformate (2.07 g, 8.0 mmol) in dioxane (20 mL) was added to a suspension of the **28b** (2.16 g, 8.0 mmol) in dioxane (20 mL) and 10% aqueous Na_2CO_3 (20 mL) at 0°C. The mixture was stirred for 1 h at 0 °C and then for 1 h at room temperature. The reaction mixture was poured into water and washed with ether (2×30 mL). The aqueous phase was acidified with concentrated aqueous HCl, and extracted with portions of ethyl acetate (3×50 mL). The combined organic phases were dried over anhydrous sodium sulfate, and evaporated under vacuum to afford the product **29b** as a white solid, which was used directly without further purification. To a solution of **15j** (247 mg, 1.0 mmol), **29b** (820 mg, 1.8 mmol), 4-dimethylpyridine (49 mg, 0.4 mmol) in pyridine (10 mL) was added DCC (412 mg, 2.0 mmol). The mixture was stirred at room temperature for 3 h until the start material disappeared as monitored by TLC. The reaction temperature was raised to 50°C for 6 h, and a major yellow spot could

be observed by TLC. After the reaction mixture was evaporated under vacuum, the residue was diluted with ethyl acetate (40 mL) and filtered to remove the side product DCU. The filtrate was evaporated and purified by silica gel column chromatography (petroleum ether/EtOAc, 5:1) to afford the product **30** as a yellow solid. Yield: 24%; purity: 99%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.31 (br s, 1H), 8.40 (dd, J = 8.0, 1.7 Hz, 1H), 7.78 (d, J = 3.2 Hz, 1H), 7.68 (ddd, J = 8.6, 7.1, 1.7 Hz, 1H), 7.61 – 7.52 (m, 2H), 7.41 (t, J = 5.2 Hz, 2H), 7.39 – 7.31 (m, 3H), 4.27 (s, 2H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 175.53, 160.31, 157.61, 157.04, 143.45, 142.66, 141.87, 134.16, 128.58×2, 126.88, 125.68, 125.65, 124.26, 123.19, 122.38, 121.44, 120.08, 117.61, 113.23, 109.68, 29.95; HRMS (ESI) m/z calcd $C_{22}H_{14}F_3N_2O_2S^+$ $[M+H]^+$ 427.0723, found 427.0716.

Protein expression and purification. The expression and purification of PDE5A were carried out similar to our previously published protocols.[31] Briefly, the catalytic domain coding (535-860) of PDE5A was cloned to vector pET-15b, and then, the cDNA was transferred to *E. coli* strain BL21 (CodonPlus, Stratagene) for overexpression. When the cell carrying the plasmid was cultivated in LB medium at 37 °C to OD_{600}= 0.7, 0.1 mM isopropyl b-D-thiogalactopyranoside (IPTG) was added to induce PDE5A expression for an additional 40 h of growth at 15 °C. The PDE5A protein was purified through Ni-NTA column (ϕ=2.5 cm, 15 ml QIAGEN agarose beads), Q-column (ϕ 2.5 × 8 cm, GE Healthcare) and Superdex 200 column (ϕ 2.5 × 45 cm, GE Healthcare). A typical batch cell yielded over 10 mg of the PDE5A protein from 2 L of LB medium with a purity > 95% based on SDS-PAGE.

The catalytic domains of PDE1B (10-487), PDE2A (580-919), PDE3A (679-1087), PDE4D (86-413), PDE6A (484-817), PDE7A (130-482), PDE9A (181-506), PDE10A (449-770) were purified using a similar protocol. PDE8A (480-820) was expressed and purified according to a previously published protocol.[31,46]

PDE enzymatic assays. Enzymatic activity assays of PDEs were performed similar to our

previously published protocol.[31] The assays were measured using corresponding ^{3}H-cGMP or ^{3}H-cAMP as the substrate in an assay buffer containing 50 mM Tris-HCl (pH 8.0), 10 mM $MgCl_2$ or 4 mM $MnCl_2$, and 1 mM DTT. The reaction was carried out at room temperature for 15 minutes and terminated by adding 0.2 N $ZnSO_4$ and $Ba(OH)_2$. The reaction product was concentrated to a precipitate, and the unreacted substrate remained in the supernatant. The radioactivity of the supernatant was measured in 2.5 mL of Ultima Gold liquid scintillation cocktails using a liquid scintillation counter. The inhibitors were screened at a concentration of 100 nM, and the IC_{50} values of the inhibitors were measured at more than seven suitable concentrations at least three times. The IC_{50} values were calculated using nonlinear regression. Sildenafil citrate served as the reference compound with an IC_{50} of 5.1 nM for PDE5.

***In Vivo* Pharmacokinetics Analysis.** The pharmacokinetic properties of **2** were analyzed by the Medicilon Company, Shanghai, China. Six male SD rats with a body weight of 230−260 g were purchased from Shanghai SIPPR-BK LAB Animal Ltd., Shanghai, China, and used for the pharmacokinetic analysis of **2**. **2** was dissolved/suspended in 5% DMSO, 10% Solutol, and 85% water for intravenous administration (IV) and oral administration (PO). A final dosage of 2.5 and 5 mg/kg rat of the formulated compounds was administered for IV and PO purposes, respectively, and the blood samples were taken at various time points during a 24 h period. The concentration of the compounds in the blood was analyzed by LC−MS/MS (Shimadzu liquid chromatographic system and API4000 mass spectrometer, Applied Biosystems, Ontario, Canada).

Pharmacodynamics Effects of Compound 2 against PAH in Animals. All animal care and experimental protocols were in accordance with the "Guide for the Care and Use of Laboratory Animals" (National Institutes of Health Publication, revised 1996, No.86-23, Bethesda, MD) and approved by the Institutional Ethical Committee for Animal Research of Sun Yat-sen University. Forty-eight Wister rats (8 weeks, 180−220 g), which were purchased from the Laboratory Animal Center of Southern Medical

University (Guangzhou, China), were used to evaluate the pharmacodynamics effects of **2** on PAH. The rats were randomly divided into four groups as follows: control group, model, compound **2** (5.0 mg/kg), and positive (sildenafil citrate, 10 mg/kg). The rats were maintained on a 12 h light/dark cycle (light from 7:00 to 19:00) at 24±1 °C and 60−70% relative humidity. Sterile food and water were provided according to the institutional guidelines. Prior to each experiment, the rats were fasted overnight and allowed free access to water. All the rats were administered with MCT 60 mg/kg, except the group control. Then, the rats were orally treated with the drug vehicle (control and model groups), compound **2** (5.0 mg/kg) and sildenafil citrate (10 mg/kg) for 3 weeks. Compound **2** and sildenafil citrate were dissolved in 5% DMSO/10% Solutol/ 85% water solution and orally administered 0.4 mL per 100 g weight. The right cardiac catheter method was applied to measure the pulmonary artery pressure, and the mean pulmonary artery pressure (mPAP) was used to conduct statistics. Then, the rats were killed, and the hearts were dissected into right ventricle (RV) and left ventricle and interventricular septum (LV+S). Then, the 2 parts of the heart were weighed using electronic scales, and the value of RV/(LV+S) was used to conduct statistics.

Biopharmaceutical Profiling (hERG inhibition and human CYP450 inhibition). The assays were performed by the Medicilon Company, Shanghai, China. hERG inhibition was performed using an automated patch clamp electrophysiology measurement in CHO-hERG cells.[47] Several human hepatic CYP enzymes play a dominant role in the metabolism of drugs and other xenobiotics.[43, 44] The CYP450 inhibition assay was performed by incubating compound **2** with human liver microsomes and NADPH in the presence of the CYP450-isoform specific probe substrate.[48, 49] The IC_{50} values of compound **2** for seven CYP isoenzymes (i.e., CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, and CYP3A4) were determined.

Stability of compound 2 in the Rat and Human Liver Microsomes. The assays were performed

at the Medicilon Company, Shanghai, China. The experimental procedures were similar to those in our previous study[31]. Compound **2** was dissolved in 100% DMSO to prepare a 10 mM stock solution and diluted to a final concentration of 0.5 μM for the experiments. Midazolam (Sigma, St. Louis, MO, USA) was used as the positive control.

Acute Toxicity of compound 2. The acute toxicity was tested according to similar protocols that were described in our previous study. Thirty KM mice (22 days, 18−20 g), which were purchased from the Laboratory Animal Center of Sun Yat-Sen University (Guangzhou, China), were used to evaluate the acute toxicity of **2**. Mice were randomly divided into three groups, and each group was given in single oral dose of 0, 1000, or 1500 mg/kg **2** on the first day of the experiment. Mice were maintained on a 12 h light/dark cycle (light from 7:00 to 19:00) at room temperature and 60−70% relative humidity. Sterile food and water were provided according the institutional guidelines. Prior to each experiment, mice were fasted overnight and allowed free access to water. Compound **2** was dissolved in 5% DMSO/10% Solutol/ 85% water solution and orally administered. Mice were observed for any abnormal behavior and mortality and weighed four hours after **2** was administered and then every 24 h for 14 days. Animals were sacrificed on the 14th day, and tissue samples of the heart, liver, and kidney were macroscopically examined for possible damage.

Short-Term (2-Week) Toxicity of Compound 2. The short-term toxicity of **2** was determined by the Medicilon Company, Shanghai, China. Twenty-four SD rats (50% male and 50% female) with body weights of 180−220 g were purchased from Shanghai SIPPR-BK LAB Animal Ltd., Shanghai, China, and used to evaluate the long-term toxicity of **2**. All animals were given a thorough physical examination prior to the administration of **2** and randomly divided into four groups (each group includes three male rats and three female rats). Each group was given a single oral dose of 0, 30, 100, or 300 mg/kg **2** for the daily experiments. The rats were maintained on a 12 h light/dark cycle (light from 7:00

to 19:00) at room temperature and 40−70% relative humidity. Sterile food and water were provided according the institutional guidelines. Prior to each experiment, the rats were fasted overnight and allowed free access to water. Compound **2** was dissolved in 0.5% CMC-Na/99.5% water solution, which was orally administered. During the experiment, all animals were observed at least two times a day (morning and afternoon) including but not limited to morbidity, mortality, damage, and water supply. Animals were sacrificed on the 14th day, and tissue samples of the heart, liver, and kidney were macroscopically examined for possible damage.

Molecular Docking. The Accelrys Discovery Studio 2.5.5 software was used for molecular docking studies. Hydrogen atoms and charges were added to the crystal structure of PDE5A (PDB entry code 4MD6) [24] with **1** bound using the CHARMM force field and the Momany-Rone partial charge method. All ionizable residues in the systems were set to their protonation states at a neutral pH. The zinc and magnesium ions were assigned with a charge of +2. Bound ligand **1** was used to define the active site of PDE5A, and the radius of the input sphere was set to 10 Å from the center of the binding site. To determine the optimal parameters for a reliable docking method, the original inhibitors were extracted from the crystal structures (PDB ID: 4MD6) and redocked back into the crystal structure. The CDOCKER method embedded in Accelrys Discovery Studio 2.5.5 was suitable for PDE5A. Average RMSD values less than 1.0 Å for the bound ligand **1** between the original X-ray pose and the top 10 docking poses were desirable for 4MD6. Then, the identical parameters were used for the docking screening of the designed molecules. Fifty conformations were prepared for each molecule.

Molecular dynamics simulations. The protocol for the molecular dynamics simulations in this study is the same as that used in our previous study.[50, 51]

Statistical Analysis. All experiments were performed in triplicate and repeated at least twice. Representative data were selected to generate the figures. The significant difference between treatments

and controls was analyzed using Student's t-test. $p \leq 0.05$ was considered statistically significant.

ASSOCIATED CONTENT

Supporting Information Available

^{1}H NMR, ^{13}C NMR, high-resolution mass spectra (HRMS) data for tested compounds, and metabolic stability of compounds **1** and **17j**. This material is available free of charge via the Internet at http://pubs.acs.org.

AUTHOR INFORMATION

Corresponding Author

*To whom correspondence should be addressed. Phone: +86-20-39943031. Fax: +86-20-39943000. E-mail: luohb77@mail.sysu.edu.cn (H.-B. Luo) and 573016880@qq.com (J. Chen).

Author Contributions

# These authors contributed equally to this work.

ACKNOWLEDGEMENTS

This work was supported by the Natural Science Foundation of China (81522041, 21572279, 81373258, and 81602968), Science Foundation of Guangdong Province (2014A020210009 and 2016A030310144), and Guangdong Province Higher Vocational Colleges & Schools Pearl River Scholar Funded Scheme (2016). We wish to thank the Special Program for Applied Research on Super Computation of the NSFC-Guangdong Joint Fund (the second phase) under Grant No. U1501501 for providing supercomputing services. We also wish to thank Prof. H. Ke from the Department of Biochemistry and

Biophysics at the University of North Carolina, Chapel Hill, for his help with molecular cloning, expression, purification, crystal structure determination, and bioassaying the PDEs.

ABBREVIATIONS USED

AUC, area under the curve; cGMP, cyclic guanosine monophosphate; Cl_{app}, apparent clearance; Cl_h, hepatic clearance; Cl_{int}, intrinsic clearance; C_{max}, peak concentration; CYP, cytochrome P450s; DCC, Dicyclohexylcarbodiimide; DCM, dichloromethane; DIPEA, ethyldiisopropylamine; DMAP, 4-Dimethylaminopyridine; DMSO, dimethyl sulfoxide; E_h, hepatic extraction ratio; FDA, Food and Drug Administration; Fmoc-Cl, fluorenylmethoxycarbonyl chloride; HATU, O-(7-azabenzotriazol-1-yl)uronium hexafluorophosphate; hERG, the human *Ether-a-go-go*-Related Gene; MD, molecular dynamics; MRT, mean residence time; NADPH, Nicotinamide adenine dinucleotide phosphate; NBS, N-bromosuccinimide; PAH, Pulmonary arterial hypertension; PDB, protein data bank; PDE, phosphodiesterase; PDE5, phosphodiesterase 5; PO, oral administration; PVR, pulmonary vascular resistance; IV, intravenous administration; SAR, structure-activity relationship; SD rats, Sprague Dawley rats; $t_{1/2}$, half time; TFA, trifluoroacetic acid; THF, tetrahydrofuran; TLC, thin-layer chromatography; t_{max}, peak time; TMS, tetramethylsilane.

REFERENCES

1. Voelkel, N. F.; Quaife, R. A.; Leinwand, L. A.; Barst, R. J.; McGoon, M. D.; Meldrum, D. R.; Dupuis, J.; Long, C. S.; Rubin, L. J.; Smart, F. W.; Suzuki, Y. J.; Gladwin, M.; Denholm, E. M.; Gail, D. B. Right Ventricular Function and Failure. *Circulation* **2006**, *114*, 1883-1891.

2. McLaughlin, V. V.; Archer, S. L.; Badesch, D. B.; Barst, R. J.; Farber, H. W.; Lindner, J. R.; Mathier, M. A.; McGoon, M. D.; Park, M. H.; Rosenson, R. S.; Rubin, L. J.; Tapson, V. F.; Varga, J. ACCF/AHA 2009 Expert Consensus Document on Pulmonary Hypertension. *Circulation* **2009**, *119*, 2250-2294.

3. Ghofrani, H. A.; Wilkins, M. W.; Rich, S. Uncertainties in the Diagnosis and Treatment of Pulmonary Arterial Hypertension. *Circulation* **2008**, *118*, 1195-1201.

4. Thenappan, T.; Shah, S. J.; Rich, S.; Gomberg-Maitland, M. A USA-Based Registry for Pulmonary Arterial Hypertension: 1982–2006. *Eur. Respir. J.* **2007**, *30*, 1103-1110.

5. Raja, S. G.; Raja, S. M. Treating Pulmonary Arterial Hypertension: Current Treatments and Future Prospects. *Ther. Adv. Chronic Dis.* **2011**, *2*, 359-370.

6. Badesch, D. B.; Abman, S. H.; Simonneau, G.; Rubin, L. J.; McLaughlin, V. V. Medical Therapy for Pulmonary Arterial Hypertension: Updated ACCP Evidence-Based Clinical Practice Guidelines. *Chest* **2007**, *131*, 1917-1928.

7. Hamet, P.; Tremblay, J. Platelet cGMP-binding Phosphodiesterase. *Methods Enzymol.* **1988**, *159*, 710-722.

8. Francis, S. H.; Corbin, J. D. Purification of cGMP-Binding Protein Phosphodiesterase from Rat Lung. *Methods Enzymol.* **1988**, *159*, 722-729.

9. Wallis, R. M.; Corbin, J. D.; Francis, S. H.; Ellis, P. Tissue Distribution of Phosphodiesterase Families and the Eeffects of Sildenafil on Tissue Cyclic Nucleotides, Platelet Function, and the Contractile Responses of Trabeculae Carneae and Aortic Rings In Vitro. *Am. J. Cardiol.* **1999**, *83*, 3-12.

10. Lin, C. S. Tissue Expression, Distribution, and Regulation of PDE5. *Int. J. Impotence Res.* **2004**, *16*, S8-S10.

11. Kouvelas, D.; Goulas, A.; Papazisis, G.; Sardeli, C.; Pourzitaki, C. PDE5 Inhibitors: In Vitro and In Vivo Pharmacological Profile. *Curr. Pharm. Des.* **2009**, *15*, 3464-3475.

12. Mohamed, H. A.; Girgis, N. M. R.; Wilcken, R.; Bauer, M. R.; Tinsley, H. N.; Gary, B. D.; Piazza, G. A.; Boeckler, F. M.; Abadi, A. H. Synthesis and Molecular Modeling of Novel Tetrahydro-β-carboline Derivatives with Phosphodiesterase 5 Inhibitory and Anticancer Properties. *J. Med. Chem.* **2011**, *54*, 495-509.

13. Wang, G.; Liu, Z.; Chen, T.; Wang, Z.; Yang, H.; Zheng, M.; Ren, J.; Tian, G.; Yang, X.; Li, L.; Li, J.; Suo, J.; Zhang, R.; Jiang, X.; Terrett, N. K.; Shen, J.; Xu, Y.; Jiang, H. Design, Synthesis, and Pharmacological Evaluation of Monocyclic Pyrimidinones as Novel Inhibitors of PDE5. *J. Med. Chem.* **2012**, *55*, 10540-10550.

14. Wyatt, T. A.; Naftilan, A. J.; Francis, S. H.; Corbin, J. D. ANF Elicits Phosphorylation of the cGMP Phosphodiesterase in Vascular Smooth Muscle Cells. *Am. J. Physiol.* **1998**, *274*, H448-H455.

15. Giordano, D.; De Stefano, M. E.; Citro, G.; Modica, A.; Giorgi, M. Expression of cGMP-Binding cGMP-Specific Phosphodiesterase (PDE5) in Mouse Tissues and Cell Lines Using an Antibody Against the Eenzyme Aamino-Terminal Domain. *Biochim. Biophys. Acta, Mol. Cell Res.* **2001**, *1539*, 16-27.

16. Rawson, D. J.; Ballard, S.; Barber, C.; Barker, L.; Beaumont, K.; Bunnage, M.; Cole, S.; Corless, M.; Denton, S.; Ellis, D.; Floc'h, M.; Foster, L.; Gosset, J.; Holmwood, F.; Lane, C.; Leahy, D.; Mathias, J.; Maw, G.; Million, W.; Poinsard, C.; Price, J.; Russel, R.; Street, S.; Watson, L. The Discovery of UK-369003, a Novel PDE5 Inhibitor with the Potential for Oral Bioavailability and Dose-Proportional Pharmacokinetics. *Bioorg. Med. Chem.* **2012**, *20*, 498-509.

17. Oh, T. Y.; Kang, K. K.; Ahn, B. O.; Yoo, M.; Kim, W. B. Erectogenic Effect of the Selective Phosphodiesterase Type 5 Inhibitor, DA-8159. *Arch. Pharm. Res.* **2000**, *23*, 471-476.

18. Rotella, D. P. Phosphodiesterase 5 Inhibitors: Current Status and Potential Applications. *Nat. Rev. Drug Discovery* **2002**, *1*, 674-682.

19. Keating, G. M.; Scott, L. J. Vardenafil. *Drugs* **2003**, *63*, 2673-2702.

20. Jung, J. Y.; Kim, S. K.; Kim, B. S.; Lee, S. H.; Park, Y. S.; Kim, S. J.; Choi, C.; Yoon, S. I.; Kim, J. S.; Cho, S. D. The Penile Erection Efficacy of a New Phosphodiesterase Type 5 Inhibitor, Mirodenafil (SK3530), in Rabbits with Acute Spinal Cord Injury. *J. Vet. Med. Sci.* **2008**, *70*, 1199-204.

21. Kotera, J.; Mochida, H.; Inoue, H.; Noto, T.; Fujishige, K.; Sasaki, T.; Kobayashi, T.; Kojima, K.; Yee, S.; Yamada, Y. Avanafil, a Potent and Highly Selective Phosphodiesterase-5 Inhibitor for Erectile Dysfunction. *J. Urol.* **2012**, *188*, 668-674.

22. Galiè, N.; Ghofrani, H. A.; Torbicki, A.; Barst, R. J.; Rubin, L. J.; Badesch, D.; Fleming, T.; Parpia, T.; Burgess, G.; Branzi, A.; Grimminger, F.; Kurzyna, M.; Simonneau, G. Sildenafil Citrate Therapy for Pulmonary Arterial Hypertension. *New Engl. J. Med.* **2005**, *353*, 2148-2157.

23. Udeoji, D. U.; Schwarz, E. R. Tadalafil as Monotherapy and in Combination Regimens for the Treatment of Pulmonary Arterial Hypertension. *Ther. Adv. Respir. Dis.* **2012**, *7*, 39-49.

24. Rotella, D. P.; Sun, Z.; Zhu, Y.; Krupinski, J.; Pongrac, R.; Seliger, L.; Normandin, D.; Macor, J. E. Optimization of Substituted N-3-Benzylimidazoquinazolinone Sulfonamides as Potent and Selective PDE5 Inhibitors. *J. Med. Chem.* **2000**, *43*, 5037-5043.

25. Tollefson, M. B.; Acker, B. A.; Jacobsen, E. J.; Hughes, R. O.; Walker, J. K.; Fox, D. N. A.; Palmer, M. J.; Freeman, S. K.; Yu, Y.; Bond, B. R. 1-(2-(2,2,2-Trifluoroethoxy)ethyl-1H-pyrazolo[4,3-d]pyrimidines as Potent Phosphodiesterase 5 (PDE5) Inhibitors. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 3125-3128.

26. Wang, H.; Liu, Y.; Huai, Q.; Cai, J.; Zoraghi, R.; Francis, S. H.; Corbin, J. D.; Robinson, H.; Xin, Z.; Lin, G.; Ke, H. Multiple Conformations of Phosphodiesterase-5: Implications for Enzyme Function and Drug Development. *J. Biol. Chem.* **2006**, *281*, 21469-21479.

27. Palmer, M. J.; Bell, A. S.; Fox, D. N.; Brown, D. G. Design of Second Generation Phosphodiesterase 5 Inhibitors. *Curr. Top. Med. Chem.* **2007**, *7*, 405-419.

28. Schwartz, B. G.; Kloner, R. A. Drug Interactions with Phosphodiesterase-5 Inhibitors Used for the Treatment of Erectile Dysfunction or Pulmonary Hypertension. *Circulation* **2010**, *122*, 88-95.

29. Faris Azzouni, M. F.; Samra, K. A. Are Phosphodiesterase Type 5 Inhibitors Associated with Vision-Threatening Adverse Events? A Critical Analysis and Review of the Literature. *J. Sex. Med.* **2011**, *8*, 2894-2903.

30. Khan, A. S.; Sheikh, Z.; Khan, S.; Dwivedi, R.; Benjamin, E. Viagra Deafness—Sensorineural Hearing Loss and Phosphodiesterase-5 Inhibitors. *Laryngoscope* **2011**, *121*, 1049-1054.

31. (a) Shang, N.-N.; Shao, Y.-X.; Cai, Y.-H.; Guan, M.; Huang, M.; Cui, W.; He, L.; Yu, Y.-J.; Huang, L.; Li, Z.; Bu, X.-Z.; Ke, H.; Luo, H.-B. Discovery of 3-(4-Hydroxybenzyl)-1-(thiophen-2-yl)chromeno[2,3-c]pyrrol-9(2H)-one as a Phosphodiesterase-5 Inhibitor and Its Complex Crystal Structure. *Biochem. Pharmacol.* **2014**, *89*, 86-98. (b) Shao, Y.X.; Huang, M.; Cui, W.; Feng, L.J.; Wu, Y.; Cai, Y.; Li, Z.; Zhu, X.; Liu, P.; Wan, Y.; Ke, H.; Luo, H.-B. Discovery of a Phosphodiesterase-9A Inhibitor as a Potential Hypoglycemic Agent. *J. Med. Chem.* **2014**, ***57,*** 10304-130313.

32. Park, J. H.; Lee, S. U.; Kim, S. H.; Shin, S. Y.; Lee, J. Y.; Shin, C.-G.; Yoo, K. H.; Lee, Y. S. Chromone and Chromanone Derivatives as Strand Transfer Inhibitors of HIV-1 Integrase. *Arch. Pharm. Res.* **2008**, *31*, 1-5.

33. Yu, Y.; Hu, Y.; Shao, W.; Huang, J.; Zuo, Y.; Huo, Y.; An, L.; Du, J.; Bu, X. Synthesis of Multi-Functionalized Chromeno[2,3-c]pyrrol-9(2H)-ones: Investigation and Application of Baker–

Venkataraman Rearrangement Involved Reactions Catalyzed by 4-(Dimethylamino)pyridine. *Eur. J. Org. Chem.* **2011**, *2011*, 4551-4563.

34. Pierson, P. D.; Fettes, A.; Freichel, C.; Gatti-McArthur, S.; Hertel, C.; Huwyler, J.; Mohr, P.; Nakagawa, T.; Nettekoven, M.; Plancher, J.-M.; Raab, S.; Richter, H.; Roche, O.; Rodríguez Sarmiento, R. M.; Schmitt, M.; Schuler, F.; Takahashi, T.; Taylor, S.; Ullmer, C.; Wiegand, R. 5-Hydroxyindole-2-carboxylic Acid Amides: Novel Histamine-3 Receptor Inverse Agonists for the Treatment of Obesity. *J. Med. Chem.* **2009**, *52*, 3855-3868.

35. Li, C.; Dickson, H. D. A Mild, One-Pot Preparation of 1,3,4-Oxadiazoles. *Tetrahedron Lett.* **2009**, *50*, 6435-6439.

36. Tuley, A.; Wang, Y.-S.; Fang, X.; Kurra, Y.; Rezenom, Y. H.; Liu, W. R. The Genetic Incorporation of Thirteen Novel Non-Canonical Amino Acids. *Chem. Commun.* **2014**, *50*, 2673-2675.

37. Jørgensen, M. R.; Olsen, C. A.; Mellor, I. R.; Usherwood, P. N. R.; Witt, M.; Franzyk, H.; Jaroszewski, J. W. The Effects of Conformational Constraints and Steric Bulk in the Amino Acid Moiety of Philanthotoxins on AMPAR Antagonism. *J. Med. Chem.* **2005**, *48*, 56-70.

38. Borkin, D.; Pollock, J.; Kempinska, K.; Purohit, T.; Li, X.; Wen, B.; Zhao, T.; Miao, H.; Shukla, S.; He, M.; Sun, D.; Cierpicki, T.; Grembecka, J. Property Focused Structure-Based Optimization of Small Molecule Inhibitors of the Protein–Protein Interaction between Menin and Mixed Lineage Leukemia (MLL). *J. Med. Chem.* **2016**, *59*, 892-913.

39. An, P.; Yu, Z.; Lin, Q. Design and Synthesis of Laser-Activatable Tetrazoles for a Fast and Fluorogenic Red-Emitting 1,3-Dipolar Cycloaddition Reaction. *Org. Lett.* **2013**, *15*, 5496-5499.

40. Jonathan B. B.; Georgina A. H. New Substructure Filters for Removal of Pan Assay Interference Compounds (PAINS) from Screening Libraries and for Their Exclusion in Bioassays. *J. Med. Chem.* **2010**, *53*, 2719-2740.

41. Walker, D. K. Pharmacokinetics and Metabolism of Sildenafil in Mouse, Rat, Rabbit, Dog and Man. *Xenobiotica* **1999**, *29*, 297-310.

42. Wright, P. J. Comparison of Phosphodiesterase Type 5 (PDE5) Inhibitors. *Int. J. Clin. Pract.* **2006**, *60*, 967-975.

43. Guengerich, F. P. Role of Cytochrome P450 Enzymes in Drug-Drug Interactions. *Adv. Pharmacol*. **1997**, *43*, 7-35.

44. Zhou, S.; Gao, Y.; Jiang, W.; Huang, M.; Xu, A.; Paxton, J. W. Interactions of Herbs with Cytochrome P450. *Drug Metab. Rev.* **2003**, *35*, 35-98.

45. Sanguinetti, M. C.; Tristani-Firouzi, M. hERG Potassium Channels and Cardiac Aarrhythmia. *Nature* **2006**, *440*, 463-469.

46. Wang, H.; Yan, Z.; Yang, S.; Cai, J.; Robinson, H.; Ke, H. Kinetic and Structural Studies of Phosphodiesterase-8A and Implication on the Inhibitor Selectivity. *Biochemistry* **2008**, *47*, 12760-12768.

47. Sorota, S.; Zhang, X. S.; Margulis, M.; Tucker, K.; Priestley, T. Characterization of a hERG Screen Using the IonWorks HT: Comparison to a hERG Rubidium Efflux Screen. *Assay Drug Dev. Technol.* **2005**, *3*, 47-57.

48. Moody, G. C.; Griffin, S. J.; Mather, A. N.; McGinnity, D. F.; Riley, R. J. Fully Automated Analysis of Activities Catalysed by the Major Human Liver Cytochrome P450 (CYP) Enzymes: Assessment of Human CYP Inhibition Potential. *Xenobiotica* **1999**, *29*, 53-75.

49. Zhang, L.; Wei, M. J.; Zhao, C. Y.; Qi, H. M. Determination of the Inhibitory Potential of 6 Fluoroquinolones on CYP1A2 and CYP2C9 in Human Liver Microsomes. *Acta Pharmacol. Sin.* **2008**, *29*, 1507-1514.

50. Li, Z.; Wu, Y., Feng, L.J.; Wu, R.; Luo, H.-B. *Ab Initio* QM/MM Study Shows a Highly Dissociated

S_N2 Hydrolysis Mechanism for the cGMP-Specific Phosphodiesterase-5. *J. Chem. Theory Comput.* **2015**, *10*, 5448-5457.

51. Huang, Y.Y.; Li, Z.; Cai, Y.H.; Feng, L.J.; Wu, Y.; Li, X.; Luo, H.-B. The Molecular Basis for the Selectivity of Tadalafil toward Phosphodiesterase 5 and 6: A modeling Study. *J. Chem. Inf. Model.* **2013**, *53*, 3044-3053.

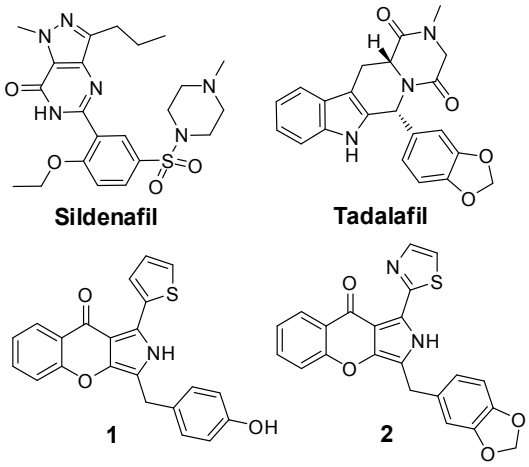


Figure 1. Chemical structures of PDE5 inhibitors.

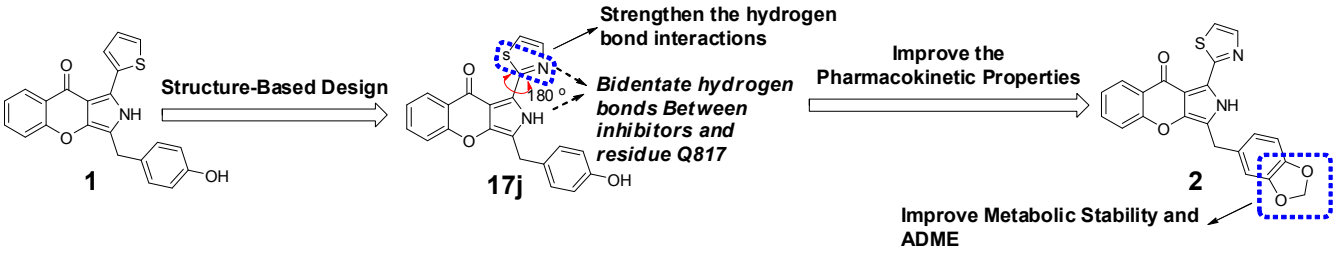


Figure 2. Structure-based design and optimization of chromeno[2,3-*c*]pyrrol-9(2*H*)-ones as novel PDE5 inhibitors with improved pharmacokinetic profiles.

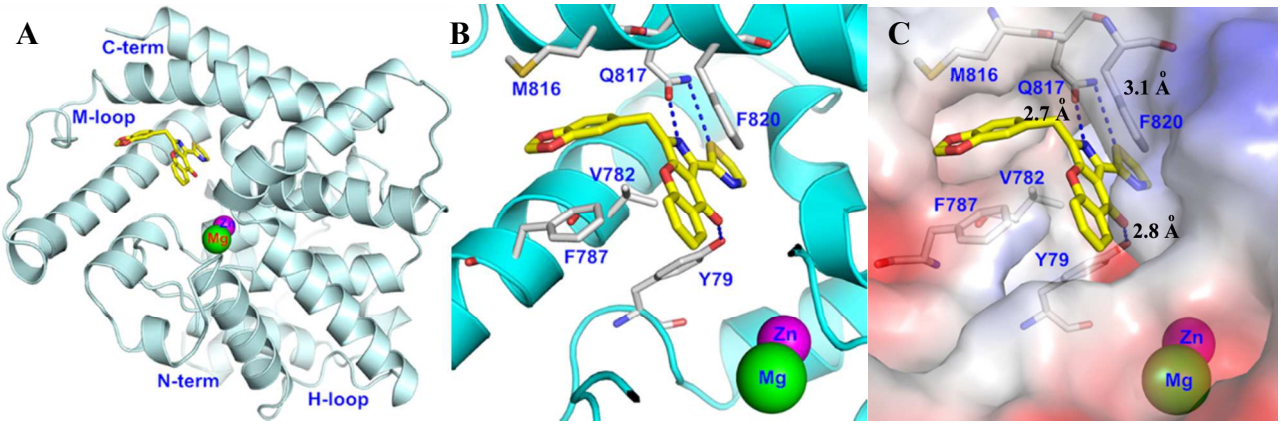


Figure 3. Binding of PDE5 in complex with **2** after 20 ns MD simulations. (A) and (B) Ribbon representation of the PDE5 catalytic domain in complex with **2** (yellow stick). (C) Surface model for compound **2** (yellow sticks) binding. The dotted lines represent hydrogen bonds.

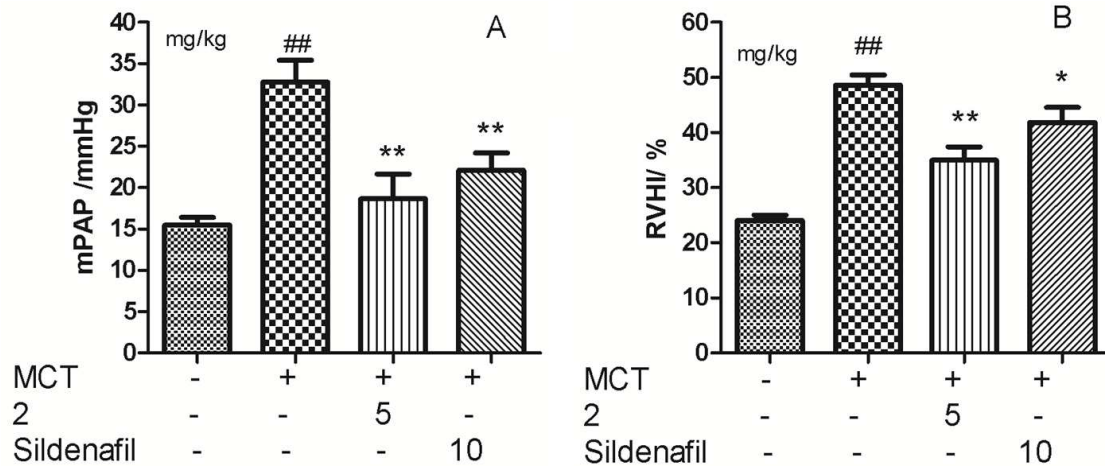


Figure 4. Effects of compound **2** and sildenafil citrate on the rats with PAH. (A) Effects of compound **2** and sildenafil on mPAP of the rats. (B) Effects of compound **2** and sildenafil on RVHI of the rats. The data are reported as the mean ± S.E.M. (n = 6 / group). ## p<0.01: compared to the control group (first column); * p<0.05, ** p<0.01: compared to the model group (second column).

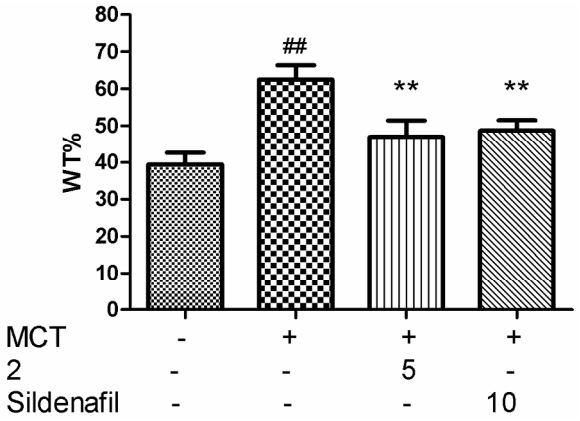
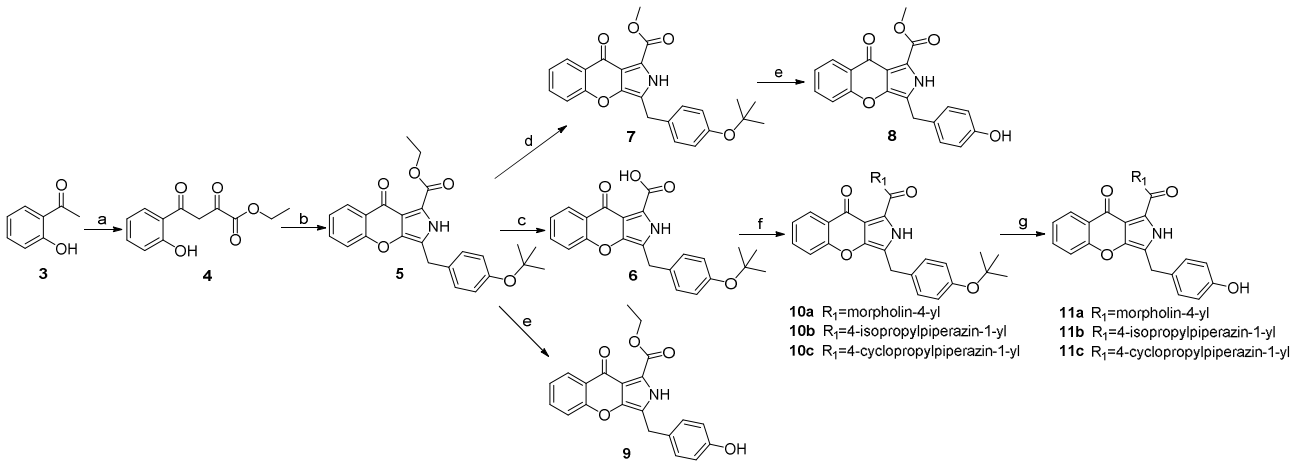


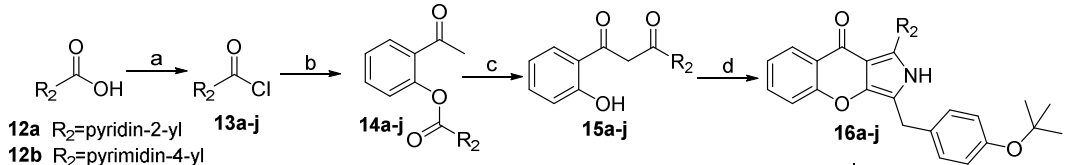
Figure 5. Effects of compound **2** (5 mg/kg) and sildenafil citrate (10 mg/kg) on the thickness of the small pulmonary arteries in the rats with PAH. Comparison of WT % between groups. ## p<0.01: compared to the control group (first column); **p<0.01: compared to the model group (second column).

Scheme 1. Syntheses of dihydrochromeno[2,3-*c*]pyrroles-1-carboxylates **8-9**, (1-carbonyl)chromeno [2,3-*c*]pyrrol-9(2*H*)-ones **11a-11c** [a]



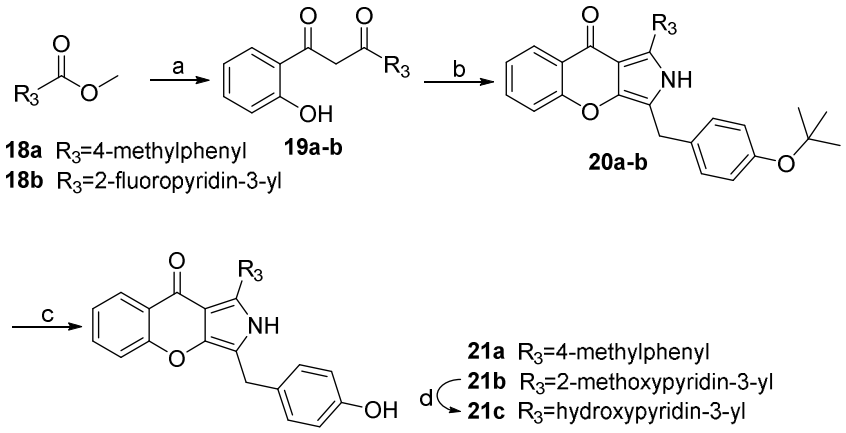
[a]Reagents and conditions: (a) diethyl oxalate, sodium hydride, toluene, 60 °C, 2 h; (b) Fmoc-O-tert-butyl-L-tyrosine, DCC, DMAP, pyridine, rt 3 h to 50°C 6 h; (c) KOH, THF, H_2O, 60 °C, 12 h; (d) MeOH, LiOH, rt, 12 h; (e) TFA, DCM, rt, 2 h; (f) amines, HATU, DIPEA, DCM, rt, 12 h; (g) TFA, DCM, rt, 2 h; (h) acethydrazide, HATU, DIPEA, DCM, rt, 12 h; (i) $POCl_3$, 60°C, 30 min.

Scheme 2. Syntheses of (1-aryl)chromeno[2,3-*c*]pyrrol-9(2*H*)-ones **17a-17j**[a]



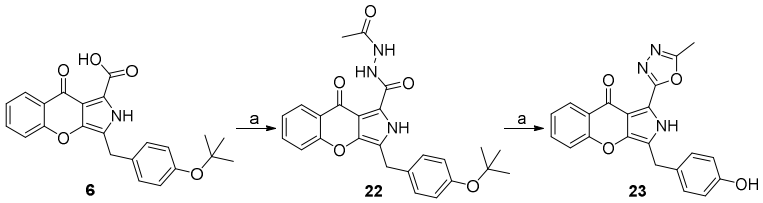
[a]Reagents and conditions: (a) $SOCl_2$, ref. 12 h; (b) **3**, pyridine, DCM, 0°C 0.5 h to rt 2 h; (c) t-BuOK, THF, rt, 12 h; (d) Fmoc-O-tert-butyl-L-tyrosine, DCC, DMAP, pyridine, rt 3 h to 50°C 6 h; (e) TFA, DCM, rt, 2 h.

Scheme 3. Syntheses of (1-Aryl)chromeno[2,3-*c*]pyrrol-9(2*H*)-ones **21a**-**21d**[a]



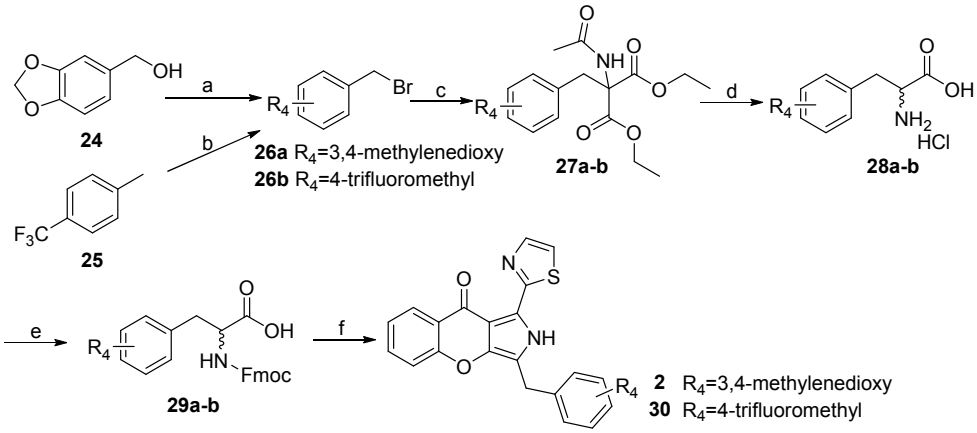
[a]Reagents and conditions: (a) **3**, NaH, toluene, 60°C, 2 h; (b) Fmoc-O-tert-butyl-L-tyrosine, DCC, DMAP, pyridine, rt 3 h to 50°C 6 h; (c) TFA, DCM, rt, 2 h; (d) BBr_3, DCM, -20°C, 2 h.

Scheme 4. Syntheses of 3-(4-hydroxybenzyl)-1-(5-methyl-1,3,4-oxadiazol-2-yl)chromeno[2,3-*c*]pyrrol-9(2*H*)-one **23**[a]



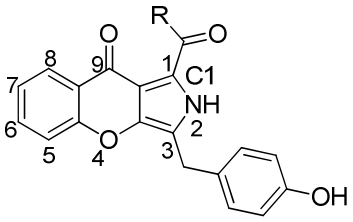
[a] Reagents and conditions: (a) acethydrazide, HATU, DIPEA, DCM, rt, 12 h; (b) $POCl_3$, 60°C, 30 min.

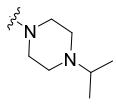
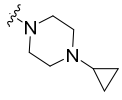
Scheme 5. Syntheses of 1-(Thiazol-2-yl)chromeno[2,3-*c*]pyrrol-9(2*H*)-ones **2** and **30**[a]



[a] Reagents and conditions: (a) PBr_3, DCM, rt, 4 h; (b) NBS, BPO, CCl_4, ref. 6 h; (c) diethyl acetamidomalonate, KI, K_2CO_3, CH_3CN, 80°C, 12 h; (d) 3 M HCl, H_2O, ref. 16 h; (e) FmocCl, Na_2CO_3, 1,4-dioxane, H_2O, 0°C 1 h, rt 4 h; (f) **15j**, DCC, DMAP, pyridine, rt 3 h to 50°C 6 h.

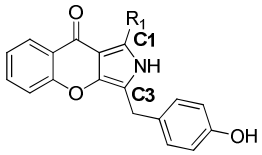
Table 1. SAR of Substituent Groups at the C1 Position of Carboxylates and Amides.



Compound	R	IC_{50} (nM)
8	OMe	31 ± 3
11a		> 100
11b		> 100
11c		> 100

***** Sildenafil citrate serves as the reference compound with an IC_{50} of 5.1 nM.

Table 2. SAR of Substituent Groups at the C1 position.



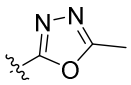
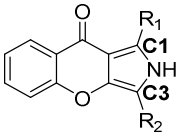
Compound	R_1	IC_{50} (nM)
17a	pyridin-2-yl	21 ± 2
17b	pyrimidin-4-yl	56 ± 6
17c	pyridin-3-yl	77 ± 8
17d	pyridin-4-yl	> 100
17e	4-chloropyridin-2-yl	> 100
17f	5-chloropyridin-2-yl	50 ± 8
17g	2-chloropyridin-3-yl	> 100
17h	3-chloropyridin-4-yl	> 100
17i	2-fluorophenyl	16 ± 2
17j	thiazol-2-yl	5.4 ± 0.7
21a	4-methylphenyl	> 100
21b	2-methoxypyridin-3-yl	> 100
21c	hydroxypyridin-3-yl	> 100
23		> 100

Table 3. SARs of Different Substitutions at the C3 Position.



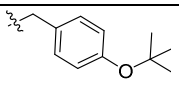
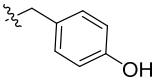
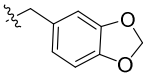
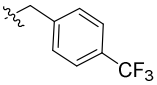
Compound	R_1	R_2	IC_{50} (nM)
16j	thiazol-2-yl		> 100
17j	thiazol-2-yl		5.4 ± 0.7
2	thiazol-2-yl		5.6 ± 0.3
30	thiazol-2-yl		30 ± 4

Table 4. Pharmacokinetic Profile of Compound 2 in Rats

	$t_{1/2}$	t_{max}	C_{max}	$AUC_{(0-t)}$	$AUC_{(0-\infty)}$	$MRT_{(0-t)}$	F（%）
	h	h	ng/mL	ng·h/mL	ng·h/mL	h	
PO	5.17±0.40	4.00±0.10	368±42	1997±164	2031±164	4.76±0.11	63.4±5.1
IV	6.02±0.12	-	2359±533	1703±94	1732±90	2.14±0.64	-

Table 5. Selectivity of Compound 2 across PDE Families

Proteins	IC_{50} (nM)	Selectivity index
PDE5A1 (535-860)	5.6 ± 0.3	-
PDE1B (10-487)	>10000	>1700
PDE2A (580-919)	362 ± 24	65
PDE3A (679-1087)	>10000	>1700
PDE4D2 (86-413)	2769 ± 440	494
PDE6A (484-817)	58 ± 7	10
PDE7A1 (130-482)	>10000	>1700
PDE8A1 (480-820)	6223 ± 884	1111
PDE9A2 (181-506)	>10000	>1786
PDE10A (449-770)	153 ± 25	27

Table 6. Metabolic Stability of 2 in Rat and Human Liver Microsomes

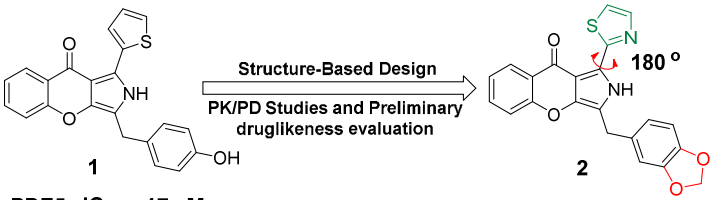
Compound		k	$t_{1/2}$ (min)	Cl_{int} (mL/min/mg)	Cl_{app} (mL/min/kg)	Cl_h (mL/min/kg)	E_h (%)
Midazolam*	rat	0.2877	2.4	0.5754	1035.66	53.23	95%
	human	0.2532	2.7	0.5064	488.27	19.21	96%
2	rat	0.0281	24.7	0.0561	101.00	35.61	65%
	human	0.0124	56.0	0.0248	23.89	10.89	54%

*, Midazolam was the positive control. Cl_{int}, intrinsic clearance, Cl_{app}: apparent clearance, Cl_h: hepatic clearance, and E_h: hepatic extraction ratio.

Table 7. Inhibition of Compound 2 against Seven Cytochrome P450s

	CYP1A2	CYP2B6	CYP2C8	CYP2C9	CYP2C19	CYP2D6	CYP3A4
IC_{50}	7.6 μM	>25 μM	>25 μM	>25 μM	> 20 μM	>25 μM	>25 μM

Table of Contents graphic



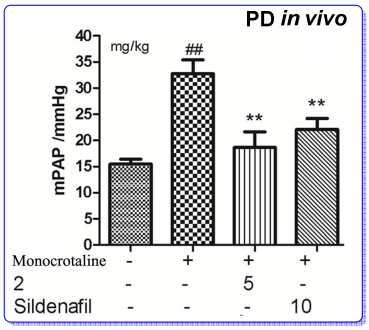
PDE5 IC_{50} = 17 nM

F = 4.9%

PDE5 $IC_{50} = 5.6 \pm 0.3$ nM

F = 63.4%, $t_{1/2}$ = 5.17 h (Oral)

hERG IC_{50} > 10 μM



CYP1A2 IC_{50} = 7.61μM; CYP2B6, 2C8, 2C9, 2C19, 2D6, 3A4 IC_{50} > 20 μM

Acute toxicity > 1.5 g/kg; Short-Term (2-Week) toxicity > 300 mg/kg/d

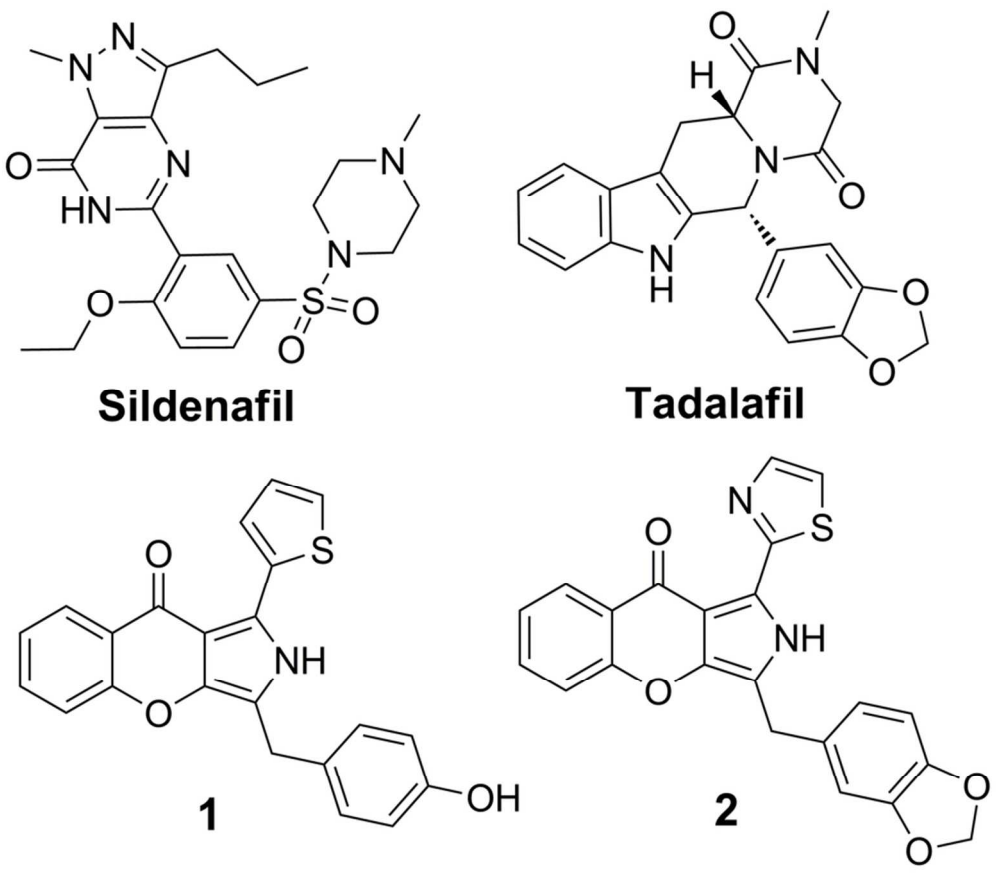


Figure 1. Chemical structures of PDE5 inhibitors.

82x71mm (300 x 300 DPI)

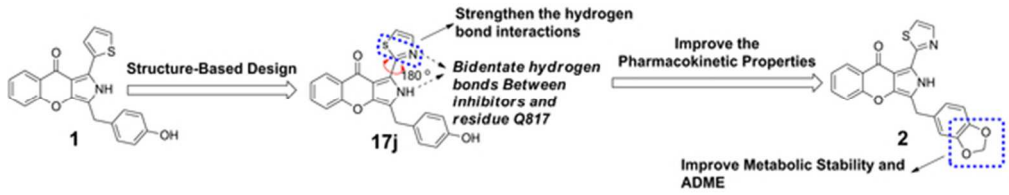
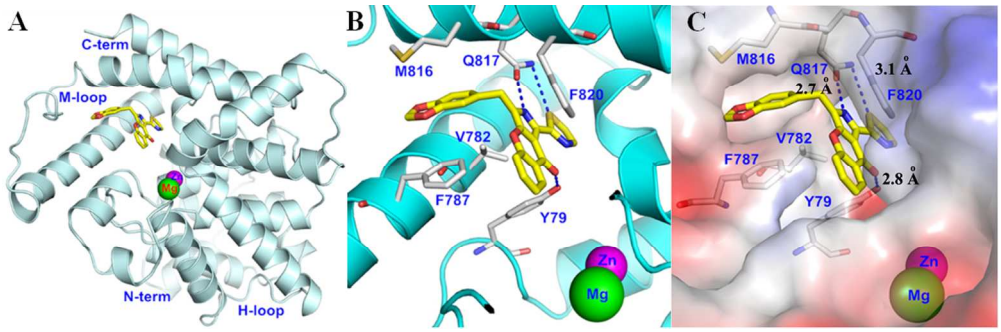


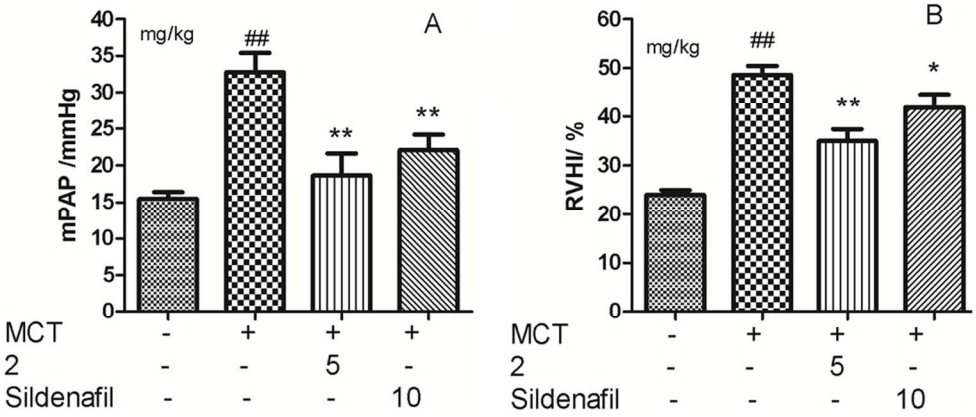
Figure 2. Structure-based design and optimization of chromeno[2,3-c]pyrrol-9(2H)-ones as novel PDE5 inhibitors with improved pharmacokinetic profile.

54x10mm (300 x 300 DPI)



Binding of PDE5 in complex with 2 after 20 ns MD simulations. (A) and (B) Ribbon representation of the PDE5 catalytic domain in complex with 2 (yellow stick). (C) Surface model for compound 2 (yellow sticks) binding. The dotted lines represent hydrogen bonds.

333x107mm (96 x 96 DPI)



Effects of compound 2 and sildenafil citrate on the rats with PAH. (A) Effects of compound 2 and sildenafil on mPAP of the rats. (B) Effects of compound 2 and sildenafil on RVHI of the rats. The data are reported as the mean ± S.E.M. (n = 6 / group). ## $p<0.01$: compared to the control group (first column); * $p<0.05$, ** $p<0.01$: compared to the model group (second column).

300x127mm (96 x 96 DPI)

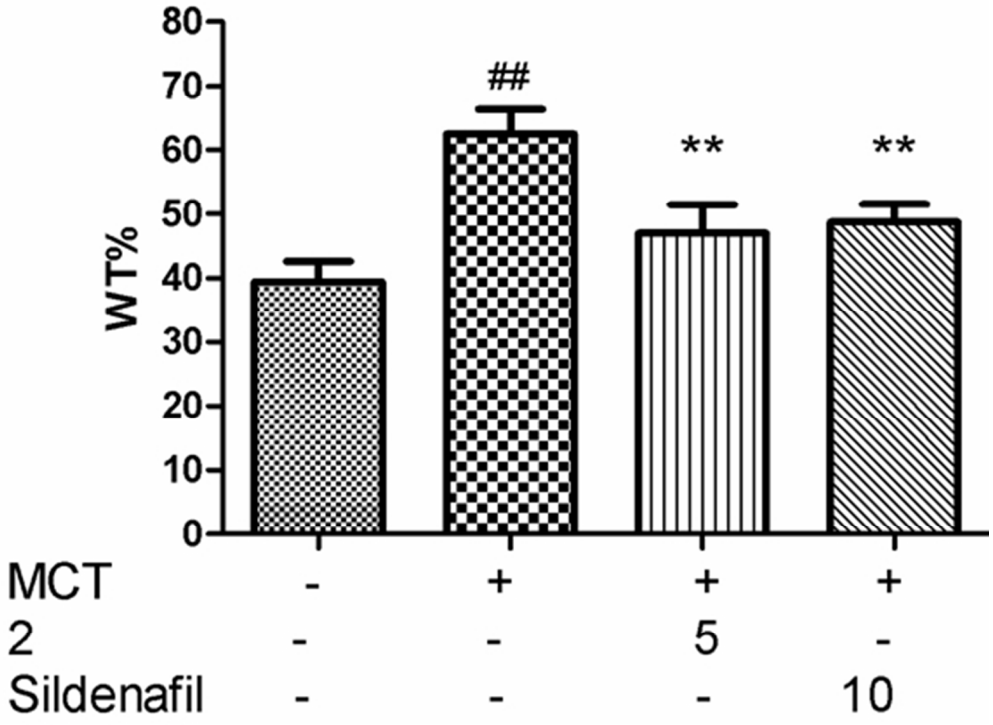


Figure 5. Effects of compound 2 (5 mg/kg) and sildenafil citrate (10 mg/kg) on the thickness of the small pulmonary arteries in the rats with PAH. Comparison of WT % between groups. ## $p<0.01$: compared to the control group (first column); **$p<0.01$: compared to the model group (second column).

152x114mm (96 x 96 DPI)