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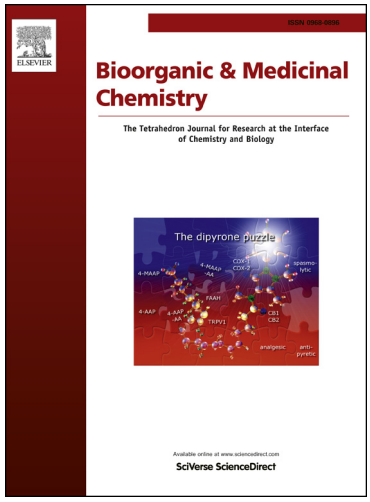

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Synthesis and Biological Evaluation of 3',5'-Dichloro-2,6-dihydroxy-biphenyl-4-yl)-aryl/alkyl-methanone Selective CB2 Inverse Agonist

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Abstract: Cannabinoid receptor 2 (CB2) selective agonists and inverse agonists possess significant potential as therapeutic agents for regulating inflammation and immune function. Although CB2 agonists have received the greatest attention, it is emerging that inverse agonists also manifest anti-inflammatory activity. In process of designing new cannabinoid ligands we discovered that the 2,6-dihydroxy-biphenyl-aryl methanone scaffold imparts inverse agonist activity at CB2 receptor without functional activity at CB1. To further explore the scaffold we synthesized a series of 3',5'-dichloro-2,6-dihydroxy-biphenyl-4-yl)-aryl/alkyl-methanone analogs and evaluated the CB1 and CB2 affinity, potency, and efficacy. The studies reveal that an aromatic C ring is required for inverse agonist activity and that substitution at the 4 position is optimum. The resorcinol moiety is required for optimum CB2 inverse agonist activity and selectivity. Antagonist studies against CP 55,940 demonstrate that the compounds **41** and **45** are noncompetitive antagonists at CB2.

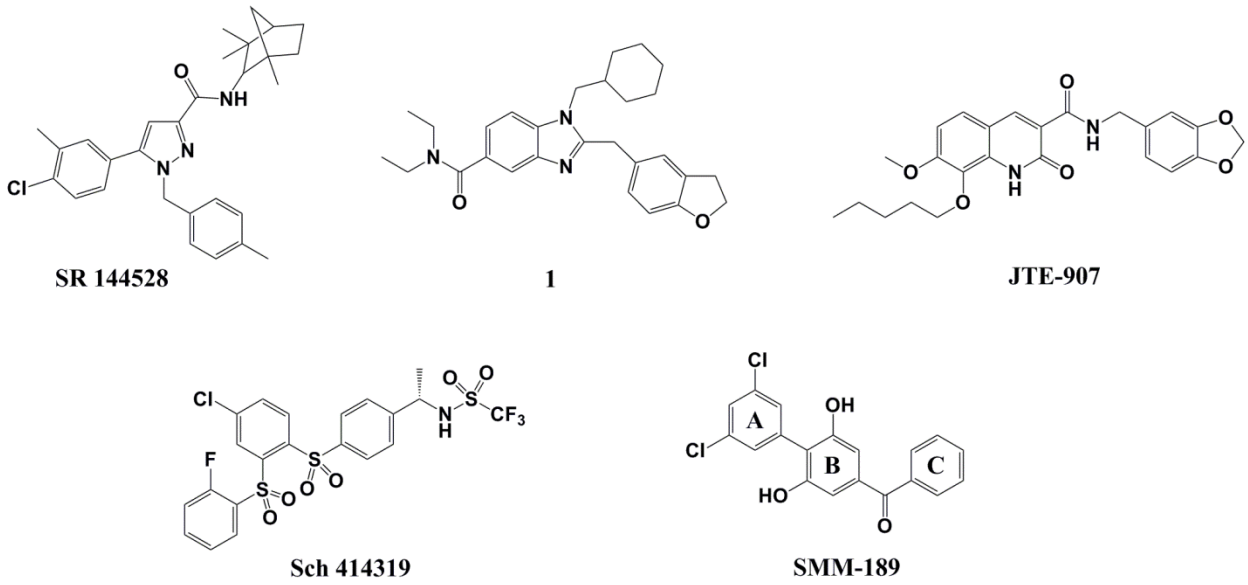
Keywords: Cannabinoid; Inverse agonist; CB2 receptor, cAMP stimulation, ACTOne Assay

1. Introduction

The scope of therapeutic potential of cannabinoid based drugs has evolved rapidly following the identification of the cannabinoid receptors 1 and 2 (CB1 and CB2).[1-3] Both receptors, either individually or in combination, have been identified as potential targets for intervention of human diseases including neurodegenerative[4-6] and cardiovascular disease,[7-9] diabetes,[10-12] and cancer.[13-16] Within this spectrum of diseases, intervention in neurodegenerative disorders using CB2 agonists and inverse agonists is arguably one of the most exciting developments in the cannabinoid field. Specifically, microglia, the resident immune and inflammatory mediators in the central nervous system (CNS), express CB2 in Alzheimer's[17, 18] and Parkinson's disease[19] and amyotrophic lateral sclerosis.[6, 20] The CB2 is also up-regulated during microglial migration, bacterial insult, and in response to CNS trauma.[21-23] The apparent selective up-regulation of CB2 on microglia in response to insult indicates that CB2 ligands would provide selective effects in only the damaged CNS tissue. In fact, both agonists and inverse agonists of CB2 have been evaluated for efficacy in ameliorating disease progression in *in vivo* models of traumatic brain injury,[24, 25] Alzheimer's [17, 18, 26-30] and Parkinson's disease, [19, 31, 32] amyotrophic lateral sclerosis, [6, 20] and multiple sclerosis.[5, 33-39] The comparable efficacy of agonists and inverse agonists highlights one of the major unanswered questions in this field, what is the optimum functional activity of CB2 ligands?

Agonists of CB2 have received the greatest attention in terms ligand design and evaluation as therapeutic agents, a recent review by Jones and colleagues highlights the progress in this area.[40] A review by Lunn however highlights the emerging interest in the development of CB2 inverse agonists.[41] The prototypical examples of a CB2 inverse agonist are the biaryl pyrazoles, and closely related triazoles, exemplified by SR144528 (Figure 1). Compounds based on the indole, benzimidazole, and isatin scaffold have also been described such as compound **1.**[42] CB2 inverse agonist activity has been achieved using a 2- (JTE-907) and 4-oxoquinoline, and related 2-oxopyrine based derivatives.[43] The triaryl bis-sulfones (Sch 414319), and our 2,6-dihydroxy-biphenyl-aryl-methanone analog (SMM-189), represent additional scaffolds that are being investigated as CB2 inverse agonists.[41, 44] Raloxifen, himbacine and cannabidiol have also been reported to function as inverse agonist of CB2.[45-47] Undoubtedly the diversity of CB2 inverse agonist scaffolds will continue to expand owing to the as yet untapped potential in treating inflammation and immune system disorders.

Figure 1. Structures of Selected CB2 Inverse Agonists



We previously reported the synthesis, and CB1 and CB2 receptor binding, of a small group of triaryl based cannabinoid ligands.[48] In the evaluation of the functional activity of the compounds it was discovered that SMM-189 exhibited selective inverse agonist activity at CB2.[44] Based on the potential of CB2 inverse agonist in treating CNS diseases, we demonstrated that SMM-189 beneficially down-regulates cytokine and chemokine production in LPS stimulated primary human microglia.[25, 44] Subsequent testing of SMM-189 in the murine model of mild traumatic brain injury (mTBI), and pre-clinical evaluation of biopharmaceutical properties, demonstrated the protective effects of SMM-189 in mTBI and indicated that the 2,6-dihydroxy-biphenyl-aryl-methanone scaffold has acceptable drug-like properties to warrant further investigation.[25] The potential of the scaffold for developing selective CB2 inverse agonist prompted us to investigate the functional group requirements required for activity. A set of compounds were synthesized and evaluated for CB1 and CB2 receptor binding in ACTOne membrane preparations and functional activity using the ACTOne cAMP assay. The determination of affinity, potency, and efficacy revealed that the resorcinol group and 4 substitutions are required for optimum CB2 inverse agonist activity. Furthermore, compounds **41** and **45** function as noncompetitive antagonists when assayed against CP 55,940 at CB2.

2. Results and Discussion

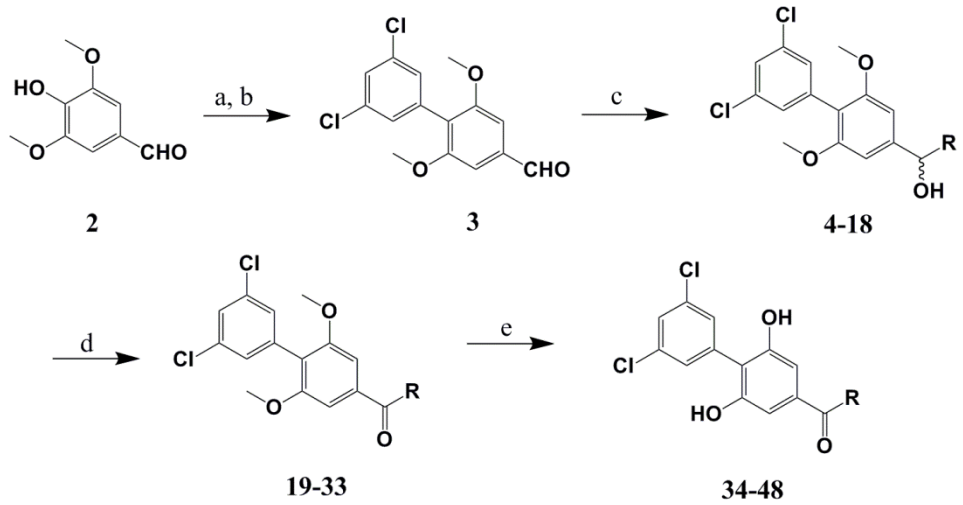
2.1 Chemistry

The design of the 2,6-dihydroxy-biphenyl-aryl-methanone scaffold arose from a combination of our work on the C1'-aryl substituted classical cannabinoids [49] and the biaryl ligands reported by Makriyannis.[50] The hypothesis was that the hexyl group in the *gem*-dimethylheptyl side chain could be replaced by a phenyl ring thus increasing CB2 selectivity. Unexpectedly, the deprotected intermediate (SMM-189) wherein the *gem*-dimethyl group was replaced by a ketone, yielded a CB2 inverse agonist with no functional activity at CB1. The original design hypothesized an overlapping binding pocket with classical and non-classical cannabinoids which was supported by the fact that SMM-189 is a orthosteric antagonist of CP 55,940.[44] Therefore, aliphatic substitution of the phenyl ring in SMM-189 was predicted increase affinities for CB2, as was observed in the C1' 3- and 4-alkylphenyl substituted analogs of Δ^8-THC.[49] To test this hypothesis a series of alkylphenyl analogs of SMM-189 were synthesized. The effect of hydrogen bonding and/or electron density on affinity and potency was evaluated by introducing hydroxyl, chloro, and trifluoromethyl groups. Furthermore, it is well established that *gem*-dimethylheptyl and *gem*-dimethylcycloalkyl side chains afford high affinity and potency in classical and non-classical CB1 and CB2 ligands.[51, 52] Thus, the heptan-1-one and cyclohexylmethanone functional groups were introduced to determine if the aromatic ring was required for affinity and potency. The requirement of the resorcinol functional group was also examined by testing hydroxymethoxy and dimethoxy analogs. We took advantage of the by-products isolated from efforts to optimize the boron tribromide deprotection of **21**, **30**, and **33** to test the resorcinol functional group requirement.

The synthetic methodology for the 3',5'-dichloro-2,6-dihydroxy-biphenyl-4-yl)-aryl/alkyl-methanone analogs was revised from the previous synthesis due to low yields in the Suzuki–Miyaura cross-coupling, final deprotection step, and to reduce cost. Formation of the trifluoro-methanesulfonic acid ester of syringe aldehyde (**2**) was conducted using dichloromethane/pyridine as described by McLure and Young (Scheme 1).[53] The modification was aimed at reducing cost by substituting pyridine for collidine. The microwave cross-coupling did not scale nor did the addition of LiCl improve the reaction.[54] An increase in yields to over 80 percent was achieved using a modification of the Suzuki–Miyaura cross-coupling described by Leblond *et al.*[55] The order of addition of the reagents proved to be important wherein the sequential addition of triflate, boronic acid, water, and finally sodium carbonate provided optimum yields. However, the reaction did not scale beyond 1 gram thus reactions were conducted in parallel as opposed to investigating alternative Pd(0) catalysts. The 2,6-dimethoxy protected intermediates were prepared by reacting **3** with the appropriately substituted Grignard reagent followed by PCC oxidation. The oxidation reaction was modified from our previous method to reduce reaction time and simplify workup. Specifically, the reaction was accelerated by first heating the PCC/dichloromethane to reflux and cooling before addition of the alcohol. Addition of 1 N sodium hydroxide for workup yielded a clear almost colorless organic phase, green aqueous, without confounding tarry residues previously obtained. Deprotection of the dimethoxy intermediates with 2.5 equivalents boron tribromide yielded starting material, mono- and di-deprotected products. Extending the reaction time and increasing the equivalents of boron tribromide, to compensate for coordination to the additional Lewis bases, decreased the yield of the desired product.

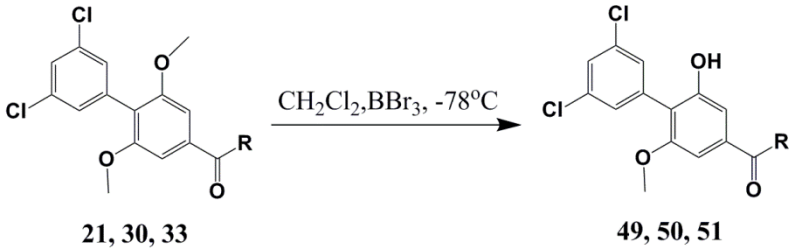
The mono-deprotected products (**49-51**) from optimization reactions on the p-chloro, p-methyl, and p-butyl were isolated (Scheme 2), albeit in low yields, and subsequently used to examine the effect of methoxy group(s) on receptor affinity and potency**.** Deprotection was achieved using molten pyridine hydrochloride [56] providing the desired resorcinol in a relatively short reaction time (2 hours) in good to high yields.

Scheme 1.



Reagents and Conditions: (a) CH_2Cl_2, pyridine, triflic anhydride, 0°C; (b) 3,5-dichlorophenylboronic acid, $Pd(PPh_3)_4$, DMF, H_2O, Na_2CO_3, 40°C; (c) aryl/alkyl bromide, Mg, THF; (d) PCC, CH_2Cl_2; (e) pyridine HCl, 220°C

Scheme 2.



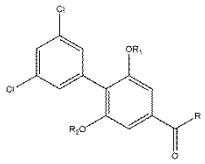
2.2 Receptor Binding and Functional Assays

The CB1 and CB2 receptor affinities, potency, and efficacy of compounds **34-51** and SMM-189, using the ACTOne functional assay cell lines and membranes preparations derived from these cells. We have previously reported this method as part of our pre-clinical evaluation of SMM-189.[44] The method provided good correlation between the K_i and EC_{50} values; however, the K_i deviated significantly from the previous

determination [48] in part due to differences in K_d and B_{max} of the preparations. A second consideration stemmed from the identification that SMM-189 is a non-competitive antagonist against CP 55,940, thus indicating an overlapping binding site. This prompted us to consider the possibility that the initial binding is to a ground state receptor and inverse agonist activity is induced by ligation. Thus binding of different ligands, and the subsequent conformational change, could lead to one or more inactive conformations with differing efficacy in G-protein coupling.[57, 58] The identification of ligand functional groups that affect G-protein coupling, and ultimately efficacy, is an important component in the drug design process. However, elucidating these effects *in vitro* can be challenging depending on the assay format and sensitivity. Our approach to this challenge was to minimize potential variables, *e.g.* affinities determined using CHO cell preparation and functional activity determined in HEK cells, by employing a single background to determine our compound affinity, potency, and efficacy at CB1 and CB2.

Using membrane preparation for the CB1 and CB2 lines, binding affinities were determined by measuring the displacement of [^{3}H]CP 55,940 by increasing concentrations of the of the compounds. The first question to be addressed was the effect hexyl and cyclohexyl substitution affinities for CB1 and CB2. The hexyl analog (**34**) exhibited no affinity (>10 μM, Table 1) for the receptors compared to the *gem*-dimethylheptyl analog (K_i = 2.6 nM and 0.6 nM, CB1 and CB2, respectively) reported by Makriyannis.[50] Considering our initial hypothesis of an overlapping ligand binding pocket (LBP) with classical cannabinoids this is not unexpected considering a keto group at C1' is disfavored in the classical scaffold.[59-61] It was therefore surprising that the cyclohexyl group in **35** improved CB1 and CB2 affinities (K_is = 298.6 and 1589 nM, respectively). However, compound **35** potency at CB1 and CB2 was >10 μM and 2.54 μM, respectively, without a significant difference in efficacy relative to controls (see below). This may suggest an off-target effect at high concentrations of **35**. Notwithstanding, this is intriguing in that cyclohexyl group is sterically comparable to the C ring phenyl yet the cyclohexyl ring introduction does not result in inverse agonist activity at CB2 and yields an antagonist at CB1.

Table 1.



Compound number	R_1	R_2	R	Affinity - Ki (nM)		CB1:CB2 ratio	Potency EC_{50} (nM)	Efficacy (% max)	Potency EC_{50} (nM)	Efficacy (% max)
				CB1	CB2		CB1		CB2	
34	H	H	*n*-hexyl	>10,000	>10,000	1	-	NS	-	NS
35	H	H	cyclohexyl	298.6 ± 53.75	1589 ± 438.4	0.19	>5,000	NS	2540 ± 42.4	NS
36	H	H	4-methylphenyl	9429 ± 364.8	257.6 ± 70.75	36.6	-	NS	118± 5.05	230 ± 5.23^{+++}
37	H	H	3-methylphenyl	>10,000	2618 ± 368.8	3.82	-	NS	-	NS
38	H	H	3,5-dimethylphenyl	>10,000	>10,000	1	-	NS	-	NS
39	H	H	4-trifluoromethylphenyl	>10,000	>10,000	1	>5,000	37.0 ± 1.55^{+++}	>5,000	49.7 ± 2.93^{+++}
40	H	H	3-trifluoromethylphenyl	4134 ± 767.5	>10,000	0.41	-	NS	-	NS
41	H	H	4-ethylphenyl	1138 ± 216.2	37.56 ± 4.91	30.3	-	NS	29.5 ± 4.83	52.2 ± 2.13^{+++}
42	H	H	3-ethylphenyl	>10,000	1717 ± 376.5	5.82	-	NS	>5,000	184 ± 7.81^{+++}
43	H	H	4-propylphenyl	>10,000	59.91 ± 5.43	166.9	-	NS	28.8 ± 3.84	50.0 ± 3.49^{++}
44	H	H	4-isopropylphenyl	3326 ± 1088	160.7 ± 39.1	20.7	-	NS	182 ± 9.38	56.0 ± 3.66^{++}
45	H	H	4-*n*-butylphenyl	>10,000	464.4 ± 75.9	21.5	-	NS	254 ± 3.79	176 ± 5.19^{+++}
46	H	H	4-*tert*-butylphenyl	2529 ± 1185	3166 ± 418.9	0.8	-	NS	3970 ± 158	275 ± 23.3^{+++}
47	H	H	4-hydroxyphenyl	>10,000	>10,000	1	-	NS	>5,000	75.6 ± 7.96^{+++}
48	H	H	4-chlorophenyl	4645 ± 2540	120.7 ± 19	38.5	3280 ± 82	169 ± 14.6^{+++}	89.7 ± 9.09	119 ± 5.49^{+++}
49	CH_3	H	4-methylphenyl	>10,000	>10,000	1	-	NS	>5,000	129 ± 7.15^{+++}
50	CH_3	H	4-*n*-butylphenyl	>10,000	>10,000	1	-	NS	>5,000	108 ± 6.19^{+++}
51	CH_3	H	4-chlorophenyl	>10,000	>10,000	1	2880 ± 30.4	71.3 ± 3.79^{+++}	>5,000	97.5 ± 5.46^{+++}
21	CH_3	CH_3	4-methylphenyl	>10,000	>10,000	1	-	NS	-	NS
30	CH_3	CH_3	4-*n*-butylphenyl	>10,000	>10,000	1	-	NS	-	NS
33	CH_3	CH_3	4-chlorophenyl	>10,000	>10,000	1	-	NS	-	NS

SMM-189	4778 ± 246	121.3 ± 20.6	39.4	-	NS	153 ± 22.3	54.8 ± 3.23†
SR 144528	>10,000	18.65 ± 1.43	536.2	-	NS	11.5 ± 3.29	80.8 ± 3.18††

$^{\dagger}p < 0.05$, $^{\dagger\dagger}p < 0.01$, $^{\dagger\dagger\dagger}p < 0.001$ in comparison to parental HEK-CN

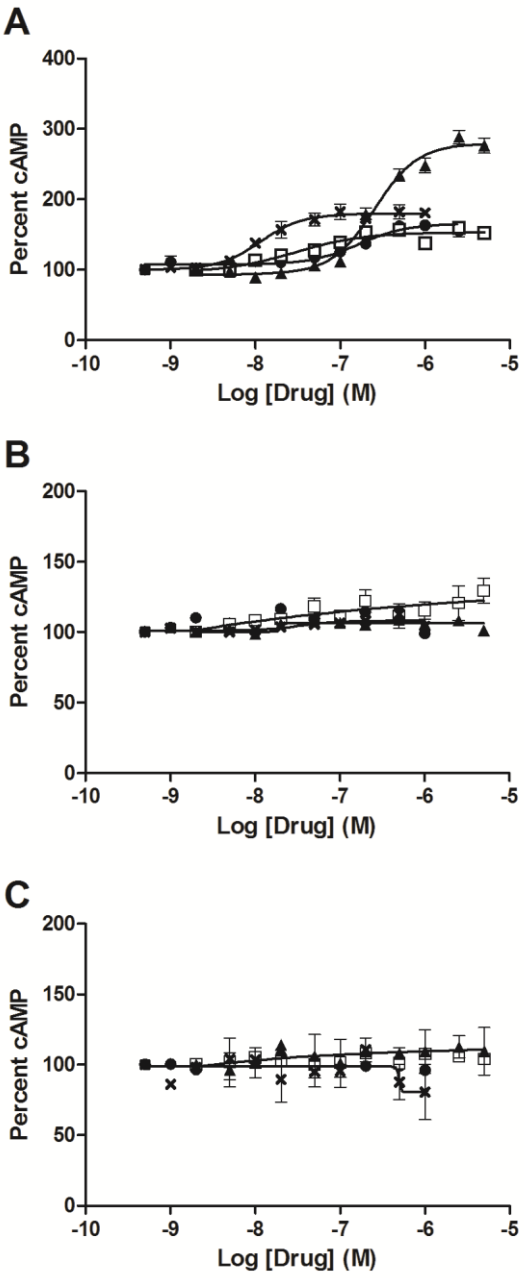
The introduction of substituents in the 3, 4, and 3,5 positions of the C ring phenyl group significantly altered CB2 affinities (K_is = 37.6 nM to >10 μM). The substituent effects were not nearly as pronounced for CB1, although substitution at the 3 and 4 positions did yield 8 compounds with micromolar affinities. In terms of alkyl groups, the 4-alkylphenyl analogs exhibited superior affinity for CB1 and CB2 compared to the to the 3-alkylphenyl while the 3,5-dimehtylphenyl (**38**) abolished affinity. Increased CB2 affinity, relative to SMM-189 (K_i = 121.3 nM), was observed in the 4-methyl (**36**), 4-ethyl (**41**), 4-propyl (**43**), and isopropyl (**44**) analogs demonstrating K_i values of 257, 37.6, 59.9, and 160 nM, respectively. Selectivity for the CB2 receptor peaked with compound **43** (ratio = 167) but was 3 fold lower than our reference inverse agonist SR 144528 (ratio = 536). Compound **41** had comparable CB2 affinity to SR 144528 (K_i = 18.7 nM) but increased CB1 affinity imparted by the ethyl group decreased the ratio to 30. The affinity of these compounds for CB1, while not optimal, revealed a modest preference for bulky versus linear aliphatic groups, *e.g.* 4-*n*-butyl (**45**, K_i > 10 μM) versus 4-*tert*-butyl (**46,** Ki = 2.53 μM). Introduction of a methyl (**37**) or ethyl (**42**) group into the 3 position blocked binding to CB1. CB2 receptor affinity was impacted similarly wherein a 10 and 46 fold reduction in the K_i occurred relative to the 4 position isomers (**36** and **41**). Modification of the resorcinol group was not tolerated as demonstrated by absence of affinity for CB1 and CB2 in the hydroxymethoxy (**49, 50, 51**) or dimethoxy (**21, 30, 33**) analogs.

The 4-hydroxyl, 3- and 4-tifluoromethyl, and 4-chloro groups were introduced to examine the contribution of electron density and hydrogen bonding to receptor affinity. Surprisingly, the 4-chloro analog (**48**) had a 2 fold higher CB2 affinity than the 4-methyl analog (**36**, K_is = 121 and 257 nM, respectively) while the 4-hydroxy (**47**) and 4-trifluoromethyl (**39**) demonstrated no affinity for either receptor. The functional groups have comparable van der Waal radii, *i.e.* 1.4 to 2.0 Å, thus steric interactions are unlikely to be responsible for reduced affinity. The charge density rank order of CF_3 > Cl > OH disfavors an effect due to repulsive interactions. The formation of a classical hydrogen bond would be predicted to increase affinity however this is not observed with 4-hydroxyl or 4-trifluoromethyl, although the existence of an intermolecular F to H bond in a drug-protein complex is rare based on the analysis reported by Dunitz and Taylor.[62] This raises the intriguing possibility that a multipolar interaction of a carbonyl oxygen with the σ hole in the aromatic chloro enhances receptor affinity.[63] It is possible for trifluoromethyl to interact analogous to chloro; however, the preferred parallel C-Cl to O=C interaction may be favorable over the orthogonal O=C to C-CF_3 interaction.[64] The CB1 affinities for the 4-hydroxy (**47**) and 4-trifluoromethyl (**39**) analogs were equally low; however, the K_is for the 4-chloro (**48**) and 3-trifluoromethyl (**40**) derivatives were improved to 4.13 and 4.64 μM.

The potency and efficacy of the compounds were determined using the CB1 and CB2 ACTOne assay system (for selected compounds **41**, **45**, SMM-189, and SR 144528, see Figure 2 A, B and C). The assay employs a cyclic nucleotide gated (CNG) ion channel which opens in response to intracellular cAMP levels. The assay permits real time monitoring of intracellular cyclic nucleotides using a fluorescent membrane potential dye to detect polarization or depolarization associated with closing or opening of the channel. CB1 and CB2 agonists trigger a decrease in cAMP driven fluorescent signal via activation of $G_{i/o}$, antagonists yield no response, and inverse agonists cause an increase in fluorescence. At CB1 all

but three compounds, the 4-trifluoromethyl (**39**), 4-chloro (**48**), and mono methoxy 4-chloro (**51**) analogs, failed to significantly alter the fluorescence signal above HEK-CNG controls (Table 1). The potency of the three active compounds was low (EC_{50} 2.8 to > 5 μM) yet a significant increase in cAMP production was detected with efficacies from 37 to 169 percent maximal. Interestingly, compound **48** had comparable K_i and EC_{50} values and good efficacy compared to the indeterminate affinity and potency of **39** and **51.** The indeterminate K_i for these compounds likely reflects poor binding interactions that are not sufficient to displace CP 55,940 at CB1 and CB2. However, in the absence of a high affinity ligand the weak ligand-receptor interactions are sufficient to produce a significant functional response, albeit with low potency.

Figure 2. Representative Functional Activity Graphs for A) CB2, B) CB1, and C) CNG. White squares are **41**, black triangles are **45**, black circles are SMM-189, and Xs are SR 144528. N = 6-17, error is SEM.

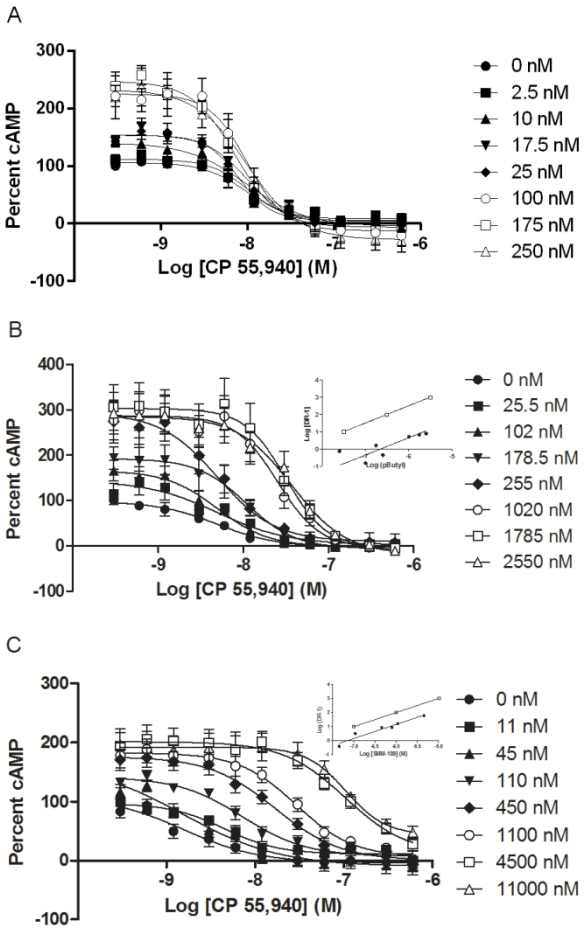


It was noted in the discussion of affinities, the cyclohexyl analog (**35**) demonstrated no functional activity. While this may be related to the low affinity (K_i = 1.59 µM), it is not consistent with the comparable K_i (3.17 µM) and EC_{50} (3.97 µM) of the *tert*-butyl analog (**46**) – which possess the highest efficacy – thus indicating **35** may be a neutral antagonist. The progression from cyclohexyl to

substituted phenyl yielded inverse agonists with potencies ranging from 28.8 to 3,968 nM. The 4-ethyl (**41**) and 4-propyl (**43**), EC_{50} values 29.5 and 28.8 nM, manifest the highest potency and were comparable to the 11.5 nM potency of the standard inverse agonist SR144528 (Table 1, Figure 2A -X); however, efficacy of **41** (Figure 2A – white boxes) and **43** were significantly lower than SR 144528 (mean 50 versus 81 percent). In contrast the effect of compounds **36**, **42**, **45** (Figure 2A – black triangles), **46**, and **48** on cAMP stimulation was significantly higher (119 to 230 percent) than SR 144528 despite significantly lower potencies. A linear analysis of the K_i versus EC_{50} of **36**, **41**, **44**, **45**, **48** and SMM-189 (compounds **39, 42,** and **47** were excluded due to overweighting of the regression) revealed a modest correlation (R^2 = 0.69) while potency versus efficacy did not correlate (R^2 = 0.18). The hydroxymethoxy compounds (**49**, **50**, **51),** 4-trifluoromethyl (**39**), and 4-hydroxy (**47**) are interesting in that affinity and potency is poor yet a significant increases in cAMP production occurs (49 to 130 percent). The basis for this effect is not clear, but is very interesting, thus prompting us to begin to investigate the effects of mono-alkylation of the resorcinol to further elucidate structure activity relationships.

Compounds **41** (EC_{50} = 29.5 nM, %max = 52.2) , **45** (EC_{50} = 254 nM, %max = 176), and SMM-189 (EC_{50} = 153 nM, %max = 54.8) were further evaluated in competition assays against CP 55,940 to determine the mode of inhibition at CB2. The study was designed to examine the three clusters of functional activities, *i.e.* high potency-modest efficacy, modest potency-high efficacy, and modest potency-modest efficacy to determine if the activities correlated with mode of inhibition. All the compounds exhibited profiles consistent with noncompetitive antagonism (Figure 3), although only **45** and SMM-189 were orthosteric antagonists as well according to Schild analysis (Figure 3B and C insets). This raised an intriguing question regarding the presence of overlapping yet unique ligand binding sites in CB2. Specifically, agonists couple to G-proteins via an active receptor conformation while inverse agonists signal through an inactive state. One possible interpretation of the result, and the absence of correlation between potency and efficacy, may be derived from the studies of on G-protein coupling of CB1 and conformational states of ligand binding.[57, 58] It was suggested that ligands induce distinct conformational states resulting in different efficacies in G-protein coupling. One possible interpretation is that ligands bind to a ground state receptor which then induces a ligand dependent change in conformation. If induction of confirmation is occurring in CB2 then this may suggest that initial binding is to a ground state, *i.e.* correlation between K_i and EC_{50}, but the efficacy in coupling to G-proteins is dependent on substituent induced inactive conformations of CB2. While speculative, functional group modification to induce unique conformations with varying efficacy in G-protein coupling may be valuable in the future development of CB2 ligands.

Figure 3. Antagonist studies of selected triaryl analogs at CB2. A) **41** B) **45** C) SMM-189 versus CP 55,940. Insets are Schild analyses of antagonist interactions (black circles) with a reference line with the slope of 1 denoting orthosteric interactions (white squares). N = 6, error is SEM.



3. Conclusions

A series of alkyl and substituted phenyl analogs of SMM-189 were synthesized and evaluated for CB1 and CB2 affinity, potency, and efficacy. The *n*-hexyl (**34**) analog was inactive at CB1 and CB2 while the cyclohexyl (**35**) exhibited neutral antagonist activity. Substitution of the cyclohexyl with an aromatic ring provided compounds with inverse agonist activity thus demonstrating the requirement for an aromatic C ring in the biaryl methanone scaffold. Affinity of the aromatic analogs for CB1 was poor while affinity for CB2 was highly dependent on phenyl ring substitution. The introduction of alkyl groups at the 4 position was optimal with the ethyl (**41**) and propyl (**43**) groups yielding the highest affinity and potency. With the exception of the 4-chloro (**48**) analog, electron density on the phenyl ring was not tolerated and significantly decreased activity. Interestingly the addition of larger alkyl groups, *e.g.* **45** and **46**, at the 4 position of the phenyl ring decreased potency but increased efficacy. We hypothesize this effect

may be related to induction of unique inactive state conformations resulting different efficacies in G-protein coupling. The resorcinol ring is required for optimum inverse agonist activity at CB2. The introduction of the monomethoxy (**49**, **50**, **51**) significantly decreased affinity and potency but interestingly the compounds manifest good efficacy. The same was not true for the dimethoxy analogs in that no activity was observed at the receptors. We believe that continued development of the 3',5'-dichloro-2,6-dihydroxy-biphenyl-4-yl)-aryl –methanone scaffold will yield not only valuable information on the structure activity relationship but also provide new probes for mechanistic studies on CB2 receptor conformation and G-protein coupling.

4. Experimental

4.1 Reagents and supplies

All chemicals and reagents were purchased from Sigma–Aldrich or Fisher Scientific Inc. Anhydrous solvents were prepared by distillation over sodium metal or calcium hydride prior to use. Moisture sensitive reactions were carried out using oven dried glassware under dry conditions under an argon atmosphere. Analytical thin layer chromatography was performed on Silica G plates Sorbent Technologies (Atlanta, GA) and was visualized by fluorescence quenching under UV light. Compound purification was performed on a Biotage SP1 Flash Chromatography Purification System (Charlotte, NC) using Grace Reveleris columns. NMR spectra were acquired on a Bruker Ascend 400 (Billerica, MA) spectrometer (400 MHz for ^{1}H and 100 MHz for ^{13}C). Coupling constants (*J*) are expressed in hertz (Hz) and chemical shifts are reported in ppm on the δ scale and referenced to the appropriate solvent peak. Routine mass spectra were collected on a Brucker ESQUIRE electrospray/ion trap instrument (Billerica, MA). High resolution mass spectrometer (HRMS) data were acquired on a Waters Xevo G2+S QTOF (Milford, MA) system. Preparative HPLC was performed on a Gilson system (Middleton, WI) equipped with a 322 pump, 155 UV-Vis detector, and a 215 liquid handler, using a Phenomenex Luna PFP 100A 250 X 21.2 mm column and isocratic elution acetonitrile:water (9:1) flow rate 10 ml/min. Analytical HPLC was performed on a Waters e2695 HPLC (Milford, MA) with a 2998 photodiode array detector and Waters Bridge C18 5□m 4.6 X 150 mm column and isocratic elution acetonitrile:water (9:1) flow rate 0.3 ml/min. All target compounds were found to be ≥ 95% pure by analytical HPLC.

4.2 Synthesis

<u>Trifluoro-methanesulfonic acid 4-formyl-2,6-dimethoxy-phenyl ester</u>

The starting material syringaldehyde (**2**, 23 g, 126 mmol) was dissolved in dry dichloromethane (200 mL) to which 36 ml dry pyridine (0.46 mol) was added and the solution was cooled to 0 °C. To the cooled solution was added triflic anhydride (40g, 140 mmol) dropwise, when addition was complete the reaction was allowed to warm to ambient temperature then stirred for 2 hours. To the reaction mixture was added 600 mL of ethyl acetate followed by extraction with 1 N HCl (2 X 200 mL), sat. $NaHCO_3$ (200 mL), and the organic layer was dried over sodium sulfate and evaporated. The red solid was dissolved in 30 mL dichloromethane, insoluble material was removed by filtration, and loaded onto a Grace Reveleris 330g silica column, and eluted with a hexane:ethyl acetate gradient (10% to 10% 2 column volumes, 10% to 80% 10 column volumes, 80% to 80% 2 column volume, flow rate 100 mL per min.). Product

fractions were pooled and dried yielding 23.7 g (60%) of as white crystals. ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.94 (s, 1H, CHO); 7.17 (s, 2H, ArH), 3.84 (s, 6H, -OCH_3).

3',5'-Dichloro-2,6-dimethoxy-biphenyl-4-carbaldehyde (**3**)

Reactions were conducted in an 8 well aluminum block in 25 X 150 test tubes and 8 reaction were pooled for workup. To degassed dimethylformamide (14 mL) was added tetrakis(triphenylphosphine)palladium(0) (184 mg, 0.16 mmol) and the mixture was heated to 40 °C for 30 minutes to dissolve the catalyst then cooled to room temperature. To the solution was added **2** (1 g, 3.18 mmol) with stirring to dissolve then 3,5-dichlorophenylboronic acid (1.21 g, 6.36 mmol), degassed water (3 mL), and sodium carbonate (675 mg, 6.36 mmol) were added sequentially. Tubes were blanked with nitrogen, sealed with a septum, and heated to 40 °C for 16 hours. Reactions were pooled and filtered through a 0.5 X 4.5 cm Celite pad and the pad washed with 40 mL ethyl acetate. To the filtrate was added 100 mL water and the mixture was extracted with ethyl acetate (3 X 40 mL). The organic phase was extracted with water (3 X 30 mL) and brine (30 mL) and dried with sodium sulfate. The residue after solvent removal was dissolved in dichloromethane (10 mL) and loaded on a Grace Reveleris 120g silica column and eluted with a hexane:ethyl acetate gradient (5% to 5% 2 column volumes, 5% to 40% 10 column volumes, 40% to 40% 2 column volume, flow rate 80 mL per min.). Product fractions were pooled and dried yielding 6.52 g (82%) of **3** as white crystals. ^{1}H NMR, (400 MHz, $CDCl_3$), δ 9.98 (s, 1H, CHO), 7.34 (t, *J* = 2.0 Hz, 1H, 4'ArH), 7.21 (d, *J* = 2.0 Hz, 2H, 2',6'ArH), 7.15 (s, 2H, 2,6ArH), 3.83 (s, 6H, OCH_3), MS: m/z (ESI, pos.) = 333.6 $[M+23]^+$

1-(3',5'-Dichloro-2,6-dimethoxy-biphenyl-4-yl)-heptan-1-ol (**4**)

Glassware and magnesium turnings were dries overnight in a 110°C oven, assembled hot, and allowed to come to ambient temperature under dry positive nitrogen pressure. To the magnesium turning (290 mg, 12.1 mmol) was added dry THF (12 mL), n-bromohexane (2 g, 12.1 mmol), and 1 drop of 1,2-dibromoethane and the mixture was heated to reflux, the reaction was determined to be complete when the magnesium turnings were consumed. The solution was allowed to cool to ambient temperature then 6.5 mL (6.5 mmol) of the Grignard reagent was added dropwise to an ice cold solution of **3** (1 g, 3.2 mmol) in dry THF (3.5 mL). The solution was allowed to warm to ambient temperature then stirred for an additional 3 hours. The reaction was quenched with saturated ammonium chloride (15 mL), layers separated, and the aqueous phase extracted with THF (2 X 15 mL), and the organic phase extracted with brine (15 mL) then dried over sodium sulfate. Solvent was removed and the residue was loaded onto a Grace Reveleris 12g silica column and eluted with a hexane:ethyl acetate gradient (5% to 5% 2 column volumes, 5% to 40% 10 column volumes, 40% to 40% 2 column volume, flow rate 36 mL per min.). Product fractions were pooled and dried yielding 1.10 g (73%) of **4** as a white solid. ^{1}H NMR, (400 MHz, $CDCl_3$), δ 7.30 (t, *J* = 2.0 Hz, 1H, 4'ArH), 7.21 (d, *J* = 2.0 Hz, 2H, 2',6'ArH), 6.62 (s, 2H, 2,6ArH), 5.30 (s, 1H, CHO*H*), 4.69 (t, 1H, C*H*OH), 3.83 (s, 6H, OCH_3), 1.78 (m, 2H, C$H_2C_5H_{11}$), 1.31 (m, 8H, C_4H_8), 0.89 (t, *J* = 7 Hz, 3H, CH_3), MS: m/z (ESI, pos.) = 419.5 $[M+23]^+$

Utilizing cyclohexylbromide or the appropriately substituted bromo-benzene derivatives the following alcohols were similarly prepared.

Cyclohexyl-(3',5'-dichloro-2,6-dimethoxy-biphenyl-4-yl)-methanol (**5**)

White solid (0.49 g, 38%) ^{1}H NMR, (400 MHz, $CDCl_3$), δ 7.29 (t, *J* = 2.0 Hz, 1H, 4'ArH), 7.23 (d, *J* = 2.0 Hz, 2H, 2',6'ArH), 6.51 (s, 2H, 2,6ArH), 4.31 (t, 1H, C*H*OH), 3.83 (s, 6H, OCH_3), 1.73 (m, 1H, C*H*C_5H_{10}), 1.61 (m, 4H, CH_2), 1.08 (m, 6H, CH_2), MS: m/z (ESI, pos.) = 417.5 $[M+23]^+$

(3',5'-Dichloro-2,6-dimethoxy-biphenyl-4-yl)-p-tolyl-methanol (**6**)

Off white solid (0.87 g, 69%) ^{1}H NMR, (400 MHz, $CDCl_3$), δ 7.32 (d, *J* = 8.0 Hz, 2H, 9,13ArH) 7.29 (t, *J* = 2.0 Hz, 1H, 4'ArH), 7.23 (d, *J* = 2.0 Hz, 2H, 2',6'ArH), 7.19 (d, J = 8.0 Hz, 2H, 10,12ArH), 6.68 (s, 2H, 2,6ArH), 5.83 (t, 1H, C*H*OH), 3.73 (s, 6H, OCH_3), 2.37 (s, 3H, $ArCH_3$), MS: m/z (ESI, pos.) = 425.4 $[M+23]^+$

(3',5'-Dichloro-2,6-dimethoxy-biphenyl-4-yl)-m-tolyl-methanol (**7**)

Off white solid (0.91 g, 70%) ^{1}H NMR, (400 MHz, $CDCl_3$), δ 7.29 (t, *J* = 2.1 Hz, 1H, 4'ArH) 7.27 (d, *J* = 7.5 Hz, 1H, 12ArH), 7.24 (m, 2H, 11,13ArH), 7.22 (d, *J* = 2.0 Hz, 2H, 2'6'ArH), 7.14 (d, J = 7.7 Hz, 1H, 9ArH), 6.68 (s, 2H, 2,6ArH), 5.82 (d, *J* = 3.3, 1H, C*H*OH), 3.73 (s, 6H, OCH_3), 2.38 (s, 3H, $ArCH_3$), MS: m/z (ESI, pos.) = 425.3 $[M+23]^+$

(3',5'-Dichloro-2,6-dimethoxy-biphenyl-4-yl)-(3,5-dimethyl-phenyl)-methanol (**8**)

Off white solid (1.25 g, 94%) ^{1}H NMR, (400 MHz, $CDCl_3$), δ 7.29 (t, *J* = 1.9 Hz, 1H, 4'ArH), 7.21 (d, *J* = 2.0 Hz, 2H, 2',6'ArH), 7.03 (s, 2H, 9,13ArH), 6.96 (s, 1H, 11ArH), 6.69 (s, 2H, 2,6ArH), 5.78 (d, *J* = 3.3 Hz, 1H, C*H*OH), 3.74 (s, 6H, OCH_3), 2.06 (s, 6H, $ArCH_3$), MS: m/z (ESI, pos.) = 439.6 $[M+23]^+$

(3',5'-Dichloro-2,6-dimethoxy-biphenyl-4-yl)-(4-trifluoromethyl-phenyl)-methanol (**9**)

White solid (1.03 g, 70%) ^{1}H NMR, (400 MHz, $CDCl_3$), δ 7.65 (d, *J* = 8.2 Hz, 2H, 10,12ArH), 7.57 (d, *J* = 8.3 Hz, 2H, 9,13ArH), 7.30 (t, *J* = 1.9 Hz, 1H, 4'ArH), 7.20 (d, *J* = 1.9 Hz, 2H, 2',6'ArH), 6.65 (s, 2H, 2,6ArH), 5.90 (d, J = 3.24 Hz, 1H, C*H*OH), 3.73 (s, 6H, OCH_3), MS: m/z (ESI, pos.) = 481.3 $[M+23]^+$

(3',5'-Dichloro-2,6-dimethoxy-biphenyl-4-yl)-(3-trifluoromethyl-phenyl)-methanol (**10**)

White solid (1.21 g, 82%) ^{1}H NMR, (400 MHz, $CDCl_3$), δ 7.76 (s, 1H, 9ArH), 7.60 (t, *J* = 7.9 Hz, 2H, 11,13ArH), 7.51 (d, *J* = 7.9Hz, 1H, 12ArH), 7.30 (t, *J* = 1.8 Hz, 1H, 4'ArH), 7.21 (d, *J* = 1.8 Hz, 2H, 2',6'ArH), 6.65 (s, 2H, 2,6ArH), 5.89 (d, J = 3.14 Hz, 1H, C*H*OH), 3.73 (s, 6H, OCH_3), MS: m/z (ESI, pos.) = 481.4 $[M+23]^+$

(3',5'-Dichloro-2,6-dimethoxy-biphenyl-4-yl)-(4-ethyl-phenyl)-methanol (**11**)

White crystals (0.80 g, 60%) ^{1}H NMR, (400 MHz, $CDCl_3$), δ 7.34 (d, *J* = 8.0 Hz, 2H, 9,13ArH), 7.29 (t, *J* = 2.0 Hz, 1H, 4'ArH), 7.22 (d, *J* = 8.0 Hz, 2H, 10,12ArH), 7.21 (d, *J* = 1.9 Hz, 2H, 2',6'ArH), 6.69 (s, 2H,

2,6ArH), 5.83 (d, J = 3.4 Hz, 1H, C*H*OH), 3.73 (s, 6H, OCH_3), 2.66 (q, *J* = 7.7 Hz, 2H, C*H*$_2$CH$_3$), 1.25 (t, *J* = 7.7 Hz, 3H, CH$_2$C*H*$_3$), MS: m/z (ESI, pos.) = 437.5 $[M+23]^+$

(3',5'-Dichloro-2,6-dimethoxy-biphenyl-4-yl)-(3-ethyl-phenyl)-methanol (**12**)

White crystals (1.24 g, 92%) ^{1}H NMR, (400 MHz, $CDCl_3$), δ 7.36 (d, *J* = 2.0 Hz 1H, 12ArH), 7.29 (t, *J* = 2.0 Hz, 1H, 4'ArH), 7.24 (t, *J* = 1.6 Hz, 1H, 9ArH), 7.22 (d, *J* = 2.0 Hz, 2H, 2',6'ArH), 7.17 (d, *J* = 7.4 Hz, 1H, 13ArH), 7.11 (d, *J* = 7.0 Hz, 1H, 11ArH), 6.69 (s, 2H, 2,6ArH), 5.83 (s, 1H, C*H*OH), 3.73 (s, 6H, OCH_3), 2.68 (q, *J* = 7.7 Hz, 2H, C*H*$_2$CH$_3$), 1.27 (t, *J* = 7.6 Hz, 3H, CH$_2$C*H*$_3$), MS: m/z (ESI, pos.) = 439.3 $[M+23]^+$

(3',5'-Dichloro-2,6-dimethoxy-biphenyl-4-yl)-(4-propyl-phenyl)-methanol (**13**)

White solid (1.15 g, 82%) ^{1}H NMR, (400 MHz, $CDCl_3$), δ 7.33 (d, *J* = 8.0 Hz, 2H, 9,13ArH), 7.29 (t, *J* = 2.1 Hz, 1H, 4'ArH), 7.21 (d, *J* = 2.0 Hz, 2H, 2',6'ArH), 7.20 (d, *J* = 8.0 Hz, 2H, 10,12ArH), 6.69 (s, 2H, 2,6ArH), 5.83 (d, *J* = 3.4 Hz, 1H, C*H*OH), 3.73 (s, 6H, OCH_3), 2.60 (t, *J* = 8.0 Hz, 2H, C*H*$_2$CH$_2$CH$_3$), 1.65 (sxt, *J* = 7.7 Hz, 2H, CH$_2$C*H*$_2$CH$_3$), 0.95 (t, *J* = 7.7 Hz, 3H, CH$_2$CH$_2$C*H*$_3$), MS: m/z (ESI, pos.) = 453.2 $[M+23]^+$

(3',5'-Dichloro-2,6-dimethoxy-biphenyl-4-yl)-(4-isopropyl-phenyl)-methanol (**14**)

White crystals (0.94 g, 68%) ^{1}H NMR, (400 MHz, $CDCl_3$), δ 7.35 (d, *J* = 8.2 Hz, 2H, 9,13ArH), 7.29 (t, *J* = 2.0 Hz, 1H, 4'ArH), 7.25 (d, *J* = 8.2 Hz, 2H, 10,12ArH), 7.21 (d, *J* = 2.0 Hz, 2H, 2',6'ArH),), 6.70 (s, 2H, 2,6ArH), 5.83 (d, *J* = 3.5 Hz, 1H, C*H*OH), 3.73 (s, 6H, OCH_3), 2.92 (spt, *J* = 7.1 Hz, 1H, C*H*$(CH_3)_2$), 1.26 (d, *J* = 7.0 Hz, 6H, CH$(CH_3)_2$), MS: m/z (ESI, pos.) = 453.8 $[M+23]^+$

(4-Butyl-phenyl)-(3',5'-dichloro-2,6-dimethoxy-biphenyl-4-yl)-methanol (**15**)

Off white crystals (1.25 g, 87%) ^{1}H NMR, (400 MHz, $CDCl_3$), δ 7.41 (d, *J* = 8.2 Hz, 2H, 9,13ArH), 7.33 (d, *J* = 8.2 Hz, 2H, 10,12ArH), 7.29 (t, *J* = 2.1 Hz, 1H, 4'ArH), 7.21 (d, *J* = 2.1 Hz, 2H, 2',6'ArH), 6.69 (s, 2H, 2,6ArH), 5.83 (s, 1H, C*H*OH), 3.73 (s, 6H, OCH_3), 2.62 (t, *J* = 7.9 Hz, 2H, C*H*$_2$CH$_2$CH$_2$CH$_3$), 1.61 (p, *J* = 8.0 Hz, 2H, CH$_2$C*H*$_2$CH$_2$CH$_3$), 1.37 (sxt, *J* = 8.0 Hz, 2H, CH$_2$CH$_2$C*H*$_2$CH$_3$), 0.94 (t, *J* = 7.5 Hz, 3H, CH$_2$CH$_2$C*H*$_2$C*H*$_3$), MS: m/z (ESI, pos.) = 467.1 $[M+23]^+$

(4-tert-Butyl-phenyl)-(3',5'-dichloro-2,6-dimethoxy-biphenyl-4-yl)-methanol (**16**)

White crystals (1.34 g, 94%) ^{1}H NMR, (400 MHz, $CDCl_3$), δ 7.41 (d, *J* = 8.2 Hz, 2H, 9,13ArH), 7.36 (d, *J* = 8.2 Hz, 2H, 10,12ArH), 7.29 (t, *J* = 2.0 Hz, 1H, 4'ArH), 7.21 (d, *J* = 2.1 Hz, 2H, 2',6'ArH), 6.71 (s, 2H, 2,6ArH), 5.84 (d, *J* = 3.0, 1H, C*H*OH), 3.74 (s, 6H, OCH_3), 1.33 (s, 9H, C$(CH_3)_3$), MS: m/z (ESI, pos.) = 467.3 $[M+23]^+$

(3',5'-Dichloro-2,6-dimethoxy-biphenyl-4-yl)-(4-methoxy-phenyl)-methanol (**17**)

White solid (0.81 g, 60%) ^{1}H NMR, (400 MHz, $CDCl_3$), δ 7.33 (d, *J* = 8.5 Hz, 2H, 9,13ArH), 7.29 (t, *J* = 2.0 Hz, 1H, 4'ArH), 7.21 (d, *J* = 2.0 Hz, 2H, 2',6'ArH), 6.90 (d, *J* = 8.6 Hz, 2H, 10,12ArH), 6.67 (s, 2H, 2,6ArH), 5.84 (d, *J* = 3.4, 1H, C*H*OH), 4.05 (s, 3H, 11ArOC*H*$_3$), 3.74 (s, 6H, 3,6ArOC*H*$_3$), MS: m/z (ESI, pos.) = 441.2 $[M+23]^+$

(4-Chloro-phenyl)-(3',5'-dichloro-2,6-dimethoxy-biphenyl-4-yl)-methanol (**18**)

White solid (1.60g, 56%) ^{1}H NMR, (400 MHz, $CDCl_3$), δ 7.37 (s, 2H, 10,12ArH), 7.36 (s, 2H, 9,13ArH), 7.29 (t, *J* = 2.0 Hz, 1H, 4'ArH), 7.20 (d, *J* = 2.1 Hz, 2H, 2',6'ArH), 6.64 (s, 2H, 2,6ArH), 5.83 (d, *J* = 3.5 Hz, 1H, C*H*OH), 3.73 (s, 6H, OCH_3). MS: m/z (ESI, pos.) = 445.6 $[M+23]^+$

1-(3',5'-Dichloro-2,6-dimethoxy-biphenyl-4-yl)-heptan-1-one (**19**)

A mixture of pyridinium chlorochromate (218 mg, 1 mmol) in dichloromethane (4 mL) in a 25 X 150 test tube was heated to reflux then cooled to ambient temperature. A solution of **4** (202 mg, 0.51 mmol) in dichloromethane (2 mL) was added dropwise and the mixture was stirred at ambient temperature for 1.5 hours. The reaction was quenched with 1N sodium hydroxide (10 mL), separated, the aqueous phase extracted with dichloromethane (2 X 10 mL), the combined organic phase dried with sodium sulfate, and the solvent removed. The residue was loaded onto a Grace Reveleris 12g silica column and eluted with a hexane:ethyl acetate gradient (5% to 5% 2 column volumes, 5% to 40% 10 column volumes, 40% to 40% 2 column volume, flow rate 36 mL per min.). Product fractions were pooled and dried yielding 95.3 mg (47%) of **19** as a white solid. ^{1}H NMR, (400 MHz, $CDCl_3$), δ 7.34 (t, *J* = 2.0 Hz, 1H, 4'ArH), 7.23 (s, 2H, 2,6ArH), 7.22 (d, *J* = 2.0 Hz, 2H, 2',6'ArH), 3.82 (s, 6H, OCH_3), 3.00 (t, *J* = 7.5 Hz, 2H, C(O)CH_2C$_5$H$_{11}$), 1.78 (p, *J* = 7.6 Hz, 2H, $CH_2CH_2C_4H_9$), 1.38 (m, 6H, $CH_2CH_2(CH_2)_3CH_3$), 0.92 (t, *J* = 7.9, 3H, $C_5H_{10}CH_3$), MS: m/z (ESI, pos.) = 417.0 $[M+23]^+$

Utilizing the appropriate alcohol the following ketones were prepared

Cyclohexyl-(3',5'-dichloro-2,6-dimethoxy-biphenyl-4-yl)-methanone (**20**)

Off white solid (123 mg, 61%) ^{1}H NMR, (400 MHz, $CDCl_3$), δ 7.34 (t, *J* = 2.0 Hz, 1H, 4'ArH), 7.22 (d, *J* = 2.0 Hz, 2H, 2',6'ArH), 7.20 (s, 2H, 2,6ArH), 3.82 (s, 6H, OCH_3), 3.25 (tt, *J* = 3.2, 11.5 Hz, 1H, C(O)CHC$_5$H$_{10}$), 1.92 (m, 2H, CH_2), 1.78(m, 2H, CH_2), 1.44 (m, 6H, C_3H_6), MS: m/z (ESI, pos.) = 415.0 $[M+23]^+$

(3',5'-Dichloro-2,6-dimethoxy-biphenyl-4-yl)-p-tolyl-methanone (**21**)

White solid (138 mg, 69%). ^{1}H NMR, (400 MHz, $CDCl_3$), δ 7.79 (d, *J* = 8.2 Hz, 2H, 9,13ArH), 7.35 (t, *J* = 1.9 Hz, 1H, 4'ArH), 7.33 (d, *J* = 8.2 Hz, 2H, 10,12ArH), 7.25 (d, *J* = 1.9 Hz, 2H, 2',6'ArH), 7.03 (s, 2H, 2,6ArH), 3.77 (s, 6H, OCH_3), 2.48 (s, 3H, $ArCH_3$). ^{13}C NMR, (100 MHz, $CDCl_3$), δ 195.9, 157.1, 143.7, 139.6, 139.2, 135.4, 134.2, 130.4, 129.3, 129.1, 125.7, 115.5, 105.8, 56.1, 21.8. HRMS: m/z calculated for $C_{22}H_{19}Cl_2O_3S$, $[M+H]^+$ 401.0711, Found: 401.0710.

(3',5'-Dichloro-2,6-dimethoxy-biphenyl-4-yl)-m-tolyl-methanone (**22**)

White solid (576 mg, 71%) ^{1}H NMR, (400 MHz, $CDCl_3$), δ 7.71 (m, 1H, 9ArH), 7.64 (d, *J* = 7.6 Hz, 1H, 13ArH), 7.44 (m, 1H, 12ArH), 7.41 (d, *J* = 7.6, 1H, 11ArH), 7.35 (t, *J* = 1.9 Hz, 1H, 4'ArH), 7.26 (d, *J* = 1.9 Hz, 2H, 2',6'ArH), 7.06 (s, 2H, 2,6ArH), 3.77 (s, 6H, OCH_3), 2.46 (s, 3H, $ArCH_3$), MS: m/z (ESI, pos.) = 423.2 $[M+23]^+$

(3',5'-Dichloro-2,6-dimethoxy-biphenyl-4-yl)-(3,5-dimethyl-phenyl)-methanone (**23**)

Light yellow solid (151 mg, 76%) ^{1}H NMR, (400 MHz, $CDCl_3$), δ 7.47 (s, 2H, 9,13ArH), 7.35 (t, *J* = 1.9 Hz, 1H, 4'ArH), 7.27 (s, 1H, 11ArH), 7.26 (d, *J* = 1.8 Hz, 2H, 2',6'ArH),), 7.05 (s, 2H, 2,6ArH), 3.77 (s, 6H, OCH_3), 2.41 (s, 6H, $ArCH_3$), MS: m/z (ESI, pos.) = 437.3 $[M+23]^+$

(3',5'-Dichloro-2,6-dimethoxy-biphenyl-4-yl)-(4-trifluoromethyl-phenyl)-methanone (**24**)

White crystals (151 mg, 76%) ^{1}H NMR, (400 MHz, $CDCl_3$), δ 7.97 (d, *J* = 8.2 Hz, 2H, 9,13ArH), 7.80 (d, *J* = 8.2 Hz, 2H, 10,12ArH), 7.36 (t, *J* = 1.8 Hz, 1H, 4'ArH), 7.25 (d, *J* = 1.8 Hz, 2H, 2',6'ArH), 7.04 (s, 2H, 2,6ArH), 3.78 (s, 6H, OCH_3), MS: m/z (ESI, pos.) = 477.0 $[M+23]^+$

(3',5'-Dichloro-2,6-dimethoxy-biphenyl-4-yl)-(3-trifluoromethyl-phenyl)-methanone (**25**)

White crystals (174 mg, 85%) ^{1}H NMR, (400 MHz, $CDCl_3$), δ 8.14 (s, 1H, 9ArH), 8.07 (d, *J* = 7.8 Hz, 1H, 11ArH), 7.90 (d, *J* = 7.8 Hz, 1H, 13ArH), 7.69 (t, *J* = 7.8 Hz, 1H, 12ArH), 7.36 (t, *J* = 1.9 Hz, 1H, 4'ArH), 7.26 (d, *J* = 1.9 Hz, 2H, 2',6'ArH), 7.04 (s, 2H, 2,6ArH), 3.78 (s, 6H, OCH_3), MS: m/z (ESI, pos.) = 477.0 $[M+23]^+$

(3',5'-Dichloro-2,6-dimethoxy-biphenyl-4-yl)-(4-ethyl-phenyl)-methanone (**26**)

White crystals (155 mg, 78%) ^{1}H NMR, (400 MHz, $CDCl_3$), δ 7.82 (d, *J* = 8.3 Hz, 2H, 9,13ArH), 7.36 (t, *J* = 1.9 Hz, 1H, 4'ArH), 7.35 (m, 2H, 10,12ArH), 7.26 (d, *J* = 1.9 Hz, 2H, 2',6'ArH), 7.05 (s, 2H, 2,6ArH), 3.78 (s, 6H, OCH_3), 2.77 (q, *J* = 7.7 Hz, 2H, CH_2CH_3), 1.32 (t, *J* = 7.6 Hz, 3H, CH_2CH_3), MS: m/z (ESI, pos.) = 437.5 $[M+23]^+$

(3',5'-Dichloro-2,6-dimethoxy-biphenyl-4-yl)-(3-ethyl-phenyl)-methanone (**27**)

Off white solid (116 mg, 56%) ^{1}H NMR, (400 MHz, $CDCl_3$), δ 7.73 (s, 1H, 9ArH), 7.68 (dt, *J* = 1.7, 7.3 Hz, 1H, 13ArH), 7.48 (m, 1H, 11ArH), 7.44 (t, *J* = 7.5, 1H, 12ArH), 7.35 (t, *J* = 2.1 Hz, 1H, 4'ArH), 7.27 (d, *J* = 2.0 Hz, 2H, 2',6'ArH), 7.06 (s, 2H, 2,6ArH), 3.78 (s, 6H, OCH_3), 2.76 (q, *J* = 7.6 Hz, 2H, CH_2CH_3), 1.30 (t, *J* = 7.7 Hz, 3H, CH_2CH_3), MS: m/z (ESI, pos.) = 437.1 $[M+23]^+$

(3',5'-Dichloro-2,6-dimethoxy-biphenyl-4-yl)-(4-propyl-phenyl)-methanone (**28**)

Off white solid (125 mg, 62%) ^{1}H NMR, (400 MHz, $CDCl_3$), δ 7.82 (d, *J* = 8.3 Hz, 2H, 9,13ArH), 7.35 (t, *J* = 2.0 Hz, 1H, 4'ArH), 7.33 (d, *J* = 8.3, 2H, 10,12ArH), 7.26 (d, *J* = 2.0 Hz, 2H, 2',6'ArH), 7.04 (s, 2H, 2,6ArH), 3.78 (s, 6H, OCH_3), 2.70 (t, *J* = 7.5 Hz, 2H, $CH_2CH_2CH_3$), 1.72 (sxt, *J* = 7.7 Hz, 2H, $CH_2CH_2CH_3$), 1.00 (t, *J* = 7.4, 3H, $CH_2CH_2CH_3$), MS: m/z (ESI, pos.) = 451.1 $[M+23]^+$

(3',5'-Dichloro-2,6-dimethoxy-biphenyl-4-yl)-(4-isopropyl-phenyl)-methanone (**29**)

White solid (123 mg, 62%) ^{1}H NMR, (400 MHz, $CDCl_3$), δ 7.83 (d, *J* = 8.3 Hz, 2H, 9,13ArH), 7.38 (d, *J* = 8.3 Hz, 2H, 10,12ArH), 7.35 (t, *J* = 2.0 Hz, 1H, 4'ArH), 7.26 (d, *J* = 2.0 Hz, 2H, 2',6'ArH), 7.05 (s, 2H, 2,6ArH), 3.78 (s, 6H, OCH_3), 3.03 (spt. *J* = 6.8 Hz, 1H, $CH(CH_3)_2$), 1.32 (d, *J* = 6.8 Hz, 6H, $CH(CH_3)_2$), MS: m/z (ESI, pos.) = 451.1 $[M+23]^+$

(4-Butyl-phenyl)-(3',5'-dichloro-2,6-dimethoxy-biphenyl-4-yl)-methanone (**30**)

White solid (460 mg, 40%). ^{1}H NMR, (400 MHz, $CDCl_3$), δ 7.82 (d, *J* = 8.0 Hz, 2H, 9,13ArH), 7.34 (d, *J* = 8.0 Hz, 2H, 10,12ArH), 7.32 (t, *J* = 2.1 Hz, 1H, 4'ArH), 7.26 (d, *J* = 2.1 Hz, 2H, 2',6'ArH), 7.05 (s, 2H, 2,6ArH), 3.78 (s, 6H, OCH_3), 2.73 (t, *J* = 8.8 Hz, 2H, $CH_2(CH_2)_2CH_3$), 1.67 (p, *J* = 8.0, 2H, $CH_2CH_2CH_2CH_3$), 1.41 (sxt. *J* = 8.4, 2H, $CH_2CH_2CH_2CH_3$), 0.97 (t, *J* = 7.6, 3H, $(CH_2)_3CH_3$). ^{13}C NMR, (100 MHz, $CDCl_3$), δ 195.8, 157.1, 148.6, 139.2, 136.3, 135.4, 129.2, 128.5, 127.3, 112.7, 105.7, 56.2, 35.8, 33.3, 22.4, 13.95. HRMS: m/z calculated for $C_{25}H_{25}Cl_2O_3$, $[M+H]^+$ 443.1181, Found: 443.1182.

(4-tert-Butyl-phenyl)-(3',5'-dichloro-2,6-dimethoxy-biphenyl-4-yl)-methanone (**31**)

White solid (146 mg, 73%) ^{1}H NMR, (400 MHz, $CDCl_3$), δ 7.84 (d, *J* = 8.6 Hz, 2H, 9,13ArH), 7.54 (d, *J* = 8.6, 2H, 10,12ArH), 7.35 (t, *J* = 2.0 Hz, 1H, 4'ArH), 7.26 (d, *J* = 2.0 Hz, 2H, 2',6'ArH), 7.05 (s, 2H, 2,6ArH), 3.78 (s, 6H, OCH_3), 1.39 (s, 9H, $C(CH_3)_3$), MS: m/z (ESI, pos.) = 465.1 $[M+23]^+$

(3',5'-Dichloro-2,6-dimethoxy-biphenyl-4-yl)-(4-methoxy-phenyl)-methanone (**32**)

White solid (140 mg, 72%) ^{1}H NMR, (400 MHz, $CDCl_3$), δ 7.89 (d, *J* = 8.8 Hz, 2H, 9,13ArH), 7.35 (t, *J* = 2.1 Hz, 1H, 4'ArH), 7.26 (d, *J* = 2.1 Hz, 2H, 2',6'ArH), 7.00 (s, 2H, 2,6ArH), 6.99 (d, *J* = 8.8 Hz, 2H, 10,12ArH), 3.78 (s, 6H, OCH_3), 4.15 (s, 3H, OCH_3), MS: m/z (ESI, pos.) = 439.8 $[M+23]^+$

(4-Chloro-phenyl)-(3',5'-dichloro-2,6-dimethoxy-biphenyl-4-yl)-methanone (**33**)

Off white solid (140 mg, 72%). ^{1}H NMR, (400 MHz, $CDCl_3$), δ 7.82 (d, *J* = 8.9 Hz, 2H, 9,13ArH), 7.50 (d, *J* = 8.9 Hz, 2H, 10,12ArH), 7.34 (t, *J* = 2.1 Hz, 1H, 4'ArH), 7.24 (d, *J* = 2.1 Hz, 2H, 2',6'ArH), 7.01 (s, 2H, 2,6ArH), 3.78 (s, 6H, OCH_3). ^{13}C NMR, (100 MHz, $CDCl_3$), δ 194.8, 157.4, 139.2, 138.5, 136.2, 135.8, 134.2, 131.4, 129.2, 128.8, 127.4, 121.0, 105.8, 56.2. HRMS: m/z calculated for $C_{21}H_{16}Cl_3O_3$, $[M+H]^+$ 421.0165, Found: 421.0163.

1-(3',5'-Dichloro-2,6-dihydroxy-biphenyl-4-yl)-heptan-1-one (**34**)

Into a 8mm X 10.2cm pressure tube (Ace Glass) with stirring bar was loaded **19** (60 mg, 0.15 mmol) and pyridine hydrochloride (520 mg, 4.6 mmol), flushed with nitrogen, sealed, and heated to 220° C in an oil bath. Immediately following the melt the mixture was shaken to thoroughly mix the reactants and returned to the bath for 2 hours. The reaction was allowed to cool to ambient temperature then water (2 mL) and diethyl ether (2 mL) were added to dissolve the brown crystalline material. Ten milliliters of water were added and the aqueous phase was extracted with diethyl ether (3 X 5 mL) and the combined organic phases were dried over sodium sulfate and the solvent removed. The residue was loaded onto a Grace Reveleris 12g silica column and eluted with a hexane:methyl t-butyl ether gradient (5% to 5% 4 column volumes, 5% to 80% 13 column volumes, 80% to 80% 2 column volume, flow rate 36 mL per min.). Product fractions were pooled and dried yielding 47.0 mg (84%) of **34** as a pink solid. A sample of **34** was recrystallized from methanol:water to yield a white solid.

Analytical HPLC retention time 7.40 min; purity 99.0%, ^{1}H NMR, (400 MHz, d_6-acetone), δ 7.44 (d, *J* = 1.9 Hz, 2H, 2',6'ArH), 7.42 (t, *J* = 1.9 Hz, 1H, 4'ArH), 7.15 (s, 2H, 2,6ArH), 2.93 (t, *J* = 7.3 Hz, 2H,

C(O)CH_2(CH_2)$_4$$CH_3$), 1.68 (p, *J* = 7.5 Hz, 2H, CH_2CH_2(CH_2)$_3$$CH_3$), 1.34 (m, 6H, CH_2CH_2(CH_2)$_3$$CH_3$), 0.89 (t, *J* = 7.2 Hz, 3H, (CH_2)$_5$CH_3). ^{13}C NMR, (100 MHz, d_6-acetone), δ 206.2, 156.5, 139.4, 138.6, 134.8, 130.5, 127.6, 118.9, 107.9, 39.1, 32.5, 25.2, 23.3, 14.0. HRMS: m/z calculated for $C_{19}H_{21}Cl_2O_3$, $[M+H]^+$ 367.0868, Found: 367.0858

Using pyridine HCl the following compounds were prepared.

Cyclohexyl-(3',5'-dichloro-2,6-dihydroxy-biphenyl-4-yl)-methanone (**35**)

Pink solid (56.2 mg, 89%). The material was future purified by preparative reverse phase HPLC. Analytical HPLC retention time 7.00 min; purity 97.5%, ^{1}H NMR, (400 MHz, d_6-acetone), δ 7.45 (d, *J* = 2.2 Hz, 2H, 2',6'ArH), 7.43 (t, *J* = 2.2 Hz, 1H, 4'ArH), 7.16 (s, 2H, 2,6ArH), 3.22 (m, 1H, C(O)C*H*C_5H_{10}), 1.85 (m, 2H, CH_2CH_2(CH_2)$_3$), 1.71 (m, 2H, CH_2CH_2(CH_2)$_3$), 1.42 (m, 6H, CH_2CH_2(CH_2)$_3$). ^{13}C NMR, (100 MHz, d_6-acetone), δ 203.8, 156.4, 139.4, 138.4, 134.5, 130.5, 127.4, 118.8, 108, 46.8, 30.5, 26.8, 26.5, HRMS: m/z calculated for $C_{19}H_{19}Cl_2O_3$, $[M+H]^+$ 365.0711, Found: 365.0707

(3',5'-Dichloro-2,6-dihydroxy-biphenyl-4-yl)-p-tolyl-methanone (**36**)

Pale pink solid solid (49.0 mg, 81%). The material was future purified by preparative reverse phase HPLC. Analytical HPLC retention time 6.64 min; purity 99.6% ^{1}H NMR, (400 MHz, d_6-acetone), δ 7.72 (d, *J* = 8.0 Hz, 2H, 9,13ArH), 7.48 (d, *J* = 1.9 Hz, 2H, 2',6'ArH), 7.43 (t, *J* = 1.9 Hz, 1H, 4'ArH), 7.38 (d, *J* = 8.0 Hz, 2H, 10,12ArH), 6.93 (s, 2H, 2,6ArH), 2.44 (s, 3H, $ArCH_3$). ^{13}C NMR, (100 MHz, $CDCl_3$), δ 195.6, 156.1, 143.9, 140.0, 138.6, 135.9, 134.7, 130.7, 130.5, 129.8, 127.43, 109.6, 109.5, 21.5. HRMS: m/z calculated for $C_{20}H_{15}Cl_2O_3$, $[M+H]^+$ 373.0398, Found: 373.0392

(3',5'-Dichloro-2,6-dihydroxy-biphenyl-4-yl)-m-tolyl-methanone (**37**)

Off white solid (49.6 mg, 52%). The material was recrystallized from methanol:water to yield a white solid. Analytical HPLC retention time 6.61 min; purity 96.5% ^{1}H NMR, (400 MHz, , d_6-acetone), δ 7.63 (m, 1H, 9ArH), 7.59 (d, *J* = 7.4 Hz, 1H, 13ArH), 7.49 (m, 1H, 12ArH), 7.48 (t, *J* = 1.9 Hz, 1H, 4'ArH), 7.47 (m, 1H, 11ArH), 7.44 (d, *J* = 1.9 Hz, 2H, 2',6'ArH), 6.95 (s, 2H, 2,6ArH), 2.43 (s, 3H, $ArCH_3$). ^{13}C NMR, (100 MHz, $CDCl_3$), δ 195.9, 156.2, 139.7, 139.0, 138.8, 138.6 , 134.8, 133.9, 130.9, 130.6, 129.1, 127.8, 127.5, 109.8, 21.4. HRMS: m/z calculated for $C_{20}H_{15}Cl_2O_3$, $[M+H]^+$ 373.0398, Found: 373.0396

(3',5'-Dichloro-2,6-dihydroxy-biphenyl-4-yl)-(3,5-dimethyl-phenyl)-methanone (**38**)

Pale pink solid (62.9 mg, 71%). The material was recrystallized from methanol:water to yield a white solid. Analytical HPLC retention time 6.98 min; purity 96.3% ^{1}H NMR, (400 MHz, d_6-acetone), δ 7.48 (d, *J* = 2.0 Hz, 2H, 2',6'ArH), 7.43 (t, *J* = 2.0 Hz, 1H, 4'ArH), 7.41 (m, 2H, 9,13ArH), 7.30 (m, 1H, 11ArH), 6.95 (s, 2H, 2,6ArH), 2.38 (s, 6H, $ArCH_3$). ^{13}C NMR, (100 MHz, d_6-acetone), δ 196.0, 156.2, 139.8, 138.8, 138.5, 134.7, 134.6, 130.5, 128.2, 127.5, 118.4, 109.7, 21.2. HRMS: m/z calculated for $C_{21}H_{17}Cl_2O_3$, $[M+H]^+$ 387.0555, Found: 387.0555

(3',5'-Dichloro-2,6-dihydroxy-biphenyl-4-yl)-(4-trifluoromethyl-phenyl)-methanone (**39**)

Pink solid (63.5 mg, 85%). The material was recrystallized from methanol:water to yield a white solid. Analytical HPLC retention time 6.80 min; purity 95.3%. ^{1}H NMR, (400 MHz, d_6-acetone), δ 8.01 (d, *J* = 8.0 Hz, 2H, 9,13ArH), 7.94 (d, *J* = 8.0 Hz, 2H, 10,12ArH), 7.49 (d, *J* = 1.9 Hz, 2H, 2',6'ArH), 7.45 (t, *J* = 1.9 Hz, 1H, 4'ArH), 7.00 (s, 2H, 2,6ArH). ^{13}C NMR, (100 MHz, d_6-acetone), δ 194.9, 156.3, 142.3, 138.6, 138.2, 134.8, 133.5, 130.9, 130.4, 127.6, 126.3, 123.6, 119.2, 109.3. HRMS: m/z calculated for $C_{20}H_{12}Cl_2F_3O_3$, $[M+H]^+$ 427.0116, Found: 427.0111

(3',5'-Dichloro-2,6-dihydroxy-biphenyl-4-yl)-(3-trifluoromethyl-phenyl)-methanone (**40**)

Pink solid (66.8 mg, 86%). The material was future purified by preparative reverse phase HPLC. Analytical HPLC retention time 6.78 min; purity 97.7%. ^{1}H NMR, (400 MHz, d_6-acetone), δ 8.11 (s, 1H, 9ArH), 8.08 (d, *J* = 7.8 Hz, 1H, 11ArH), 8.02 (d, *J* = 7.8 Hz, 1H, 13ArH), 7.84 (t, *J* = 8.1 Hz, 1H, 12ArH), 7.48 (d, *J* = 2.0 Hz, 2H, 2',6'ArH), 7.44 (t, *J* = 2.0 Hz, 1H, 4'ArH), 6.98 (s, 2H, 2,6ArH). ^{13}C NMR, (100 MHz, d_6-acetone), δ 194.5, 156.4, 139.5, 138.6, 138.3, 134.7, 134.2, 131.3, 131.0, 130.4, 129.6, 127.6, 126.8, 123.6, 109.7. HRMS: m/z calculated for $C_{20}H_{12}Cl_2F_3O_3$, $[M+H]^+$ 427.0116, Found: 427.0119

(3',5'-Dichloro-2,6-dihydroxy-biphenyl-4-yl)-(4-ethyl-phenyl)-methanone (**41**)

Pink solid (66.1mg, 78%). The material was recrystallized from methanol:water to yield a white solid. Analytical HPLC retention time 6.97 min; purity 96.5%. ^{1}H NMR, (400 MHz, d_6-acetone), δ 7.76 (d, *J* = 7.9 Hz, 2H, 9,13ArH), 7.48 (d, *J* = 2.0 Hz, 2H, 2',6'ArH), 7.43 (t, *J* = 2.0 Hz, 1H, 4'ArH), 7.41 (d, *J* = 7.9 Hz, 2H, 10,12ArH), 6.94 (s, 2H, 2,6ArH), 2.75 (q, *J* = 7.5 Hz, 2H, C*H*$_2$CH$_3$), 1.27 (t, *J* = 7.7 Hz, 3H, CH$_2$C*H*$_3$). ^{13}C NMR, (100 MHz, d_6-acetone), δ 195.4, 156.1, 150.1, 139.9, 138.5, 136.2, 134.6, 130.8, 130.5, 128.7, 127.4, 109.6, 29.6, 15.7. HRMS: m/z calculated for $C_{21}H_{17}Cl_2O_3$, $[M+H]^+$ 387.0555, Found: 387.0545

(3',5'-Dichloro-2,6-dihydroxy-biphenyl-4-yl)-(3-ethyl-phenyl)-methanone (**42**)

Pink solid (59.2 mg, 96%). The material was future purified by preparative reverse phase HPLC. Analytical HPLC retention time 7.01 min; purity 99.3%. ^{1}H NMR, (400 MHz, d_6-acetone), δ 7.67 (s, 1H, 9ArH), 7.62 (dt, *J* = 1.6, 7.6 Hz, 1H, 13ArH), 7.53 (m, 1H, 11ArH), 7.49 (d, *J* = 1.9 Hz, 2H, 2',6'ArH), 7.48 (d, *J* = 7.6, 1H, 12ArH), 7.44 (t, *J* = 1.9 Hz, 1H, 4'ArH), 6.97 (s, 2H, 2,6ArH), 2.75 (q, *J* = 7.6 Hz, 2H, C*H*$_2$CH$_3$), 1.27 (t, *J* = 7.6 Hz, 3H, CH$_2$C*H*$_3$). ^{13}C NMR, (100 MHz, d_6-acetone), δ 195.9, 156.1, 139.7, 138.8, 138.5, 134.7, 132.7, 130.5, 129.7, 129.1, 128.0, 127.4, 126.9, 126.7, 109.7, 29.6, 16.0. HRMS: m/z calculated for $C_{21}H_{17}Cl_2O_3$, $[M+H]^+$ 387.0555, Found: 387.0545

(3',5'-Dichloro-2,6-dihydroxy-biphenyl-4-yl)-(4-propyl-phenyl)-methanone (**43**)

Pink solid (65.6 mg, 85%). The material was recrystallized from methanol:water to yield a white solid. Analytical HPLC retention time 7.43 min; purity 99.1%. ^{1}H NMR, (400 MHz, d_6-acetone), δ 7.74 (d, *J* = 8.1 Hz, 2H, 9,13ArH), 7.48 (d, *J* = 1.9 Hz, 2H, 2',6'ArH), 7.43 (t, *J* = 1.9 Hz, 1H, 4'ArH), 7.39 (d, *J* = 8.1, 2H, 10,12ArH), 6.94 (s, 2H, 2,6ArH), 2.70 (t, *J* = 7.9 Hz, 2H, C*H*$_2$CH$_2$CH$_3$), 1.70 (sxt, *J* = 7.7 Hz, 2H, CH$_2$C*H*$_2$CH$_3$), 0.96 (t, *J* = 7.4, 3H, CH$_2$CH$_2$C*H*$_3$). ^{13}C NMR, (100 MHz, d_6-acetone), δ 195.4, 156.2, 148.6, 139.9, 138.5, 136.1, 134.7, 130.8, 130.5, 129.3, 127.4, 118.3, 109.6, 38.5, 25.1, 14.0. HRMS: m/z calculated for $C_{22}H_{19}Cl_2O_3$, $[M+H]^+$ 401.0711, Found: 401.0707

(3',5'-Dichloro-2,6-dihydroxy-biphenyl-4-yl)-(4-isopropyl-phenyl)-methanone (**44**)

Off white solid (39.1 mg, 70%). The material was recrystallized from methanol:water to yield a white solid. Analytical HPLC retention time 7.31 min; purity 97.7%. ^{1}H NMR, (400 MHz, d_6-acetone), δ 7.76 (d, *J* = 8.4 Hz, 2H, 9,13ArH), 7.48 (d, *J* = 2.0 Hz, 2H, 2',6'ArH), 7.45 (t, *J* = 2.0 Hz, 1H, 4'ArH), 7.43 (d, *J* = 8.4, 2H, 10,12ArH), 6.95 (s, 2H, 2,6ArH), 3.04 (spt. *J* = 6.8 Hz, 1H, C*H*$(CH_3)_2$), 1.30 (d, *J* = 6.8 Hz, 6H, CH(C*H*$_3)_2$). ^{13}C NMR, (100 MHz, d_6-acetone), δ 195.4, 156.2, 154.6, 139.9, 138.5, 136.3, 134.7, 130.9, 130.5, 127.4, 127.2, 118.3, 109.6, 34.9, 24.0. HRMS: m/z calculated for $C_{22}H_{19}Cl_2O_3$, $[M+H]^+$ 401.0711, Found: 401.0700

(4-Butyl-phenyl)-(3',5'-dichloro-2,6-dihydroxy-biphenyl-4-yl)-methanone (**45**)

Off white solid (44.1 mg, 47%). The material was recrystallized from methanol:water to yield a white solid. Analytical HPLC retention time 8.08 min; purity 99.2%. ^{1}H NMR, (400 MHz, d_6-acetone), δ 7.75 (d, *J* = 8.4 Hz, 2H, 9,13ArH), 7.48 (d, *J* = 2.0 Hz, 2H, 2',6'ArH), 7.43 (t, *J* = 2.0 Hz, 1H, 4'ArH), 7.40 (d, *J* = 8.4 Hz, 2H, 10,12ArH), 6.94 (s, 2H, 2,6ArH), 2.73 (t, *J* = 7.8 Hz, 2H, C*H*$_2(CH_2)_2CH_3$), 1.66 (p, *J* = 7.8, 2H, $CH_2CH_2CH_2CH_3$), 1.39 (sxt. *J* = 7.4, 2H, $CH_2CH_2CH_2CH_3$), 0.94 (t, *J* = 7.4, 3H, $(CH_2)_3CH_3$). ^{13}C NMR, (100 MHz, d_6-acetone), δ 195.4, 156.1, 148.9, 139.9, 138.5, 136.1, 134.7, 130.8, 130.5, 129.2, 127.4, 118.3, 109.6, 36.2, 34.2, 23.0, 14.2. HRMS: m/z calculated for $C_{23}H_{21}Cl_2O_3$, $[M+H]^+$ 415.0868, Found: 415.0865

(4-tert-Butyl-phenyl)-(3',5'-dichloro-2,6-dihydroxy-biphenyl-4-yl)-methanone (**46**)

Pale pink solid (49.8 mg, 77%). The material was recrystallized from methanol:water to yield a white solid. Analytical HPLC retention time 7.73 min; purity 98.9%. ^{1}H NMR, (400 MHz, d_6-acetone), δ 7.77 (d, *J* = 8.8 Hz, 2H, 9,13ArH), 7.61 (d, *J* = 8.8 Hz, 2H, 10,12ArH), 7.49 (d, *J* = 2.1 Hz, 2H, 2',6'ArH), 7.44 (t, *J* = 2.1 Hz, 2H, 4'ArH), 6.95 (s, 2H, 2,6ArH), 1.38 (s, 9H, C(C*H*$_3)_3$). ^{13}C NMR, (100 MHz, d_6-acetone), δ 195.4, 156.7, 156.1, 139.8, 138.5, 135.9, 134.7, 130.6, 130.5, 127.4, 126.1, 109.6, 35.6, 31.4. HRMS: m/z calculated for $C_{23}H_{21}Cl_2O_3$, $[M+H]^+$ 415.0868, Found: 415.0868

(3',5'-Dichloro-2,6-dihydroxy-biphenyl-4-yl)-(4-hydroxy-phenyl)-methanone (**47**)

Pink solid (61.0 mg, 85%). The material was future purified by preparative reverse phase HPLC. Analytical HPLC retention time 5.94 min; purity 99.4%. ^{1}H NMR, (400 MHz, d_6-acetone), δ 7.79 (d, *J* = 8.7 Hz, 2H, 9,13ArH), 7.49 (d, *J* = 1.8 Hz, 2H, 2',6'ArH), 7.44 (t, *J* = 1.8 Hz, 1H, 4'ArH), 6.99 (d, *J* = 8.6 Hz, 2H, 10,12ArH), 6.90 (s, 2H, 2,6ArH). ^{13}C NMR, (100 MHz, d_6-acetone), δ 194.4, 162.5, 156.1, 140.6, 138.6, 134.6, 133.3, 130.5, 130.0, 127.4, 117.8, 115.9, 109.4. HRMS: m/z calculated for $C_{19}H_{13}Cl_2O_4$, $[M+H]^+$ 375.0191, Found: 375.0178

(4-Chloro-phenyl)-(3',5'-dichloro-2,6-dihydroxy-biphenyl-4-yl)-methanone (**48**)

Pale pink solid (40.4mg, 89%). The material was recrystallized from methanol:water to yield a white solid. Analytical HPLC retention time 6.83 min; purity 100%. ^{1}H NMR, (400 MHz, d_6-acetone), δ 7.83 (d, *J* = 9.1 Hz, 2H, 9,13ArH), 7.61 (d, *J* = 9.1 Hz, 2H, 10,12ArH), 7.47 (d, *J* = 1.9 Hz, 2H, 2',6'ArH), 7.44 (t, *J* = 1.9 Hz, 1H, 4'ArH), 6.94 (s, 2H, 2,6ArH). ^{13}C NMR, (100 MHz, d_6-acetone), δ 194.8, 156.3, 138.7, 134.7,

134.2, 132.6, 132.2, 130.4, 129.4, 127.5, 118.8, 109.7. HRMS: m/z calculated for $C_{19}H_{12}Cl_3O_3$, $[M+H]^+$ 392.9852, Found: 392.9831

(3',5'-Dichloro-2-hydroxy-6-methoxy-biphenyl-4-yl)-p-tolyl-methanone (**49**)

A solution of **21** (21.7 mg, 54.0 μmol) in dry dichloromethane (3 mL) was cooled to -78° C followed by the dropwise addition of BBr_3 (200 μL, 1M solution, 85 μmol, 3 eq). The solution was stirred at this temperature for 1 hour then allowed to warm to ambient temperature and stirred 12 hours. The solution was cooled to -78° C and 1 piece of ice (~1 mL) was added followed by warming to ambient temperature. Five milliliters of water and 3 mL of dichloromethane were added, layers separated, aqueous phase extracted 2 X 3 mL dichloromethane, organic phases combined and dried over sodium sulfate and the solvent removed. The residue was loaded onto a Grace Reveleris 12g silica column and eluted with a hexane:methyl t-butyl ether gradient (5% to 5% 4 column volumes, 5% to 80% 13 column volumes, 80% to 80% 2 column volume, flow rate 36 mL per min.). Dark pink solid (4.7 mg, 24%).

The compound was future purified by preparative reverse phase HPLC. Analytical HPLC retention time 9.05 min; purity 100%. ^{1}H NMR, (400 MHz, $CDCl_3$), δ 7.78 (d, *J* = 8.2 Hz, 2H, 9,13ArH), 7.43 (t, *J* = 1.9 Hz, 1H, 4'ArH), 7.32 (d, *J* = 8.2 Hz, 2H, 10,12ArH), 7.31 (d, *J* = 1.9 Hz, 2H, 2',6'ArH), 7.02 (d, *J* = 1.5 Hz, 1H, 2ArH), 6.99 (d, *J* = 1.5 Hz, 1H, 6ArH), 3.80 (s, 3H, OCH_3), 2.45 (s, 3H, $ArCH_3$). ^{13}C NMR, (100 MHz, $CDCl_3$), δ 195.6, 157.3, 153.1, 143.6, 139.5, 135.5, 135.2, 134.5, 130.3, 129.1, 128.4, 118.4, 111.1, 104.4, 56.1, 21.7. HRMS: m/z calculated for $C_{21}H_{17}Cl_2O_3$, $[M+H]^+$ 387.0555, Found: 387.0556

(4-Butyl-phenyl)-(3',5'-dichloro-2-hydroxy-6-methoxy-biphenyl-4-yl)-methanone (**50**)

Dark pink solid (7.5 mg, 38%). The compound was future purified by preparative reverse phase HPLC. Analytical HPLC retention time 10.72 min; purity 100%. ^{1}H NMR, (400 MHz, $CDCl_3$), δ 7.79 (d, *J* = 8.1 Hz, 2H, 9,13ArH), 7.42 (t, *J* = 2.0 Hz, 1H, 4'ArH), 7.32 (d, *J* = 8.1 Hz, 2H, 10,12ArH), 7.31 (d, *J* = 2.0 Hz, 2H, 2',6'ArH), 7.02 (d, *J* = 1.3 Hz, 1H, 2ArH), 7.00 (d, *J* = 1.3 Hz, 1H, 6ArH), 3.79 (s, 3H, OCH_3), 2.71 (t, *J* = 8.2 Hz, 2H, $CH_2(CH_2)_2CH_3$), 1.66 (p, *J* = 7.7 Hz, 2H, $CH_2CH_2CH_2CH_3$), 1.40 (sxt. *J* = 7.7, 2H, $CH_2CH_2CH_2CH_3$), 0.96 (t, *J* = 7.2 Hz, 3H, $(CH_2)_3CH_3$). ^{13}C NMR, (100 MHz, $CDCl_3$), δ 195.6, 157.4, 153.1, 148.6, 139.5, 135.4, 134.7, 130.3, 129.1, 128.5, 128.4, 125.7, 118.5, 111.1, 104.4. HRMS: m/z calculated for $C_{24}H_{23}Cl_2O_3$, $[M+H]^+$ 429.1024, Found: 429.1025

(4-Chloro-phenyl)-(3',5'-dichloro-2-hydroxy-6-methoxy-biphenyl-4-yl)-methanone (**51**)

Dark pink solid (32.1 mg, 33%). The compound was future purified by preparative reverse phase HPLC. Analytical HPLC retention time 9.48; purity 97.8%. ^{1}H NMR, (400 MHz, $CDCl_3$), δ 7.80 (d, *J* = 8.4 Hz, 2H, 9,13ArH), 7.48 (d, *J* = 8.4 Hz, 2H, 10,12ArH), 7.42 (t, *J* = 2.1 Hz, 1H, 4'ArH), 7.29 (d, *J* = 2.1 Hz, 2H, 2',6'ArH), 6.99 (d, *J* = 1.3 Hz, 1H, 2ArH), 6.96 (d, *J* = 1.3 Hz, 1H, 6ArH), 3.78 (s, 3H, OCH_3). ^{13}C NMR, (100 MHz, $CDCl_3$), δ 194.7, 157.6, 153.3, 139.3, 138.7, 135.5, 135.4, 135.0, 131.5, 129.0, 128.8, 128.5, 118.9, 111.1, 104.3, 56.2. HRMS: m/z calculated for $C_{20}H_{14}Cl_3O_3$, $[M+H]^+$ 407.0009, Found: 407.0010

4.3 Receptor Binding

Filter plates were filled with 210 μL/well of 0.05% (w/v) polyethyleneamine in deionized water and incubated for 1 hour at room temperature. Plates were then filtered using a vacuum manifold and

washed 5 more times with 250 µL/well of deionized water prior to assay. Each well contained 125 µL binding buffer, 5 µL of [^{3}H]-CP 55,940 (final concentration 1 nM), 10 µg (in 20 µL) of membrane protein homogenized in binding buffer [see [44] for membrane preparation methods], and 50 µL test ligand in binding buffer (final concentrations: 1 nM to 10 µM). Plates were incubated at 30°C for 90 minutes. Following incubation, solutions were removed via vacuum and wells were washed 9X with 250 µL/well of binding buffer. Plate backing was then removed, vacuumed dry, and individual filters punched out using punch tips into 5 mL of Eco-lite scintillation cocktail in 7 mL scintillation vials. Vials were left overnight and read the next day on PerkinElmer Liquid Scintillation Analyzer Tri-Carb 2810TR using a 3 minute dwell time. All binding studies were carried out with a minimum of 6 biological replicates. CB1 K_d was 1.98 ± 0.6 nM, B_{max} 8.47 ± 2.35 pmol/mg. CB2 K_d was 1.65 ± 0.5 nM, B_{max} 3.18 ± 0.1 pmol/mg.

4.4 ACTOne functional assay

In order to determine functional pharmacology of the tested compound, HEK-CNG, HEK-CNG+CB1, and HEK-CNG+CB2 cells were plated at 50,000 cells/100 µL medium in clear Poly-D-lysine coated 96 well plates in DMEM 10% FBS, 1% P/S medium the day before experiments were performed. The day of the experiment, ACTOne formulation Membrane Potential Dye was warmed to 37°C and 100 µL added to each well, followed by 1 hour incubation in the dark at room temperature. Test ligands were tested at final concentrations from 5x 10^{-6} to 5 x 10^{-10} with 25 µM Ro 20-1724, and 800 nM forskolin in DPBS with 2.5% (v/v) DMSO. Once test ligands, Ro, forskolin, and buffer were added, plates were read using a BioTek (Winooski, VT) plate reader (Ex 540 nm, Em 590 nm) at 50 mins. At least six biological replicates were used for subsequent data analysis. Data analysis was done in GraphPad Prism 6.0 with non-linear analysis.

In order to determine antagonist pharmacology, 30 minutes after adding cation sensitive dye, 20 µL of 4-ethyl (**41**) or 4-*n*-butyl (**45**) in DPBS with 2.5% DMSO was added to each well and incubated for an additional 30 minutes in the dark at room temperature prior to addition of CP 55,940. Concentrations of **41** or **45** were chosen to span concentrations roughly one log above and below the previously determined EC_{50}. To initiate antagonist studies, 30 µL of CP 55,940 made in DPBS with 2.5% DMSO buffer, Ro 20-1724 (final concentration 25 µM), and forskolin (final concentration 800 nM) was added to each well, and the plate was read at 50 minutes. Six independent experiments were used for subsequent data analysis. Schild analysis was done with sigmoidal curve fit for Log (DR-1) values. Significance comparisons were done using sum of squares in comparison to the logEC_{50} from agonist alone curve.

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