Published in final edited form as:

ACS Chem Neurosci. 2019 January 16; 10(1): 518–527. doi:10.1021/acschemneuro.8b00396.

Diarylureas Containing 5-Membered Heterocycles as CB_1 Receptor Allosteric Modulators: Design, Synthesis, and Pharmacological Evaluation

Thuy Nguyen[#1], **Thomas F. Gamage**[#1], **Ann M. Decker**[1], **Nadezhda German**[1], **Tiffany L. Langston**[1], **Charlotte E. Farquhar**[1], **Terry P. Kenakin**[2], **Jenny L. Wiley**[1], **Brian F. Thomas**[1], and **Yanan Zhang**[1,*]

[1]Research Triangle Institute, Research Triangle Park, North Carolina, United States

[2]Department of Pharmacology, University of North Carolina, Chapel Hill, North Carolina, United States

[#] These authors contributed equally to this work.

Abstract

Allosteric modulators have attracted significant interest as an alternate strategy to modulate CB_1 receptor signaling for therapeutic benefits that may avoid the adverse effects associated with orthosteric ligands. Here we extended our previous structure-activity relationship studies on the diarylurea-based CB_1 negative allosteric modulators (NAMs) by introducing five-membered heterocycles to replace the 5-pyrrolidinylpyridinyl group in PSNCBAM-1 (**1**), one of the first generation CB_1 allosteric modulators. Many of these compounds had comparable potency to **1** in blocking the CB_1 agonist CP55,940 stimulated calcium mobilization and [^{35}S]GTP-γ-S binding. Similar to **1**, most compounds showed positive cooperativity by increasing [^{3}H]CP55,940 binding, consistent with the positive allosteric modulator (PAM)-antagonist mechanism. Interestingly, these compounds exhibited differences in ability to increase specific binding of [^{3}H]CP55,940 and decrease binding of the antagonist [^{3}H]SR141716. In saturation binding studies, only increases in [^{3}H]CP55,940 B_{max}, but not K_d, were observed, suggesting that these compounds stabilize low affinity receptors into a high affinity state. Among the series, the 2-pyrrolyl analogue (**13**) exhibited greater potency than **1** in the [^{35}S]GTP-γ-S binding assay and significantly enhanced the maximum binding level in the [^{3}H]CP5,5940 binding assay, indicating greater CB_1 receptor affinity and/or cooperativity.

Graphical Abstract

[*]**Corresponding Author:** yzhang@rti.org. Phone: 919-541-1235. Fax: 919-541-6499.,.

Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

ASSOCIATED CONTENT

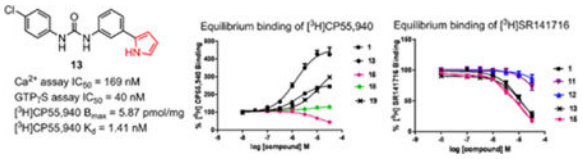
Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI:

HPLC analysis results of target compounds and agonist screens at CB_1 and CB_2 receptors.

Notes

The authors declare no competing financial interest.



Keywords

CB_1 receptor; diarylurea; allosteric modulators; five-membered heterocycles; structure-activity relationship; PSNCBAM-1

Introduction

The cannabinoid type-1 (CB_1) receptor is one of the most abundant G protein-coupled receptors (GPCRs) in the central nervous system (CNS) and has long been regarded as a promising target for the treatment of many conditions such as obesity, drug addiction, pain, inflammation, gastrointestinal diseases, multiple sclerosis, psychosis, schizophrenia, and osteoporosis.[1, 2] The CB_1 receptor antagonist/inverse agonist, rimonabant (SR141716), was previously approved in Europe for the treatment of obesity.[3] It also exhibited clinical benefits in smoking cessation,[4] and it attenuated drug seeking behaviors in animal studies,[5] supporting the notion that CB_1 antagonism may be useful as a treatment for drug addiction.[6–8] As rimonabant was subsequently withdrawn from the market due to its adverse effects, allosteric modulators have attracted attention as an alternative means to target the CB_1 signaling pathway for therapeutic benefits.[9–12]

PSNCBAM-1 (**1**) was one of the first CB_1 receptor allosteric modulators, identified from a high throughput screen in a yeast-based assay in 2007.[13] **1** shared similar in vitro pharmacological properties to another CB_1 allosteric modulator, Org27569 (**2**) such as enhancing [^{3}H]CP55,490 binding levels (positive allosteric modulation of agonist affinity) but inhibiting agonist-induced responses in functional assays (negative allosteric modulation of agonist function).[14, 15] Termed positive allosteric modulator-antagonists, or PAM-antagonists, these unique allosteric modulators increase the affinity of the agonist for the receptor but simultaneously decrease functional efficacy of the co-bound agonist. [16] As PAM-antagonists ‘seek and destroy’ agonist-activated receptors, they offer unique advantages in contrast to orthosteric and typical NAMs such as better reversal of ongoing persistent agonism and favorable target coverage in vivo.[16] In addition, unlike **2**, **1** alone did not show agonist activities in several assays tested such as ERK phosphorylation.[9, 14, 17] In vivo, **1** demonstrated efficacy in reducing food intake and body weight.[13] Recently, our group has demonstrated that **1** and an analogue with greater metabolic stability, RTICBM-74 (**3**), attenuated prime-induced reinstatement of extinguished cocaine-seeking behavior in rats.[18] Furthermore, we have shown that **1** attenuated tetrahydrocannabinol-induced anti-nociception, and its analogue RTICBM-28 (**4**) shifted the potency of THC in drug discrimination.[19] The promising results from these in vivo studies encourage further development on these diarylurea compounds for the potential use in the treatment of CB_1-related disorders (Fig. 1).

In previous studies, our group reported that the pyrrolidinyl ring of **1** could be replaced by other alkylamino groups (e.g. RTICBM-15, **5**) or abolished completely without compromising the CB_1 activity, and the entire 5-pyrrolidinylpyridinyl group could be replaced by substituted phenyl and pyridinyl rings.[18, 20] In agreement with our results, Khurana et al recently reported that analogues with a pyrimidinyl ring in place of the pyridinyl ring retained CB_1 modulatory activity (**6** and **7**, Fig. 1).[21] In order to further explore the structure-activity relationship (SAR) around this scaffold and develop potent and selective CB_1 NAMs as potential pharmacotherapies for treating CNS disorders, we designed and synthesized a series of analogues in which the 5-pyrrolidinylpyridinyl ring was replaced by other heterocyclic rings to introduce structural diversity (Fig. 2). Specifically, we have focused our effort on investigating the effects of substitution of various 5-membered heterocyclic rings in CB_1 receptor allostery, such as furan, thiophene, pyrrole, thiazole, and imidazole. These novel compounds were examined in calcium mobilization, [^{35}S]GTP-γ-S binding, [^{3}H]CP55,940 and [^{3}H]SR141716 radioligand binding assays to investigate their pharmacological properties as CB_1 allosteric modulators.

Results and Discussion

Chemistry.

Scheme 1 depicts the synthetic routes used to prepare target compounds **8**-**12** and **16**-**18**. For compounds **8**-**9**, commercially available 3-furanylboronic acid or 3-thienylboronic acid underwent Suzuki coupling with 3-bromoaniline to provide the intermediates **20a**-**b**, which reacted with 4-chlorophenyl isocyanate to afford the target compounds **8** and **9**. For target compounds **10**-**12** and **16**-**18**, the heterocyclic boronic acids were not commercially available and therefore the Suzuki coupling was carried out between the 3-nitrobenzene boronic acid and the corresponding heterocyclic bromide to give intermediates **21a**-**f**. The nitro group was then reduced to furnish the corresponding anilines **22a**-**f** by Raney-nickel catalyzed transfer hydrogenation using hydrazine in ethanol. Subsequently, anilines **22a**-**f** were coupled with 4-chlorophenyl isocyanates to give the desired products **10**-**12** and **16**-**18**.[18, 20]

Similarly, Suzuki coupling between *N*-Boc-2-pyrrole boronic acid and 1-bromo-3-nitrobenzene provided the intermediate **23**, which was then reduced to aniline **24** under transfer hydrogenation conditions. Intermediate **24** was then reacted with 4-chlorophenyl isocyanate to yield the final product **15**. Removal of the Boc group in **24** by refluxing in 5% w/v aqueous KOH solution gave the aniline **25**, which was then coupling with 4-chlorophenyl isocyanate to afford the target compound **13** (Scheme 2).[22] Notably, attempts to remove the Boc group of **24** under acidic conditions using acids such as 4N HCl in dioxane led to product degradation.

Palladium-catalyzed direct arylation of N-methylpyrrole with 3-iodoaniline gave the 3-(1-methyl-1*H*-pyrrol-2-yl)aniline **26**,[23] which was then converted to the final product **14** upon treatment with 4-chlorophenyl isocyanate (Scheme 3).

For the preparation of compound **19**, initial attempts of Suzuki coupling between 2-bromo-1*H*-imidazole and 3-nitrobenzene boronic acid failed to yield the desired product.

Hence, the imidazole ring was constructed by reaction between 3-nitrobenzonitrile and aminoacetaldehyde dimethyl acetal to give the intermediate **27**.[24] The target compound **19** was then obtained by the reduction of the nitro group leading to the aniline **28** and the subsequent reaction with 4-chlorophenyl isocyanate (Scheme 3).

Effects of compounds 8-19 in calcium mobilization assays.

All target compounds were first evaluated for their ability to inhibit the mobilization of intracellular calcium levels stimulated by CP55,940 in CHO cells overexpressing the promiscuous Gα16 protein (RD-HGA16 cells, Molecular Devices) and the human CB_1 or CB_2 receptor as previously described.[25] Table 1 lists the IC_{50} values of the synthesized compounds against the EC_{80} concentration of CP55,940 (100 nM) in the CB_1 calcium mobilization assay. **1** had an IC_{50} value of 33 nM. When the 5-pyrrolidinylpyridinyl group was replaced with five-membered heterocyclic rings such as furan, thiophene, or methylthiophene, the CB_1 modulatory potency was retained (**8**-**12**, IC_{50} = 36 - 67 nM). Replacement with pyrrole (**13**) or N-methylpyrrole (**14**) resulted in about 3-fold reduction in potencies (IC_{50} = 165-169 nM). The presence of the bulky Boc group in **15** significantly decreased the potency (IC_{50} > 3,000 nM). The three thiazolyl analogues had slightly decreased potency compared to **1** (**16**-**18**, IC_{50} = 84 - 154 nM). Lastly, the potency was dampened by around 16-fold in the presence of the more polar group, imidazolyl (**19**, IC_{50} = 529 nM). These results suggest that the CB_1 receptor can accommodate a diversity of heterocylic rings at this position.

None of the compounds showed significant CB_2 antagonist activity (< 50% inhibition of CP55,940 EC_{80} concentration at 10 μM or IC_{50} values >10 μM) (Table 1). All compounds were additionally tested for agonist activity at both CB_1 and CB_2 receptors. No significant CB_1 agonist activity (< 30% of CP55,940 E_{max}) or CB_2 agonist activity (< 30% of CP55,940 E_{max}) at concentrations of 10 μM was observed (Supporting Information).

Effects of diarylureas in [^{35}S]GTP-γ-S binding assay.

All compounds were then evaluated as NAMs at the CB_1 receptor in the [^{35}S]GTP-γ-S assay using the CB_1 agonist CP55,940 (Table 1). While 3-furanyl (**8**) showed similar potency in the calcium assay to **1**, it was ~4 fold less potent in the GTP-γ-S assay. The 3-thiophenyl analogue (**9**, IC_{50} = 164 nM) had comparable potency to **1** (IC_{50} = 115 nM), and the 2-thiophenyl analogue (**10**) was slightly weaker (IC_{50} = 573 nM). Introduction of a methyl group resulted in a 5-fold reduction in inhibitory potency in the 3-thiophenyl analogue (**11**), whereas the potency slightly increased in the 2-thiophenyl analogue (**12** vs. **10**). The lower potencies of both *N*-methylpyrrolyl (**14**) and *N*-Boc pyrrolyl (**15**) analogues were consistent with the results from the calcium assay. Similarly, the three thiazolyl analogues (**16**-**18**) shared the same trend in activities in both assays.

In general, the compounds were more potent in the calcium assay than in GTP-γ-S. However, the pyrrolyl analogue (**13**, IC_{50} = 40 nM) appeared to be more potent than **1** in the GTP-γ-S binding assay even though it was weaker in the calcium assay. Similarly, the imidazolyl analogue (**19**) demonstrated better activity in the GTP-γ-S binding assay than the calcium assay. This difference in the potency ranking order between the two assays could

result from the differences in affinity for human versus mouse CB_1 receptor and/or G protein subtype coupling involved in the response (e.g. overexpression of $G\alpha_{16}$ in the calcium assay).

Effects of diarylureas on [^{3}H]CP55,940 radioligand binding.

Effects of representative allosteric modulators on equilibrium binding of 1 nM [^{3}H]CP55,940 and 1 nM [^{3}H]SR141716 are depicted in Figure 3. The curve-fit values of all target compounds are listed in Table 2.

Similar to previously reported CB_1 allosteric modulators such as **1**, most analogues increased specific binding of [^{3}H]CP55,940, demonstrating positive cooperativity with the agonist radioligand. At the highest tested concentration of 10 μM, the 3-thiophenyl analogue (**9**) had relatively similar maximal increases in specific [^{3}H]CP55,940 binding as compared to **1**, whereas the 2-thiophenyl analogue (**10**) only exhibited approximately half the increase in specific binding. The 3-furanyl analogue (**8**) exhibited a similar increase in specific [^{3}H]CP55,940 binding as compared to the 2-thiophenyl analogue (**10**), suggesting that both the position and nature of heteroatom are important for imparting cooperativity. Introduction of a methyl at the 5 position of the 3-thiophenyl (**11**) or 2-thiophenyl analogue (**12**) resulted in a slight lower increase in [^{3}H]CP55,940 binding level compared to **9**. Within the pyrrolyl series, the unsubstituted 2-pyrrolyl analogue (**13**, maximum binding level: 458%) increased the [^{3}H]CP55,90 binding level more than 2-fold compared to **1** (maximum binding level: 256%), whereas *N*-methylpyrrol-2-yl (**14**) only slightly enhanced the level of radioligand binding. Interestingly, the *N*-Boc-2-pyrrolyl (**15**) significantly decreased the binding of the radioligand.

The trend of cooperativity in agonist binding positively correlates with their potency in the GTP-γ-S binding assay ($r(13) = 0.65$, $p<0.05$). For instance, **13** was the most potent compound of the series in the GTP-γ-S assay (IC_{50} = 40 nM), and it also increased the maximal [^{3}H]CP55,940 binding to the largest extent (458%). In contrast, consistent with its low activity in both the calcium and GTP-γ-S assays, **15** displayed no increase in [^{3}H]CP55,940 binding but instead decreased the maximal level.

Among the thiazolyl analogues (**16**-**18**), the 4-thiazolyl analogue (**16**) increased the radioligand binding level nearly as much as **1**. However, the positive effect was diminished in the case of the 5-thiazolyl (**17**), and markedly reduced in case of the 2-thiazolyl (**18**). The correlation between [^{3}H]CP55,940 binding maximum level and potency in the GTP-γ-S binding assays seen in the pyrrole series was also observed in the thiazole series. On the other hand, the imidazolyl analogue (**19**, maximum binding level 355%) demonstrated stronger binding cooperativity with [^{3}H]CP55,940 yet was less potent in the GTP-γ-S binding assay compared to **1**, which may reflect a lower affinity (e.g. pEC_{50} value appeared right shifted in comparison) despite greater cooperativity.

Effects of diarylureas on [^{3}H]SR141716 radioligand binding.

As opposed to the [^{3}H]CP55,940 binding studies where most compounds increase radioligand binding, most of the analogues reduced [^{3}H]SR141716 binding in a similar

fashion to previously reported CB_1 allosteric modulators **1** and **2**.[13, 26] These results are consistent with the PAM-antagonist mechanism.[16] Cannabinoid agonists exhibit two distinct affinities for the CB_1 receptor,[27] which reflect two receptor states, one coupled to the cognate G protein (active) and the other uncoupled (inactive). Some reports suggest that allosteric regulation by G proteins does not affect the affinity of the CB_1 antagonist/inverse agonist SR141716 for the receptor, i.e. it binds with equal affinity to either receptor state,[28, 29] and that SR141716 may sequester G proteins in a nonsignaling complex; however, Bouaboula et al. reported an increase in SR141716 affinity for the CB_1 receptor when it is uncoupled from G protein by coincubation with GTP-γ-S,[30] an effect that has been reported for other inverse agonists of G protein coupled receptors.[31] Therefore, an increase in affinity of the modulator for the agonist high-affinity receptor state (as reflected by the ability of the modulator to increase binding of [^{3}H]CP55,940) would also result in an increase in the ability of the modulator to reduce SR141716 binding. In other words, if SR141716 has a higher affinity for receptors inactive/uncoupled from G protein, then structural changes that increase the affinity of the modulator for the agonist high-affinity conformation will result in a concomitant increase in the modulator's ability to reduce [^{3}H]SR141716 binding and vice versa, since the distribution of receptor states would be shifted towards those with which SR141716 has higher affinity.

Interestingly, the 5-methylthiophenyl analogues (**11** and **12**) only exhibited a slight reduction in [^{3}H]SR141716 binding that was significantly less than observed in the rest of the series. However, as discussed above, both **11** and **12** displayed a similar ability relative to **1** in increasing [^{3}H]CP55,940 binding. It has suggested that the binding pocket for the structurally similar CB_1 allosteric modulator Org27569 overlaps with that of SR141716.[32] Thus, it is possible that the binding site for these allosteric modulators also partially overlaps with that of SR141716, with the methyl group interfering with the ability of the modulator to occupy SR141716's binding site. Alternatively, it could be that **11** and **12** have lower affinity for receptors uncoupled from their cognate G protein, for which SR141716 may have higher affinity.

Notably, calculated pIC_{50} values from [^{35}S]GTP-γ-S binding studies were positively correlated with pEC_{50} values from [^{3}H]CP55,940 equilibrium binding ($r(13) = 0.65$, $p<0.05$) (Table 2) but not pIC_{50} values from displacement binding of [^{3}H]SR141716 ($r(13) = 0.06$, $p=0.82$). This suggests that affinity for the agonist-bound conformation, but not the antagonist, predicts the allosteric modulator's inhibitory potency, which is consistent with positive cooperativity between the modulator and the probe agonist (PAM-antagonist).[19]

Effects of diarylureas on [^{3}H]CP55,940 saturation binding.

Saturation binding experiments were conducted in the absence and presence (10 μM) of a subset of synthesized modulators to determine their effects on [^{3}H]CP55,940's B_{max} and K_d values. Under the vehicle condition, [^{3}H]CP55,940 exhibited a B_{max} of 1.56 ± 0.09 pmol/mg, which was increased by co-incubation with each allosteric modulator (Table 3) as previously reported for **1**.[14] Compounds **11** and **17** exhibited comparable B_{max} and K_d values to **1**. Intriguingly, **13**, which exhibited a larger increase in specific [^{3}H]CP55,940 binding than PSNCBAM-1 (Table 2), also produced a larger B_{max} in saturation experiments

(Table 3). Additionally, **8**, which exhibited a smaller increase in [^{3}H]CP55,940 binding as compared to **1** (Table 2), also resulted in a lower B_{max} for [^{3}H]CP55,940 in saturation binding (Table 3). In contrast, K_d values for [^{3}H]CP55,940 were not significantly affected by the presence of the allosteric modulators. Considering the low range of concentrations for [^{3}H]CP55,940, it is likely the B_{max} values observed in these saturation binding plots reflect the population of high affinity receptors that are coupled to the G proteins, as CP55,940 exhibits a K_i in the 100 nM range for the uncoupled low affinity receptor.[33] Thus, the increase in B_{max} following co-incubation with allosteric modulators reflects an increase in affinity of the non-coupled receptors resulting in a B_{max} shift rather than a K_d shift. These results combined with the elevated B_{max} values suggest that these compounds are not modulating high-affinity binding but rather altering the equilibrium of low- and high-affinity receptors. The enhanced B_{max} values and unchanged K_d values of these diarylureas correspond to the same trend observed for Org27569 previously.[14] Therefore, differences in maximal increases observed in specific [^{3}H]CP55,940 binding [Table 2; Figure 3A] between allosteric modulators likely reflect the proportion of low affinity receptors shifted into a high affinity state.

Conclusions

In our previous study, we demonstrated that the 5-pyrrolidinylpyridinyl of **1** could be replaced by substituted phenyl and pyridinyl groups and modifications at this position led to CB_1 allosteric modulators with better metabolic stability and greater potency in vivo.[18] This study further elucidates the structural requirements at the place of 5-pyrrolidinylpyridinyl group of **1**. We showed that various five-membered heterocyclic rings could be accommodated at this position, resulting in the generation of interesting pharmacological activities at the CB_1 receptor. Replacement by furanyl, thiophenyl, or 5-methylthiophenyl groups (**8**-**12**) maintained similar allosteric modulating potency in the calcium mobilization assay against human CB_1 receptor. Notably, modification to 2-pyrrolyl group (**13**) significantly enhanced the potency in the GTP-γ-S assay, doubled the [^{3}H]CP55,940 binding level in the competitive binding assay and markedly increased B_{max} in the [^{3}H]CP55,940 saturation binding assay. These results indicate that **13** had greater affinity and/or cooperativity at the CB_1 receptor. The fact that K_d values in the [^{3}H]CP55,940 saturation binding assay remained unchanged suggested that the these compounds modulated the CB_1 receptor by stabilizing agonist low-affinity receptors into an agonist high-affinity state. In the competitive [^{3}H]SR141716 binding assay, while most synthesized diarylureas retained the ability to reduce SR141716 binding to CB_1 receptor as expected for PAM-antagonists, the 5-methylthiophenyl analogues (**11** and **12**) only slightly reduced [^{3}H]SR141716 binding, possibly due to the partially overlapping binding pockets.[32]

A number of CB_1 NAMs have been reported and their in vitro pharmacological properties have been thoroughly characterized to date, most of which appear to be PAM-antagonists. Since PAM-antagonists and agonists increase the other's binding affinity to the receptor, this class of NAMs "seek out and destroy" agonist-bound receptor complexes, therefore providing the unique benefit of reversing ongoing persistent agonism and resulting in favorable in vivo target coverage.[16] Despite these, the translation of in vitro to in vivo effects has not been well established with these CB_1 NAMs. For instance, the subtle attenuation of

THC-induced anti-nociception by **1** and the small reduction in THC's potency by **4** in drug discrimination assays were not seen with their structurally-related analogue **5**.[19] This may be attributed to pharmacokinetic profiles of these allosteric modulators or slower association kinetics commonly observed of allosteric modulators compared to the orthosteric ligands,[34] leading to less pronounced in vivo effects. Indeed, we have demonstrated that **3**, a more metabolically stable analogue of **1**, produced a more pronounced effect in attenuating reinstatement of cocaine self-administration.[18] By replacing the 5-pyrrolidinylpyridinyl group of **1** with a diversity of five-membered heterocycles, this work illustrated that structural modification at this position may allow further fine-tuning of pharmacological properties to achieve desired therapeutic effects. Continued SAR studies and optimization effort by improving potency and pharmacokinetic properties and/or modifying receptor binding kinetics may lead to CB_1 allosteric modulators as improved in vivo probes and potential clinical therapeutics.

EXPERIMENTAL SECTION

Chemistry.

All solvents and chemicals were reagent grade. Unless otherwise mentioned, all reagents and solvents were purchased from commercial vendors and used as received. Flash column chromatography was carried out on a Teledyne ISCO CombiFlash Rf system using prepacked columns. Solvents used include hexanes, ethyl acetate (EtOAc), dichloromethane, and methanol. Purity and characterization of compounds were established by a combination of HPLC, TLC, mass spectrometry, and NMR analyses. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker Avance DPX-300 (300 MHz) spectrometer and were determined in $CDCl_3$, DMSO-d6, or CD_3OD with tetramethylsilane (TMS) (0.00 ppm) or solvent peaks as the internal reference. Chemical shifts are reported in ppm relative to the reference signal, and coupling constant (J) values are reported in hertz (Hz). Thin layer chromatography (TLC) was performed on EMD precoated silica gel 60 F254 plates, and spots were visualized with UV light or iodine staining. Nominal mass spectra were obtained using a Waters Alliance HT/Micromass ZQ system (ESI). High resolution mass spectra were obtained using Agilent 1290 Infinity UHPLC-6230 TOF system (ESI). All final compounds were greater than 95% pure as determined by HPLC on an Agilent 1100 system using an Agilent Zorbax SB-Phenyl, 2.1 mm × 150 mm, 5 μm column using a 15 minute gradient elution of 5-95% solvent B at 1 mL/min followed by 10 minutes at 95% solvent B (solvent A, water with 0.1% TFA; solvent B, acetonitrile with 0.1% TFA and 5% water; absorbance monitored at 220 and 280 nm).

General procedure A.

To a mixture of aryl bromide or aryl iodide (1 eq), boronic acid (1.1 eq) in dimethoxyethane (0.1 M) was added 1M aqueous $NaHCO_3$ solution (3 eq) followed by $Pd(Ph_3)_4$ (0.075 eq). The reaction mixture was refluxed overnight under nitrogen atmosphere. The reaction mixture was diluted with ethyl acetate, washed with a saturated $NaHCO_3$ solution and brine. The combined organic layers were dried over anhydrous $MgSO_4$ and filtered. The filtrate was concentrated in vacuo and the residue was purified by column chromatography (SiO_2, ethyl acetate/hexanes) to give the desired product.

3-(Furan-3-yl)aniline (20a) was prepared from 3-bromoaniline (0.19 ml, 1.74 mmol) and (furan-3-yl)boronic acid (0.21 g, 1.91 mmol) following the general procedure A as white solid (0.09 g, 32%). 1H NMR (300 MHz, $CDCl_3$) δ 7.66 - 7.71 (m, 1H), 7.45 (t, J = 1.70 Hz, 1H), 7.12 - 7.20 (m, 1H), 6.90 (td, J = 1.30, 7.58 Hz, 1H), 6.81 (t, J = 1.88 Hz, 1H), 6.66 (d, J = 1.13 Hz, 1H), 6.60 (ddd, J = 0.94, 2.45, 7.91 Hz, 1H), 3.69 (br. s., 2H). MS (ESI) m/z for $C_{10}H_9NO$ [M+H]+: calcd: 160.1; found: 160.1.

3-(Thiophen-3-yl)aniline (20b) was prepared from 3-bromoaniline (0.19 ml, 1.74 mmol) and (thiophen-3-yl)boronic acid (0.25 g, 1.91 mmol) following the general procedure A as white solid (0.03 g, 10%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.38 - 7.42 (m, 1H), 7.32 - 7.37 (m, 2H), 7.15 - 7.22 (m, 1H), 7.00 (td, *J* = 1.25, 7.68 Hz, 1H), 6.92 (t, *J* = 1.88 Hz, 1H), 6.63 (ddd, *J* = 0.94, 2.26, 7.91 Hz, 1H), 3.71 (br. s., 2H). MS (ESI) *m/z* for $C_{10}H_9NS$ $[M+H]^+$: calcd: 176.1; found: 176.3.

2-(3-Nitrophenyl)thiophene (21a) was prepared from 2-bromothiophene (0.10 ml, 1.00 mmol) and 3-nitrophenylboronic acid (0.18 g, 1.10 mmol) following the general procedure A as yellow solid (0.18 g, 92%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.42 (t, *J* = 1.88 Hz, 1H), 8.06 - 8.13 (m, 1H), 7.89 (d, *J* = 7.72 Hz, 1H), 7.53 (t, *J* = 8.01 Hz, 1H), 7.42 (dd, *J* = 0.85, 3.67 Hz, 1H), 7.37 (dd, *J* = 0.75, 5.09 Hz, 1H), 7.12 (dd, *J* = 3.77, 5.09 Hz, 1H).

2-Methyl-4-(3-nitrophenyl)thiophene (21b) was prepared from 4-bromo-2-methylthiophene (0.10 ml, 1.00 mmol) and 3-nitrophenylboronic acid (0.18 g, 1.10 mmol) following the general procedure A as yellow solid (0.09 g, 42%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.37 (t, *J* = 1.88 Hz, 1H), 8.09 (td, *J* = 1.06, 8.24 Hz, 1H), 7.85 (d, *J* = 7.72 Hz, 1H), 7.52 (t, *J* = 8.01 Hz, 1H), 7.32 (d, *J* = 1.51 Hz, 1H), 7.08 (s, 1H), 2.54 (s, 1H).

2-Methyl-5-(3-nitrophenyl)thiophene (21c) was prepared from 4-bromo-2-methylthiophene (0.10 ml, 1.00 mmol) and 3-nitrophenylboronic acid (0.18 g, 1.10 mmol) following the general procedure A as yellow solid (0.09 g, 42%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.36 (t, *J* = 1.98 Hz, 1H), 8.05 (ddd, *J* = 0.94, 2.12, 8.24 Hz, 1H), 7.79 - 7.84 (m, 1H), 7.50 (t, *J* = 8.01 Hz, 1H), 7.22 (d, *J* = 3.58 Hz, 1H), 6.75 - 6.79 (m, 1H), 2.50 - 2.56 (m, 3H).

4-(3-Nitrophenyl)-1,3-thiazole (21d) was prepared from 4-bromo-1,3-thiazole (0.09 ml, 1.00 mmol) and 3-nitrophenylboronic acid (0.18 g, 1.10 mmol) following the general procedure A as yellow solid (0.12 g, 58%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.93 (d, *J* = 1.88 Hz, 1H), 8.78 (t, *J* = 1.98 Hz, 1H), 8.26 - 8.31 (m, 1H), 8.17 - 8.22 (m, 1H), 7.72 (d, *J* = 2.07 Hz, 1H), 7.62 (t, *J* = 8.01 Hz, 1H).

5-(3-Nitrophenyl)-1,3-thiazole (21e) was prepared from 5-bromo-1,3-thiazole (0.09 ml, 1.00 mmol) and 3-nitrophenylboronic acid (0.18 g, 1.10 mmol) following the general procedure A as yellow solid (0.10 g, 46%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.86 (s, 1H), 8.43 (t, *J* = 1.88 Hz, 1H), 8.17 - 8.24 (m, 2H), 7.88 - 7.94 (m, 1H), 7.58 - 7.66 (m, 1H).

2-(3-Nitrophenyl)-1,3-thiazole (21f) was prepared from 4-bromo-1,3-thiazole (0.09 ml, 1.00 mmol) and 3-nitrophenylboronic acid (0.18 g, 1.10 mmol) following the general

procedure A as yellow solid (0.08 g, 37%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.80 (t, *J* = 1.88 Hz, 1H), 8.24 - 8.32 (m, 1H), 7.87 - 7.96 (m, 2H), 7.43 - 7.47 (m, 2H).

***tert*-Butyl 2-(3-nitrophenyl)-1*H*-pyrrole-1-carboxylate (23)** was prepared from 1-bromo-3-nitrobenzene (0.20 g, 1 mmol) and *N*-Boc-2-pyrroleboronic acid (0.23 g, 1.1 mmol) following the general procedure A as yellow solid (0.15 g, 51%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.12 - 8.28 (m, 2H), 7.69 (d, *J* = 6.22 Hz, 1H), 7.47 - 7.58 (m, 1H), 7.36 - 7.44 (m, 1H), 6.22 - 6.35 (m, 2H), 1.40 (s, 9H).

2-(3-Nitrophenyl)-1*H*-imidazole (27). To a solution of 3-nitrobenzonitrile (0.50 g, 3.37 mmol) in anhydrous methanol (17 ml) was added sodium methoxide (0.18 g, 3.37 mmol). The reaction mixture was stirred at room temperature for 5 h. Acid acetic (0.39 ml, 6.82 mmol) and aminoacetaldehyde dimethyl acetal (0.37 ml, 3.37 mmol) were then added and the reaction mixture was heated at 70 °C with stirring for 1 h. After cooling to room temperature, methanol (2.25 ml) and 6N aqueous HCl (1.7 ml) solution were added to the reaction mixture. The reaction temperature was subsequently raised to 70 °C for 1 h. After cooling to room temperature, the solvent was removed under reduced pressure. Saturated aqueous potassium carbonate was added slowly until pH 8-10. The desired product precipitated and was collected by filtration as white solid (0.39 g, 61%). ^{1}H NMR (300 MHz, CD_3OD) δ 7.21 - 7.27 (m, 1H), 6.66 - 6.77 (m, 2H), 6.13 - 6.24 (m, 1H), 5.64 - 5.73 (m, 2H). MS (ESI) *m/z* for $C_9H_7N_3O_2$ $[M+H]^+$: calcd: 190.1; found: 190.3.

General procedure B.

To a solution of nitrobenzene derivative (1 eq) in ethanol (0.1 M) was added hydrazine hydrate (15 eq). The reaction was stirred at 50 °C for 15 min and an excess of Raney nickel slurry in water (1.2 eq) was added slowly. After 1 h, the bubbling ceased, the mixture was cooled to room temperature and filtered through Celite. The filtrate was condensed under reduced pressured and the residue was either used for the next step without purification or purified by column chromatography (SiO_2, ethyl acetate/hexanes) to afford the desired product.

3-(Thiophen-2-yl)aniline (22a) was prepared from **21a** (0.18 g, 0.89 mmol) following the general procedure B as yellow solid (0.10 g, 64%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.20 - 7.29 (m, 2H), 7.10 - 7.18 (m, 1H), 6.98 - 7.08 (m, 2H), 6.92 (s, 1H), 6.59 (dd, *J* = 2.07, 7.91 Hz, 1H), 3.69 (br. s., 2H). MS (ESI) *m/z* for $C_{12}H_9Cl_2N$ $[M+H]^+$: calcd: 238.0; found: 238.1.

3-(5-Methylthiophen-3-yl)aniline (22b) was prepared from **21b** (0.09 g, 0.42 mmol) following the general procedure B as white solid (0.08 g, quant.). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.12 - 7.17 (m, 2H), 6.93 - 7.02 (m, 2H), 6.87 (d, *J* = 1.88 Hz, 1H), 6.59 (dd, *J* = 1.51, 7.91 Hz, 1H), 3.68 (br. s., 2H), 2.50 (s, 3H). MS (ESI) *m/z* for $C_{11}H_{11}NS$ $[M+H]^+$: calcd: 190.1; found: 190.2.

3-(5-Methylthiophen-2-yl)aniline (22c) was prepared from **21c** (0.09 g, 0.42 mmol) following the general procedure B as colorless liquid (0.08 g, quant.). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.09 - 7.16 (m, 1H), 7.05 (d, *J* = 3.39 Hz, 1H), 6.93 - 6.99 (m, 1H), 6.86 (t, *J* =

1.88 Hz, 1H), 6.67 - 6.72 (m, 1H), 6.56 (ddd, *J* = 0.94, 2.26, 7.91 Hz, 1H), 3.65 (br. s., 2H), 2.48 (s, 2H). MS (ESI) *m/z* for $C_{11}H_{11}NS$ $[M+H]^+$: calcd: 190.1; found: 190.3.

3-(1,3-Thiazol-4-yl)aniline (22d) was prepared from **21d** (0.12 g, 0.58 mmol) following the general procedure B as colorless liquid (0.06 g, 61%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.86 (d, *J* = 1.88 Hz, 1H), 7.48 (d, *J* = 1.88 Hz, 1H), 7.26 - 7.35 (m, 2H), 6.69 (dd, *J* = 1.22, 2.35 Hz, 1H), 6.23 (dd, *J* = 2.26, 7.91 Hz, 1H), 3.71 (br. s., 2H). MS (ESI) *m/z* for $C_9H_8N_2S$ $[M+H]^+$: calcd: 177.1; found: 177.5.

3-(1,3-Thiazol-5-yl)aniline (22e) was prepared from **21e** (0.09 g, 0.46 mmol) following the general procedure B as colorless liquid (0.06 g, 73%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.72 (s, 1H), 8.03 (s, 1H), 7.13 - 7.23 (m, 1H), 6.97 (dd, *J* = 0.94, 7.72 Hz, 1H), 6.88 (d, *J* = 3.77 Hz, 1H), 6.66 (td, *J* = 1.06, 8.05 Hz, 1H), 3.80 (br. s., 2H). MS (ESI) *m/z* for $C_9H_8N_2S$ $[M+H]^+$: calcd: 177.1; found: 177.4.

3-(1,3-Thiazol-2-yl)aniline (22f) was prepared from **21f** (0.08 g, 0.37 mmol) following the general procedure B as colorless liquid (0.03 g, 46%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.84 (d, *J* = 3.39 Hz, 1H), 7.29 - 7.35 (m, 3H), 7.22 (t, *J* = 8.01 Hz, 1H), 6.72 - 6.77 (m, 1H), 3.80 (br. s., 2H). MS (ESI) *m/z* for $C_9H_8N_2S$ $[M+H]^+$: calcd: 177.1; found: 177.3.

***tert*-Butyl 2-(3-aminophenyl)-1*H*-pyrrole-1-carboxylate (24)** was prepared from **23** (0.147 g, 0.51 mmol) following the general procedure B as white solid (0.09 g, 65%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.21 - 7.37 (m, 1H), 7.06 - 7.18 (m, 1H), 6.70 - 6.78 (m, 1H), 6.57 - 6.68 (m, 2H), 6.18 (d, *J* = 10.74 Hz, 2H), 3.63 (br. s., 2H), 1.37 (s, 9H). MS (ESI) *m/z* for $C_{15}H_{18}N_2O_2$ $[M+H]^+$: calcd: 259.1; found: 259.5.

3-(1*H*-Pyrrol-2-yl)aniline (25). A solution of **24** (0.06 g, 0.23 mmol) in 5% aqueous potassium hydroxide (23 ml) was refluxed for 4 h. After cooling to room temperature, the mixture was poured into water and extracted with dichloromethane. The organic phase was dried with anhydrous magnesium sulfate, filtered, concentrated in vacuo to the desired product as white solid (0.03 g, 78%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.41 (br. s., 1H), 7.05 - 7.22 (m, 1H), 6.73 - 6.94 (m, 3H), 6.40 - 6.60 (m, 2H), 6.28 (s, 1H), 3.67 (br. s., 2H). MS (ESI) *m/z* for $C_{10}H_{10}N_2$ $[M+H]^+$: calcd: 159.1; found: 159.2.

3-(1-Methyl-1*H*-pyrrol-2-yl)aniline (26). To a solution of 3-iodoaniline (0.24 ml, 2 mmol) in N,N-dimethylacetamide (8 ml) in a sealed tube was added N-methylpyrrole (0.36 ml, 8 mmol), potassium acetate (0.39 g, 8 mmol), and palladium (II) acetate (0.005 g, 0.02 mmol). The reaction mixture was stirred at 150 °C for 20 h. The reaction mixture was then diluted with ethyl acetate, washed three times with water and once with brine. The organic layer was dried with anhydrous magnesium sulfate, filtered, and concentrated under reduced pressure. The residue was purified by column chromatography (SiO_2, ethyl acetate/ hexanes) to give the desired product was yellow liquid (0.08 g, 22%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.17 (t, *J* = 7.82 Hz, 1H), 6.76 - 6.81 (m, 1H), 6.69 (td, *J* = 2.10, 8.24 Hz, 2H), 6.61 (ddd, *J* = 0.75, 2.31, 8.05 Hz, 1H), 6.16 - 6.21 (m, 2H), 3.68 (br. s., 2H), 3.64 (s, 3H). MS (ESI) *m/z* for $C_{11}H_{12}N_2$ $[M+H]^+$: calcd: 173.1; found: 173.5.

3-(1*H*-Imidazol-2-yl)aniline (28) was prepared from **27** (0.30 g, 1.58 mmol) following the general procedure B as white solid (0.25 g, quant.). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.05 - 7.23 (m, 5H), 6.69 (d, *J* = 8.10 Hz, 1H), 3.74 (br. s., 2H). MS (ESI) *m/z* for $C_9H_9N_3$ $[M+H]^+$: calcd: 160.1; found: 160.2.

General procedure C.

To a solution of aryl amine (1 eq) in anhydrous chloroform (0.04 M) was added 4-chlorophenyl isocyanate (1eq) at room temperature. The reaction mixture was then heated at 60 °C for 16 h. The precipitated product was filtered and thoroughly washed with dichloromethane.

3-(4-Chlorophenyl)-1-[3-(furan-3-yl)phenyl]urea (8) was prepared from **20a** (0.03 g, 0.16 mmol) following the general procedure C as white solid (0.05 g, 95%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.86 (s, 1H), 8.73 (s, 1H), 8.12 (s, 1H), 7.75 (t, *J* = 1.70 Hz, 1H), 7.65 (s, 1H), 7.47 - 7.52 (m, 2H), 7.29 - 7.36 (m, 4H), 7.20 - 7.28 (m, 1H), 6.88 (d, *J* = 0.94 Hz, 1H). HRMS (ESI) *m/z* for $C_{17}H_{13}ClN_2O_2$ $[M+H]^+$: calcd: 313.0738; found: 313.0744.

3-(4-Chlorophenyl)-1-[3-(thiophen-3-yl)phenyl]urea (9) was prepared from **20b** (0.03 g, 0.15 mmol) following the general procedure C as white solid (0.03 g, 63%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.85 (s, 1H), 8.76 (s, 1H), 7.75 - 7.79 (m, 2H), 7.63 (dd, *J* = 2.92, 4.99 Hz, 1H), 7.45 - 7.51 (m, 3H), 7.30 - 7.36 (m, 5H). HRMS (ESI) *m/z* for $C_{17}H_{13}ClN_2OS$ $[M+H]^+$: calcd: 329.0510; found: 329.0504.

3-(4-Chlorophenyl)-1-[3-(thiophen-2-yl)phenyl]urea (10) was prepared from **22a** (0.10 g, 0.67 mmol) following the general procedure C as white solid (0.13 g, 57%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.84 (s, 2H), 7.82 (s, 1H), 7.55 (d, *J* = 5.09 Hz, 1H), 7.50 (d, *J* = 8.85 Hz, 2H), 7.44 - 7.47 (m, 1H), 7.28 - 7.36 (m, 5H), 7.14 (dd, *J* = 3.67, 4.99 Hz, 1H). HRMS (ESI) *m/z* for $C_{17}H_{13}ClN_2OS$ $[M+H]^+$: calcd: 329.0510; found: 329.0508.

3-(4-Chlorophenyl)-1-[3-(5-methylthiophen-3-yl)phenyl]urea (11) was prepared from **22b** (0.03 g, 0.17 mmol) following the general procedure C as white solid (0.04 g, 70%). ^{1}H NMR (300 MHz, CD_3OD) δ 7.73 (br. s., 1H), 7.61 (d, *J* = 7.54 Hz, 1H), 7.39 - 7.48 (m, 4H), 7.32 - 7.38 (m, 2H), 7.24 - 7.31 (m, 4H), 2.51 (s, 3H). HRMS (ESI) *m/z* for $C_{18}H_{15}ClN_2OS$ $[M+H]^+$: calcd: 343.0666; found: 343.0666.

3-(4-Chlorophenyl)-1-[3-(5-methylthiophen-2-yl)phenyl]urea (12) was prepared from **22c** (0.04 g, 0.19 mmol) following the general procedure C as white solid (0.05 g, 77%). ^{1}H NMR (300 MHz, CD_3OD) δ 7.71 (s, 1H), 7.44 (d, *J* = 8.85 Hz, 2H), 7.21 - 7.32 (m, 5H), 7.16 (d, *J* = 3.58 Hz, 1H), 6.74 (d, *J* = 2.64 Hz, 1H), 2.49 (s, 3H). HRMS (ESI) *m/z* for $C_{18}H_{15}ClN_2OS$ $[M+H]^+$: calcd: 343.0666; found: 343.0665.

3-(4-Chlorophenyl)-1-[3-(1H-pyrrol-2-yl)phenyl]urea (13) was prepared from **25** (0.03 g, 0.18 mmol) following the general procedure C as white solid (0.05 g, 81%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 11.26 (br. s., 1H), 8.87 (br. s., 1H), 8.67 (br. s., 1H), 7.67 (s, 1H), 7.47 - 7.56 (m, 2H), 7.30 - 7.39 (m, *J* = 5.70 Hz, 2H), 7.22 - 7.29 (m, 3H), 6.84 (s, 1H), 6.44 (s, 1H), 6.12 (s, 1H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 152.5, 139.8, 138.7, 133.6, 131.1,

129.0, 128.6, 125.3, 119.7, 119.3, 117.5, 115.9, 113.7, 109.0, 105.5. HRMS (ESI) *m/z* for $C_{17}H_{14}ClN_3O$ $[M+H]^+$: calcd: 312.0898; found: 312.0894.

3-(4-Chlorophenyl)-1-[3-(1-methyl-1*H*-pyrrol-2-yl)phenyl]urea (14) was prepared from **26** (0.08 g, 0.45 mmol) following the general procedure C as white solid (0.14 g, 98%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.86 (s, 1H), 8.77 (s, 1H), 7.56 (s, 1H), 7.50 (d, *J* = 8.85 Hz, 2H), 7.30 - 7.36 (m, 4H), 7.03 - 7.08 (m, 1H), 6.83 (t, *J* = 2.17 Hz, 1H), 6.15 (dd, *J* = 1.88, 3.58 Hz, 1H), 6.04 - 6.08 (m, 1H), 3.66 (s, 3H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 152.5, 139.6, 138.6, 133.4, 128.8, 128.6, 125.4, 124.3, 121.6, 119.8, 117.8, 116.6, 108.3, 107.3, 34.9. HRMS (ESI) *m/z* for $C_{18}H_{16}ClN_3O$ $[M+H]^+$: calcd: 326.1055; found: 326.1049.

***tert*-Butyl 2-[3-({1-[(4-chlorophenyl)amino]ethenyl}amino)phenyl]-1*H*-pyrrole-1-carboxylate (15)** was prepared from **24** (0.03 g, 0.10 mmol) following the general procedure C as white solid (0.04 g, 84%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.83 (s, 1H), 8.75 (s, 1H), 7.46 - 7.51 (m, 2H), 7.31 - 7.39 (m, 3H), 7.23 - 7.30 (m, 1H), 6.94 (d, *J* = 7.54 Hz, 1H), 6.26 - 6.30 (m, 1H), 6.22 - 6.26 (m, 1H), 1.31 (s, 9H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 152.4, 148.8, 138.9, 138.6, 134.4, 134.2, 128.6, 128.0, 125.3, 122.5, 119.7, 118.6, 117.0, 114.1, 110.7, 83.5, 27.1. HRMS (ESI) *m/z* for $C_{22}H_{22}ClN_3O_3$ $[M+H]^+$: calcd: 412.1422; found: 412.1423.

3-(4-Chlorophenyl)-1-[3-(1,3-thiazol-4-yl)phenyl]urea (16) was prepared from **22d** (0.03 g, 0.19 mmol) following the general procedure C as white solid (0.05 g, 74%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.20 (d, *J* = 1.88 Hz, 1H), 8.85 (s, 2H), 8.12 - 8.15 (m, 1H), 8.11 (d, *J* = 1.88 Hz, 1H), 7.59 (d, *J* = 7.35 Hz, 1H), 7.48 - 7.53 (m, 2H), 7.31 - 7.45 (m, 5H). HRMS (ESI) *m/z* for $C_{16}H_{12}ClN_3OS$ $[M+H]^+$: calcd: 330.0462; found: 330.0460.

3-(4-Chlorophenyl)-1-[3-(1,3-thiazol-5-yl)phenyl]urea (17) was prepared from **21e** (0.03 g, 0.16 mmol) following the general procedure C as white solid (0.04 g, 66%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.09 (s, 1H), 8.88 (s, 2H), 8.26 (s, 1H), 7.83 (s, 1H), 7.50 (d, *J* = 8.85 Hz, 2H), 7.30 - 7.41 (m, 5H). HRMS (ESI) *m/z* for $C_{16}H_{12}ClN_3OS$ $[M+H]^+$: calcd: 330.0462; found: 330.0454.

3-(4-Chlorophenyl)-1-[3-(1,3-thiazol-2-yl)phenyl]urea (18) was prepared from **22f** (0.03 g, 0.16 mmol) following the general procedure C as white solid (0.04 g, 72%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.96 (s, 1H), 8.87 (s, 1H), 8.22 (s, 1H), 7.93 (d, *J* = 3.20 Hz, 1H), 7.80 (d, *J* = 3.01 Hz, 1H), 7.48 - 7.59 (m, 3H), 7.40 - 7.47 (m, 2H), 7.30 - 7.39 (m, 2H). HRMS (ESI) *m/z* for $C_{16}H_{12}ClN_3OS$ $[M+H]^+$: calcd: 330.0462; found: 330.0464.

3-(4-Chlorophenyl)-1-[3-(1*H*-imidazol-2-yl)phenyl]urea (19) was prepared from **28** (0.13 g, 0.82 mmol) following the general procedure C as white solid (0.22 g, 86%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.52 (br. s., 1H), 8.84 (d, *J* = 11.30 Hz, 2H), 8.07 (br. s., 1H), 7.52 (br. s., 3H), 7.44 (br. s., 1H), 7.34 (d, *J* = 7.16 Hz, 3H), 7.03 - 7.20 (m, 2H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 152.4, 145.5, 139.8, 138.7, 131.4, 129.1, 128.6, 125.4, 119.7, 118.6, 118.0, 115.1. HRMS (ESI) *m/z* for $C_{16}H_{13}ClN_4O$ $[M+H]^+$: calcd: 313.0851; found: 313.0846.

Calcium Mobilization Assay.

CHO-RD-HGA16 cells (Molecular Devices, CA) stably expressing the human CB_1 receptor were plated into 96-well black-walled assay plates at 25,000 cells/well in 100 μL of Ham's F12 (supplemented with 10% fetal bovine serum, 100 units of penicillin/streptomycin, and 100 μg/mL Normocin) and incubated overnight at 37 °C, 5% CO_2. Calcium 5 dye (Molecular Devices, CA) was reconstituted according to the manufacturer's instructions. The reconstituted dye was diluted 1:40 in prewarmed (37 °C) assay buffer (1x HBSS, 20 mM HEPES, 2.5 mM probenecid, pH 7.4 at 37 °C). Growth medium was removed, and the cells were gently washed with 100 μL of prewarmed (37 °C) assay buffer. The cells were incubated for 45 min at 37 °C, 5% CO_2 in 200 μL of the diluted Calcium 5 dye solution. For antagonist assays to determine IC_{50} values, the EC_{80} concentration of CP55,940 was prepared at 10x the desired final concentration in 0.25% BSA/0.5% DMSO/0.5% EtOH/ assay buffer, aliquoted into 96-well polypropylene plates, and warmed to 37 °C. Serial dilutions of the test compounds were prepared at 10x the desired final concentration in 2.25% BSA/4.5% DMSO/4.5% EtOH/assay buffer. After the dye loading incubation period, the cells were pretreated with 25 μL of the test compound serial dilutions and incubated for 15 min at 37 °C. After the pretreatment incubation period, the plate was read with a FLIPR Tetra (Molecular Devices, CA). Calcium-mediated changes in fluorescence were monitored every 1 s over a 90 s time period, with the Tetra adding 25 μL of the CP55,940 EC_{80} concentration at the 10s time point (excitation/emission: 485/525 nm). Relative fluorescence units (RFU) were plotted against the log of compound concentrations. For agonist screens, the above procedure was followed except that cells were pretreated with 2.25% BSA/4.5% DMSO/4.5% EtOH/assay buffer and the Tetra added single concentration dilutions of the test compounds prepared at 10x the desired final concentration in 0.25% BSA/0.5% DMSO/ 0.5% EtOH/assay buffer. Test compound RFUs were compared to the CP55,940 E_{max} RFUs to generate % E_{max} values. For the CB_2 agonist and antagonist assays, the same procedures were followed except that stable human CB_2-CHO-RD-HGA16 cells were used.

Membrane preparation.

Cerebella from male ICR mice (6-8 weeks old; Enviga International, Indianapolis, IN) were dissected on ice, flash frozen in liquid N_2 and stored at −80°C until the day of the experiment. On the day of the experiment, cerebella were mechanically homogenized (Brinkmann Polytron) in membrane buffer (50 mM Tris, 3 mM MgCl2, 0.2 mM EGTA, 100 mM NaCl, pH 7.4) on ice, and then centrifuged for 10 min at 40,000 *g* at 4°C. The supernatant was discarded and the pellet was suspended in membrane buffer, homogenized, and centrifuged again for 10 min at 40,000 *g*. The pellet was resuspended in membrane buffer and protein quantified by Bradford method.

Radioligand Binding.

For equilibrium radioligand binding, reactions were carried out in assay buffer (membrane buffer containing 1 mg/ml bovine serum albumin; BSA) and initiated by addition of cerebellar membranes (10 μg protein) and incubated for 90 min at 30°C. For all radioligand binding experiments, non-specific binding was determined by excess cold ligand (1 μM). Total bound of radioligand was less than 10% of total added (minimal ligand depletion). For

competition curves, either 1 nM [^{3}H]SR141716 (NIDA Drug Supply Program, Bethesda, MD) or 1 nM [^{3}H]CP55,940 (NIDA) were incubated with multiple concentrations of the allosteric modulators. For Saturation binding of [^{3}H]CP55,940, reactions were carried out by incubating the radioligand at nominal concentrations of 0.032, 0.1, 0.32, 0.56, 1, 3.2, and 5.6 nM with a fixed concentration (10 μM) of allosteric modulator. Radioligand binding reactions were terminated by vacuum filtration through Perkin Elmer GF/C 96 well filter plates followed by 3 rinses of wash buffer.

Agonist-stimulated [^{35}S]GTPγS binding.

For receptor signaling, membranes (10 μg protein) were preincubated in assay buffer for 10 min with 3 units/ml adenosine deaminase then incubated for 60 min at 30°C with 30 μM GDP and 0.1 nM [^{35}S]GTPγS (Perkin Elmer Life Sciences, Boston, MA). Non-specific binding was determined by adding 30 μM unlabeled GTPγS. Concentration response curves for allosteric modulators were conducted in the presence of CP55,940 (100 nM) to calculate IC_{50} values.

Data Analysis

For calcium mobilization experiments, data were fit to a three-parameter logistic curve to generate IC_{50} values (GraphPad Prism 6.0, CA). For [^{35}S]GTP-γ-S experiments, data were normalized to maximal CP55,940 (100 nM) stimulation in the absence of test compound (i.e., vehicle = 100%). Curve fits were accomplished using GraphPad Prism 6.0 and data were fit to three-parameter nonlinear regression, with bottom and top constrained to >0 and = 100, respectively, for IC_{50} calculation. For equilibrium radioligand binding data, concentration response curves for the allosteric modulators using a single fixed concentration of radioligand were fit to 3 parameter non-linear regression for determination of EC_{50} and curve top ([^{3}H]CP55,940 binding) and IC_{50} ([^{3}H]SR141716 binding). For saturation binding, data were fit to a one-site specific binding using GraphPad Prism 6.0 to calculate [^{3}H]CP55,940's B_{max} and K_d.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Funding Sources

This work was supported by National Institute on Drug Abuse, National Institutes of Health, U.S. (Grants DA040693 to Y.Z., DA040460 to B.F.T., DA03672 to J.L.W., and DA045752 to T.F.G.) and National Natural Science Foundation of China (81473089).

ABBREVIATIONS

CB_1	cannabinoid 1 receptor
CB_2	cannabinoid 2 receptor
CNS	central nervous system

FLIPR	fluorometric imaging plate reader
GPCR	G-protein-coupled receptor
HPLC	high performance liquid chromatography
IC_{50}	half-maximum inhibitory concentration
MS	mass spectrometry
NAM	negative allosteric modulator
NMR	nuclear magnetic resonance
PAM-Antagonist	positive allosteric modulator - Antagonist
SAR	structure–activity relationship
TLC	thin-layer chromatography

References

(1). Mackie K (2006) Cannabinoid receptors as therapeutic targets. Annu. Rev. Pharmacol. Toxicol. 46, 101–122. [PubMed: 16402900]

(2). Pertwee RG (2005) The therapeutic potential of drugs that target cannabinoid receptors or modulate the tissue levels or actions of endocannabinoids. AAPS J. 7, E625–654. [PubMed: 16353941]

(3). Pi-Sunyer FX, Aronne LJ, Heshmati HM, Devin J, Rosenstock J, and Group RI-NAS (2006) Effect of rimonabant, a cannabinoid-1 receptor blocker, on weight and cardiometabolic risk factors in overweight or obese patients: RIO-North America: a randomized controlled trial. JAMA 295, 761–775. [PubMed: 16478899]

(4). Cahill K, and Ussher MH (2011) Cannabinoid type 1 receptor antagonists for smoking cessation. Cochrane Database Syst Rev, CD005353. [PubMed: 21412887]

(5). De Vries TJ, Shaham Y, Homberg JR, Crombag H, Schuurman K, Dieben J, Vanderschuren LJ, and Schoffelmeer AN (2001) A cannabinoid mechanism in relapse to cocaine seeking. Nat. Med. 7, 1151–1154. [PubMed: 11590440]

(6). Maldonado R, Valverde O, and Berrendero F (2006) Involvement of the endocannabinoid system in drug addiction. Trends Neurosci. 29, 225–232. [PubMed: 16483675]

(7). Carai MA, Colombo G, and Gessa GL (2005) Rimonabant: the first therapeutically relevant cannabinoid antagonist. Life Sci. 77, 2339–2350. [PubMed: 15935395]

(8). Fattore L, Spano MS, Deiana S, Melis V, Cossu G, Fadda P, and Fratta W (2007) An endocannabinoid mechanism in relapse to drug seeking: a review of animal studies and clinical perspectives. Brain Res Rev 53, 1–16. [PubMed: 16839608]

(9). Nguyen T, Li JX, Thomas BF, Wiley JL, Kenakin TP, and Zhang Y (2017) Allosteric Modulation: An Alternate Approach Targeting the Cannabinoid CB1 Receptor. Med. Res. Rev. 37, 441–474. [PubMed: 27879006]

(10). Dopart R, Lu D, Lichtman AH, and Kendall DA (2018) Allosteric modulators of cannabinoid receptor 1: developing compounds for improved specificity. Drug Metab. Rev. 50, 3–13. [PubMed: 29355030]

(11). Khurana L, Mackie K, Piomelli D, and Kendall DA (2017) Modulation of CB1 cannabinoid receptor by allosteric ligands: Pharmacology and therapeutic opportunities. Neuropharmacology 124, 3–12. [PubMed: 28527758]

(12). Kulkarni AR, Garai S, Janero DR, and Thakur GA (2017) Design and Synthesis of Cannabinoid 1 Receptor (CB1R) Allosteric Modulators: Drug Discovery Applications. Methods Enzymol. 593, 281–315. [PubMed: 28750808]

(13). Horswill J, Bali U, Shaaban S, Keily J, Jeevaratnam P, Babbs A, Reynet C, and In PWK (2007) PSNCBAM-1, a novel allosteric antagonist at cannabinoid CB1 receptors with hypophagic effects in rats. Br. J. Pharmacol. 152, 805–814. [PubMed: 17592509]

(14). Baillie GL, Horswill JG, Anavi-Goffer S, Reggio PH, Bolognini D, Abood ME, McAllister S, Strange PG, Stephens GJ, Pertwee RG, and Ross RA (2013) CB1 Receptor Allosteric Modulators Display Both Agonist and Signaling Pathway Specificity. Mol. Pharmacol. 83, 322–338. [PubMed: 23160940]

(15). Cawston EER, William J; Breen Courtney M.; Grimsey Natasha L.; Connor Mark; Glass Michelle. (2013) Real-time characterization of cannabinoid receptor 1 (CB1) allosteric modulators reveals novel mechanism of action. Br. J. Pharmacol. 170, 893–907. [PubMed: 23937487]

(16). Kenakin T, and Strachan RT (2018) PAM-Antagonists: A Better Way to Block Pathological Receptor Signaling? Trends Pharmacol. Sci. 39, 748–765. [PubMed: 29885909]

(17). Khajehali E, Malone DT, Glass M, Sexton PM, Christopoulos A, and Leach K (2015) Biased agonism and biased allosteric modulation at the CB1 cannabinoid receptor. Mol. Pharmacol. 88, 368–379. [PubMed: 26044547]

(18). Nguyen T, German N, Decker AM, Langston TL, Gamage TF, Farquhar CE, Li JX, Wiley JL, Thomas BF, and Zhang Y (2017) Novel Diarylurea Based Allosteric Modulators of the Cannabinoid CB1 Receptor: Evaluation of Importance of 6-Pyrrolidinylpyridinyl Substitution. J. Med. Chem. 60, 7410–7424. [PubMed: 28792219]

(19). Gamage TF, Farquhar CE, Lefever TW, Thomas BF, Nguyen T, Zhang Y, and Wiley JL (2017) The great divide: Separation between in vitro and in vivo effects of PSNCBAM-based CB1 receptor allosteric modulators. Neuropharmacology 125, 365–375. [PubMed: 28803965]

(20). German ND, Ann M; Gilmour, Brian P; Gay, Elaine A; Wiley, Jenny L; Thomas, Brian F; Zhang, Yanan. (2014) Diarylureas as Allosteric Modulators of the Cannabinoid CB1 Receptor: Structure-Activity Relationship Studies on 1-(4-Chlorophenyl)-3-{3-[6-(pyrrolidin-1-yl)pyridin-2-yl]phenyl}urea (PSNCBAM-1). J. Med. Chem. 57, 7758–7769. [PubMed: 25162172]

(21). Khurana L, Fu BQ, Duddupudi AL, Liao YH, Immadi SS, Kendall DA, and Lu D (2017) Pyrimidinyl Biphenylureas: Identification of New Lead Compounds as Allosteric Modulators of the Cannabinoid Receptor CB1. J. Med. Chem. 60, 1089–1104. [PubMed: 28059509]

(22). Marzaro G, Coluccia A, Ferrarese A, Brun P, Castagliuolo I, Conconi MT, La Regina G, Bai R, Silvestri R, Hamel E, and Chilin A (2014) Discovery of biarylaminoquinazolines as novel tubulin polymerization inhibitors. J. Med. Chem. 57, 4598–4605. [PubMed: 24801610]

(23). Beladhria A, Beydoun K, Ben Ammar H, Ben Salem R, and Doucet H (2012) Palladium-Catalysed Direct Arylation of Heteroaromatics Using Unprotected Iodoanilines with Inhibition of the Amination Reaction. Synthesis-Stuttgart 44, 2264–2276.

(24). Hah JM, Yoo KH, Sim TB, Oh CH, Lee JH, Yu H, and Kim H (2011) Imidazole derivatives and compositions for treating melanoma, Korea Institute Of Science And Technology, WO 2011049274 A1.

(25). Zhang Y, Gilliam A, Maitra R, Damaj MI, Tajuba JM, Seltzman HH, and Thomas BF (2010) Synthesis and biological evaluation of bivalent ligands for the cannabinoid 1 receptor. J. Med. Chem. 53, 7048–7060. [PubMed: 20845959]

(26). Price MR, Baillie GL, Thomas A, Stevenson LA, Easson M, Goodwin R, McLean A, McIntosh L, Goodwin G, Walker G, Westwood P, Marrs J, Thomson F, Cowley P, Christopoulos A, Pertwee RG, and Ross RA (2005) Allosteric Modulation of the Cannabinoid CB1 Receptor. Mol. Pharmacol. 68, 1484–1495. [PubMed: 16113085]

(27). Kearn CS, Greenberg MJ, DiCamelli R, Kurzawa K, and Hillard CJ (1999) Relationships between ligand affinities for the cerebellar cannabinoid receptor CB1 and the induction of GDP/GTP exchange. J. Neurochem. 72, 2379–2387. [PubMed: 10349847]

(28). Houston DB, and Howlett AC (1998) Differential receptor-G-protein coupling evoked by dissimilar cannabinoid receptor agonists. Cell. Signal. 10, 667–674. [PubMed: 9794249]

(29). Rinaldi-Carmona M, Pialot F, Congy C, Redon E, Barth F, Bachy A, Breliere JC, Soubrie P, and Le Fur G (1996) Characterization and distribution of binding sites for [3H]-SR 141716A, a

selective brain (CB1) cannabinoid receptor antagonist, in rodent brain. Life Sci. 58, 1239–1247. [PubMed: 8614277]

(30). Bouaboula M, Perrachon S, Milligan L, Canat X, Rinaldi-Carmona M, Portier M, Barth F, Calandra B, Pecceu F, Lupker J, Maffrand JP, Le Fur G, and Casellas P (1997) A selective inverse agonist for central cannabinoid receptor inhibits mitogen-activated protein kinase activation stimulated by insulin or insulin-like growth factor 1. Evidence for a new model of receptor/ligand interactions. J. Biol. Chem. 272, 22330–22339. [PubMed: 9268384]

(31). Barker EL, Westphal RS, Schmidt D, and Sanders-Bush E (1994) Constitutively active 5-hydroxytryptamine2C receptors reveal novel inverse agonist activity of receptor ligands. J. Biol. Chem. 269, 11687–11690. [PubMed: 7909313]

(32). Shore DMB, Gemma L; Hurst Dow H.; Navas Frank III; Seltzman Herbert H.; Marcu Jahan P.; Abood Mary E.; Ross Ruth A.; Reggio Patricia H. (2014) Allosteric Modulation of a Cannabinoid G Protein-coupled Receptor. J. Biol. Chem. 289, 5828–5845. [PubMed: 24366865]

(33). Devlin MG, and Christopoulos A (2002) Modulation of cannabinoid agonist binding by 5-HT in the rat cerebellum. J. Neurochem. 80, 1095–1102. [PubMed: 11953460]

(34). Kenakin T, and Miller LJ (2010) Seven transmembrane receptors as shapeshifting proteins: the impact of allosteric modulation and functional selectivity on new drug discovery. Pharmacol. Rev. 62, 265–304. [PubMed: 20392808]

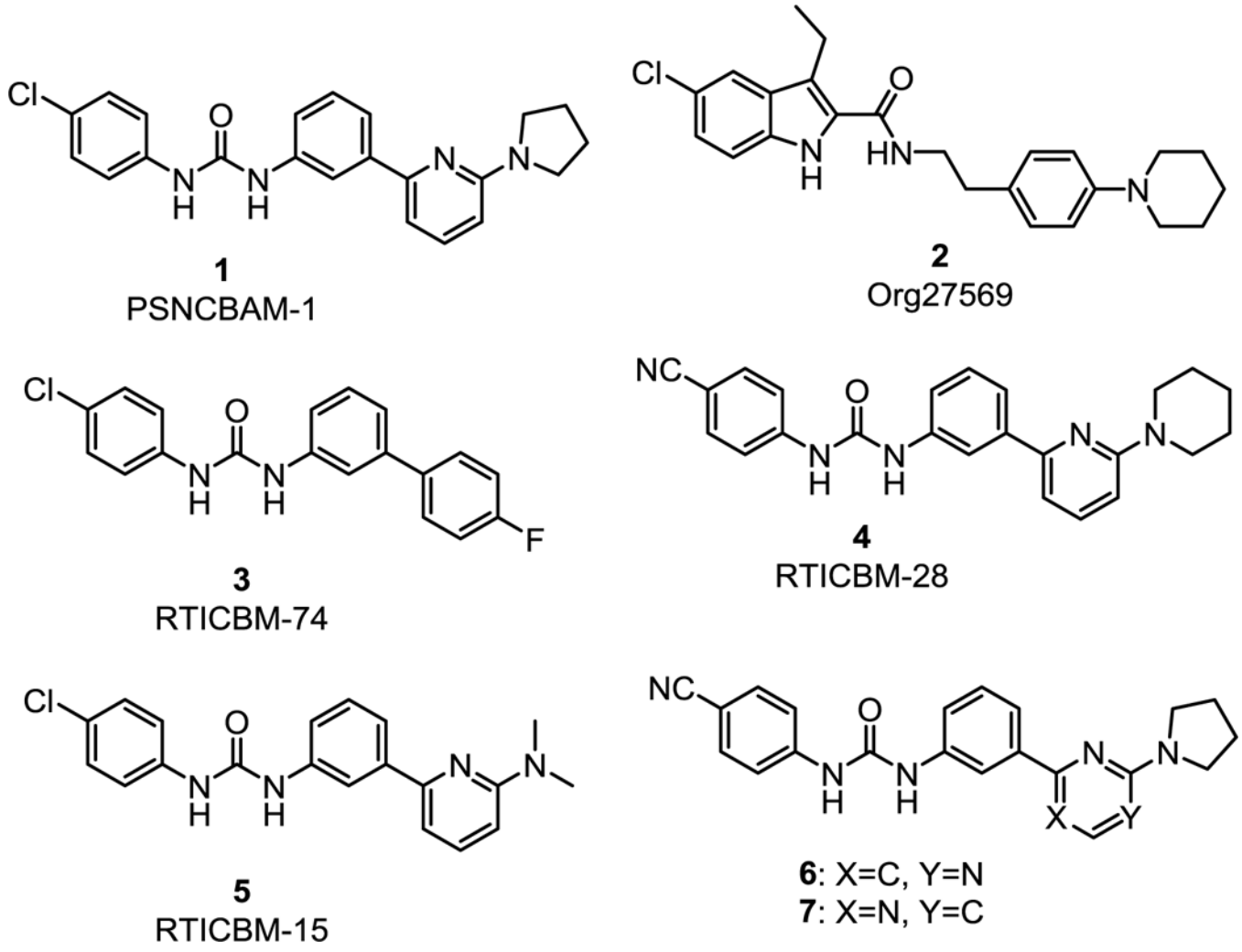


Figure 1.
Structures of representative CB_1 negative allosteric modulators

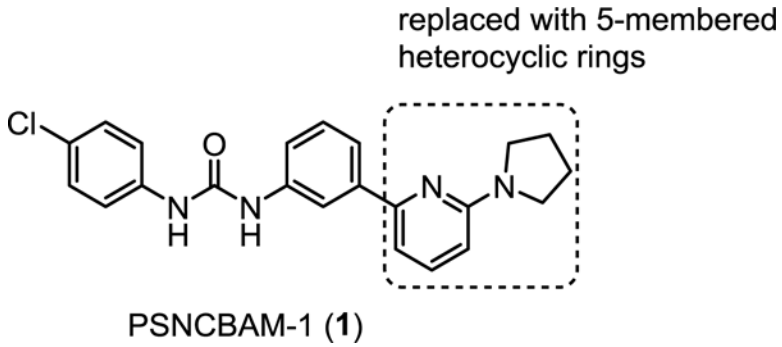


Figure 2.
Structural modifications of the diarylurea template in this study

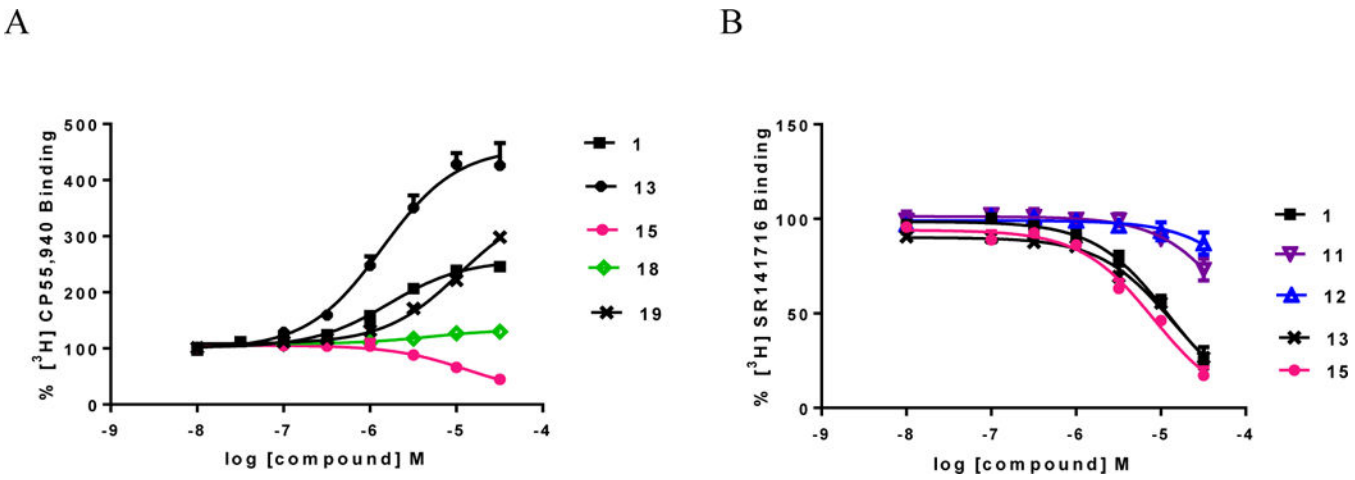
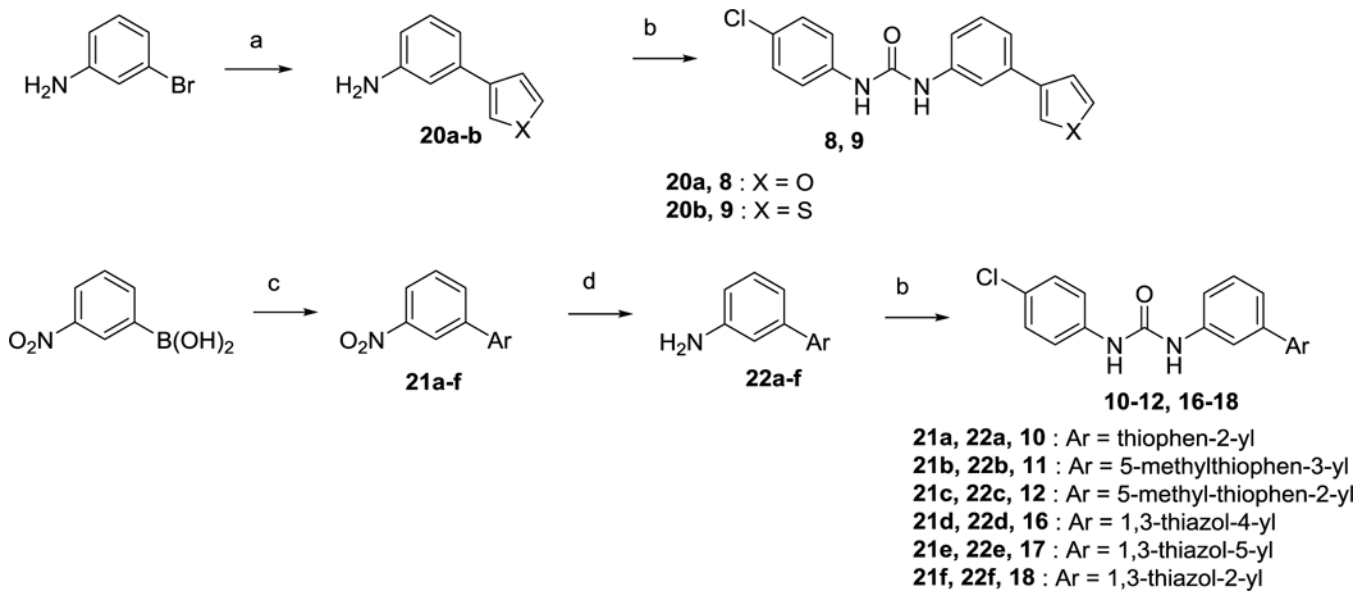


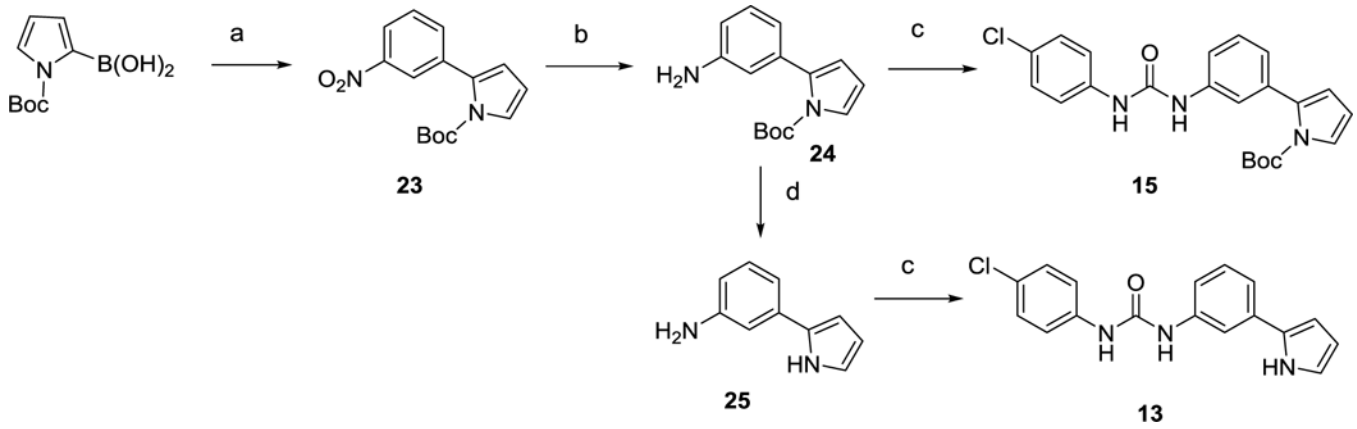
Figure 3.

(A) Effects of representative diarylureas on the equilibrium binding levels of 1 nM of [^{3}H]CP55,940, and (B) Effects of representative diarylureas on the equilibrium binding levels of 1 nM of [^{3}H]SR141716.



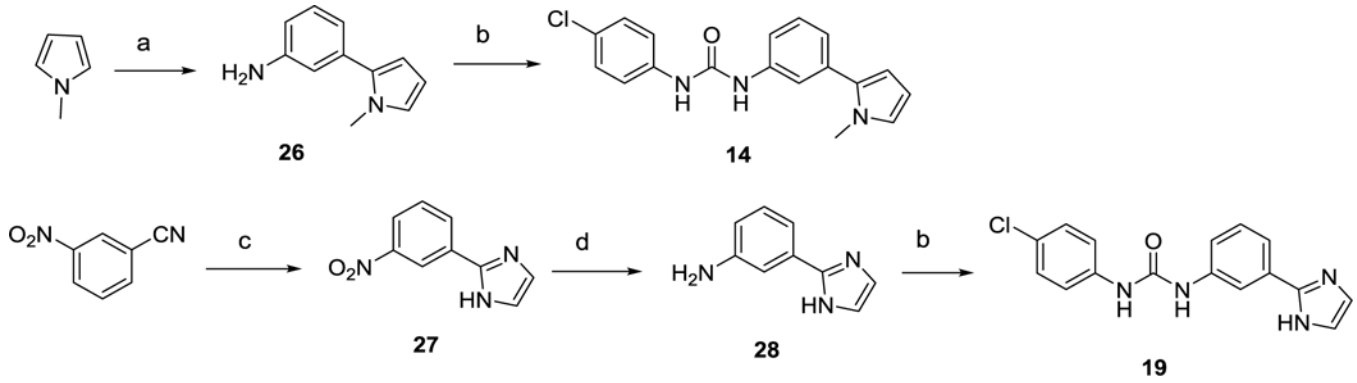
Scheme 1.

Reagents and conditions (a) aryl boronic acid, $Pd(PPh_3)_4$, DME, $NaHCO_3$, reflux, 16 h, 10-32% (b) 4-chlorophenyl isocyanate, chloroform, 50 °C, 16 h, 57-95% (c) aryl bromide, $Pd(PPh_3)_4$, DME, $NaHCO_3$, reflux, 16 h, 37-92% (d) hydrazine hydrate, Raney Ni, ethanol, 50 °C, 2 h, 46%-quant. yield.



Scheme 2.

Reagents and conditions (a) 1-Bromo-3-nitrobenzne, $Pd(PPh_3)_4$, DME, $NaHCO_3$, reflux, 16 h, 51% (b) hydrazine hydrate, Raney Ni, ethanol, 50 °C, 2 h, 65% (c) 4-chlorophenyl isocyanate, chloroform, 50 °C, 16 h, 81-84% (d) aq. 5% KOH, reflux, 4 h, 78%.

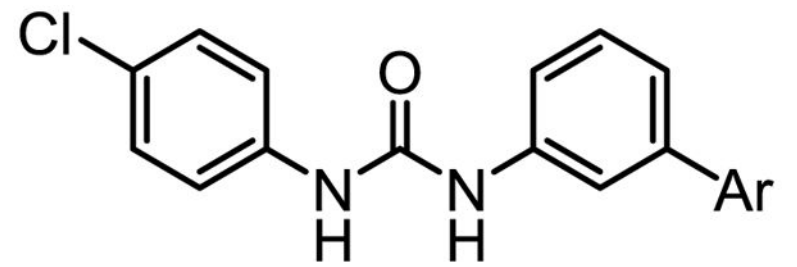


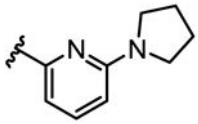
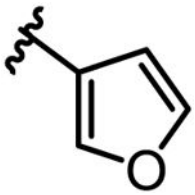
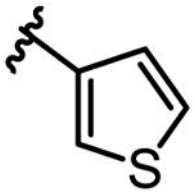
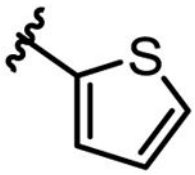
Scheme 3.

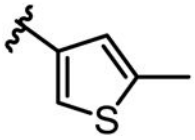
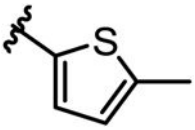
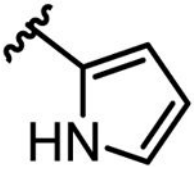
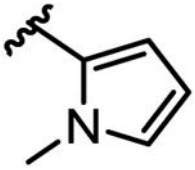
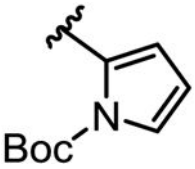
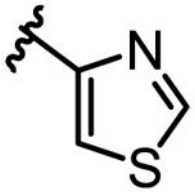
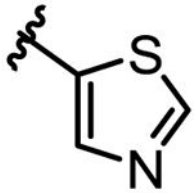
Reagents and conditions (a) 3-iodoaniline, AcOK, $Pd(OAc)_2$, $AcNMe_2$, sealed tube, 150 °C, 20 h, 22% (b) 4-chlorophenyl isocyanate, chloroform, 50 °C, 16 h, 86-98% (c) (i) NaOMe, MeOH, rt, 5 h (ii) $(MeO)_2CHCH_2NH_2$, AcOH, 70 °C, 1 h (iii) aq. 6N HCl, MeOH, 70 °C, 3 h, 61% (d) hydrazine hydrate, Raney Ni, ethanol, 50 °C, 2 h, quant. yield.

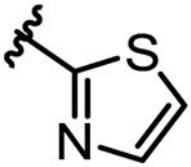
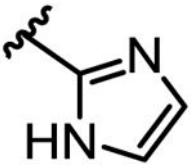
Table 1.

Allosteric modulatory activities of compounds **8**-**19** in CB_1 and CB_2 calcium mobilization and CB_1 [^{35}S]GTP-γ-S binding assays.



Compound	Ar	CB_1		CB_2
		Calcium assay IC_{50} (nM)[a]	[^{35}S]GTPγS binding assay IC_{50} (nM)[b]	Antagonist screen in calcium assay (%CP55,940 E_{max})[c]
1		33 ± 8	115 (87, 155)	*d*
8		41 ± 9	441 (339, 574)	*d*
9		36 ± 1	164 (113, 239)	*d*
10		67 ± 6	573 (272, 1205)	*d*

Compound	Ar	CB_1		CB_2
		Calcium assay IC_{50} (nM)[a]	[^{35}S]GTPγS binding assay IC_{50} (nM)[b]	Antagonist screen in calcium assay (%CP55,940 E_{max})[c]
11		40 ± 4	776 (324, 1858)	*d*
12		42 ± 6	372 (182, 759)	*d*
13		169 ± 34	40 (29, 55)	IC_{50} > 10 μM [e]
14		165 ± 14	665 (501, 883)	*d*
15		3444 ± 451	3236 (1480, 7070)	*d*
16		84 ± 6	251 (118, 530)	*d*
17		94 ± 9	331 (215, 509)	*d*

Compound	Ar	CB_1		CB_2
		Calcium assay IC_{50} (nM)[a]	[^{35}S]GTPγS binding assay IC_{50} (nM)[b]	Antagonist screen in calcium assay (%CP55,940 E_{max})[c]
18		154 ± 14	1259 (562, 2818)	*d*
19		529 ± 98	343 (224, 524)	*d*

[a] Against EC_{80} (100 nM) of CP55,940 in stable human CB_1-CHO-RD-HGA16 cells. Values are the mean ± SEM of at least three independent experiments in duplicate.

[b] Tested in mouse cerebellar membranes against CP55,940 (100 nM). Values are expressed as mean (95% confidence interval) from at least three independent experiments in duplicate.

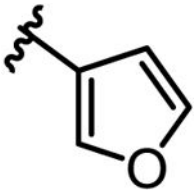
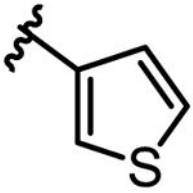
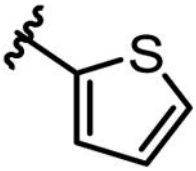
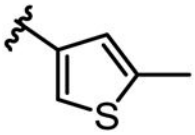
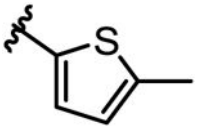
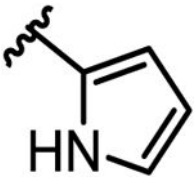
[c] Against EC_{80} (100 nM) of CP55,940 in stable human CB_2-CHO-RD-HGA16 cells. Compounds were tested at 10 μM final concentration in two independent experiments in duplicate.

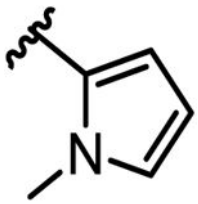
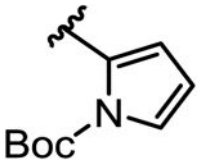
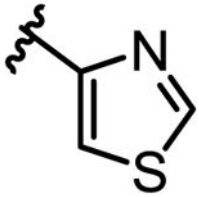
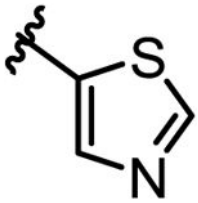
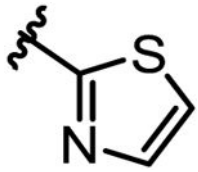
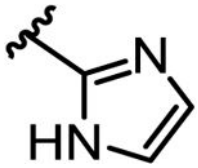
[d] < 50% inhibition.

[e] Compound showed > 50% inhibition and was tested for potency.

Table 2.

Allosteric modulatory activities of diarylureas **1** and **3**-**14** in the CB_1 [^{3}H]CP55,940 and [^{3}H]SR141716 binding assays [a]

Compound	Ar	[^{3}H]CP55,940 pEC_{50} ± S.E.	[^{3}H] CP55,940 Curve-fit top ± S.E.	[^{3}H]SR141716 pIC_{50} ± S.E.	%[^{3}H]SR141716 Binding [a,b] ± S.E.
1		5.69 ± 0.08	256.0 ± 5.59	4.91 ± 0.11	20.42 ± 3.05
8		5.13 ± 0.18	174.1 ± 8.86	4.76 ± 0.14	34.87 ± 2.43
9		5.34 ± 0.01	264.6 ± 9.81	4.95 ± 0.15	20.44 ± 2.13
10		5.24 ± 0.14	199.8 ± 8.78	4.54 ± 0.21	45.54 ± 0.15
11		5.41 ± 0.08	226.0 ± 5.82	4.08 ± 0.83	73.19 ± 5.77
12		4.88 ± 0.18	233.3 ± 20.53	4.64 ± 0.74	86.90 ± 5.87
13		5.86 ± 0.09	458.3 ± 16.25	4.85 ± 0.18	26.60 ± 5.68

Compound	Ar	[^{3}H]CP55,940 pEC_{50} ± S.E.	[^{3}H] CP55,940 Curve-fit top ± S.E.	[^{3}H]SR141716 pIC_{50} ± S.E.	%[^{3}H]SR141716 Binding [a,b] ± S.E.
14		5.25 ± 0.19	164.5 ± 8.61	4.75 ± 0.20	41.41 ± 3.26
15		4.83 ± 0.27	15.5 ± 24.12	5.07 ± 0.11	17.20 ± 4.12
16		4.97 ± 0.17	224.2 ± 15.85	4.59 ± 0.17	44.34 ± 1.42
17		5.19 ± 0.17	181.2 ± 8.15	5.53 ± 0.11	50.58 ± 2.04
18		5.29 ± 0.27	134.2 ± 4.47	4.78 ± 0.17	37.68 ± 0.76
19		4.99 ± 0.12	355.7 ± 27.39	3.91 ± 0.79	34.83 ± 8.94

[a]Compounds tested in presence of 1 nM [^{3}H]CP55,940 or 1 nM [^{3}H]SR141716.

[b]percent specific [^{3}H]SR141716 binding at 32 μM of test compound.

Table 3.

Allosteric effects of diarylureas on [^{3}H]CP55,940 saturation binding at 10 μM of test compound

Compound	[^{3}H]CP55,940 B_{max} (pmol/mg) ± S.E.	B_{max} (pmol/mg) 95% CI	[^{3}H]CP55,940 K_d (nM) ± S.E.	K_d (nM) 95% CI
Vehicle	1.56 ± 0.09	1.38 to 1.74	1.92 ± 0.30	1.31 to 2.52
1	4.53 ± 0.29	3.95 to 5.11	1.93 ± 0.33	1.27 to 2.60
8	3.22 ± 0.19	2.82 to 3.62	2.85 ± 0.41	1.98 to 3.71
11	3.64 ± 0.17	3.28 to 4.00	2.63 ± 0.31	1.98 to 3.29
13	5.87 ± 0.15	5.55 to 6.19	1.41 ± 0.10	1.20 to 1.63
17	3.57 ± 0.25	3.05 to 4.10	3.10 ± 0.51	2.02 to 4.17