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Exploring the substituent effects on a novel series of C1′-dimethyl-aryl Δ^8-tetrahydrocannabinol analogs

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ABSTRACT

The synthesis and characterization of novel C1′-phenyl-substituted Δ^8-THC analogs were previously reported by our laboratory. Within this small series of compounds, the C1′-dimethyl phenyl group was found to impart 13.5-fold selectivity for the CB2 receptor with a K_i 0.91 nM. The current study expands on the previous report by evaluating the effects of aromatic ring substitution on CB1 and CB2 receptor subtype binding and selectivity. The ring substituents synthesized in this study include aliphatic, halogen, nitrile, and acetamido functional groups. In addition, the isosteric replacement of the phenyl group by thiophene was evaluated. The anti-glioma activities of selected compounds were evaluated in vitro and compared to the lead compound **2**.

1. Introduction

The hemp plant, *Cannabis sativa*, is the major source of tricyclic terpenoids with a core benzopyran structure that are referred to as classical cannabinoids. The natural product Δ^9-THC, and its isomer Δ^8-THC (**1**, Fig. 1) are the prototypical members of this class of cannabinoids. They bind to the CB1 and CB2 cannabinoid receptors and act as agonists or partial agonists.[1] Compounds such as Δ^8-THC and its synthetic analogs have potential indication in the treatment of diverse pathological conditions ranging from cancer,[2] inflammation,[3] glaucoma,[4] epilepsy,[5,6] and pain,[7] thus kindling extensive research in this field.

Efforts directed at elucidating the SAR of classical cannabinoids have focused on the tricyclic ring structure and the C-3 alkyl side chain. The different side chain modifications that have been reported so far include branched chain alkyls,[8,9] unsaturated alkyls,[10–12] alkyls containing 1′,1′-cyclic functionality,[13] adamantyl,[14] and cycloalkyl side chains containing 1′,1′-dimethyl and 1′,1′-dithiolane functionalities.[15] These modifications have been directed at improving binding affinities as well as the CB1/CB2 selectivity. We had previously reported a series of Δ^8-THC analogs with a phenyl side chain and with differing functionalities at the 1′ position including dimethyl, dithiolane, methylene, and ketone groups. In this series, the analog with a 1′,1′-dimethyl functionality (**2**) displayed greatly enhanced binding affinities for both the CB1 and the CB2 receptor (CB1 K_i = 12.3 nM, CB2 K_i = 0.91 nM) when compared to Δ^8-THC (CB1 K_i = 28.5 nM and CB2 K_i = 25.0 nM) as well as 13-fold selectivity for the CB2 receptor when compared to Δ^8-THC.

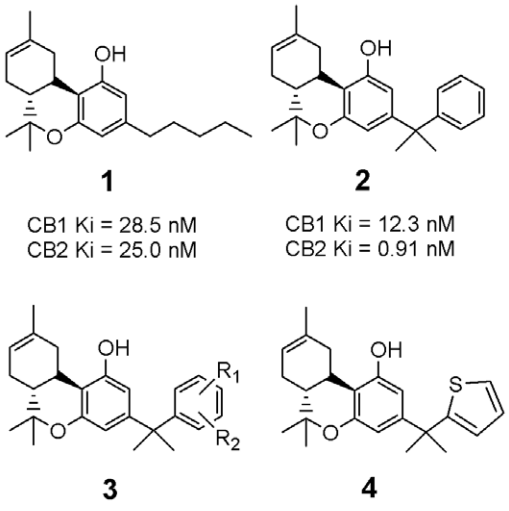

Figure 1.

The high CB2-binding affinity as well as modest CB2 selectivity exhibited by compound **2** prompted us to further explore the SAR of the phenyl-substituted side chain series of Δ^8-THC analogs. The objectives of this study include an improved understanding of the functional group requirements of the ligand-binding pocket (LBP) of the CB2 receptor as well as augmentation of CB2 affinity and selectivity.

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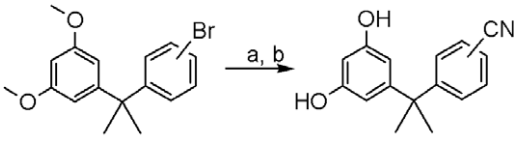
To probe potential hydrophobic, electrostatic, and hydrogen-bonding interactions with the amino acid residues present in the LBP of the receptor, substituents were introduced that include methyl, ethyl, propyl, dimethyl, fluoro, chloro, bromo, nitrile, and amide at the *para*, *meta*, and *ortho*-positions of the side chain phenyl ring (**3**) or a combination thereof. The choice of substituents not only enables the probing of different binding interactions with the receptor but also is compatible with the existing synthetic strategy. The effects of replacing the phenyl ring with the bioisosteric replacement 2-thiophene (**4**) on the binding affinities for the CB1 and the CB2 receptor were investigated. Selected compounds were also evaluated for cytotoxic effects on human glioma cell lines.

2. Results and discussion

2.1. Chemistry

The synthesis of the phenyl-substituted side chain analogs of Δ^8-THC utilized 3,5-dimethoxy benzaldehyde as the starting material. This was reacted with the appropriately substituted Grignard reagents to afford the corresponding alcohols (Scheme 1).[16] In cases where the Grignard reagents were not commercially available, the Grignard reagents were prepared by reacting the substituted aromatic or heteroaromatic bromides with magnesium turnings. Oxidation of the alcohols with PCC afforded the diphenyl ketone intermediates **5**–**17**, which were then reacted with dimethyl zinc and titanium (IV) chloride to yield the corresponding C1′-dimethyl-substituted compounds **18**–**30**. The 3,5-dimethoxyphenyl intermediates **18**–**30** were converted to resorcinols **31**–**43** using boron tribromide.[17] Intermediates **28** and **29** were converted to nitriles using copper (I) cyanide[18] and then deprotected using boron tribromide to yield resorcinols **44** and **45** (Scheme 2). Palladium-catalyzed amination strategy[19] was employed to effect the conversion of **29** to the amine which was then reacted with acetyl chloride to yield amide **46** (Scheme 3).

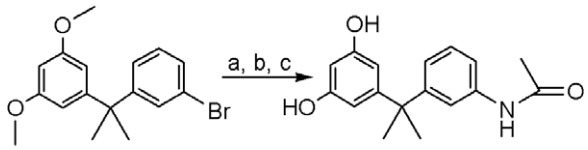
Synthesis of the Δ^8-THC generally employs an acid-catalyzed ring closure between the resorcinol and (+)-Δ^2-*p*-menthene-1, 8-diol or (+)-$\Delta^{2,8}$-*p*-menthadien-1-ol. The later compound was synthesized using the method of Rickards[20] starting from *trans*-(*R*)-(+)-limonene oxide. The published procedure utilized a 50:50 mixture of *cis*- and *trans*-(+)-limonene oxide in a *trans*-diaxial epoxide ring opening utilizing sodium phenyl selenide. Although this is an effective strategy for preparing (+)-*cis*-$\Delta^{2,8}$-*p*-menthadien-1-ol, only one half of the selenide is productively consumed. To address this issue, *trans*-(*R*)-(+)-limonene oxide was obtained from a 50:50 mixture of limonene oxide isomers (**47**) by Steiner's kinetic separation technique using aqueous pyrazole (Scheme 4).[21] The *trans*-limonene oxide (**48**) was converted to the selenide by reaction with diphenyl diselenide under reducing conditions followed by oxidation using hydrogen peroxide.[20] The selenoxide was then decomposed in refluxing chloroform to yield (+)-$\Delta^{2,8}$-*p*-menthadien-1-ol (**49**). The Δ^8-THC analogs **4** and **50**–**64** were then obtained by reacting resorcinols **31**–**46** with (+)-$\Delta^{2,8}$-*p*-menthadien-1-ol or (+)-Δ^2-*p*-menthene-1,8-diol in the presence of *p*-toluene sulfonic acid (Scheme 5).



28, 4-bromo
29, 3-bromo

44, 4-cyano
45, 3-cyano

Scheme 2. Reagents and conditions: (a) CuCN, DMF, reflux, 6 h; (b) BBr_3, −78 °C, 12 h.

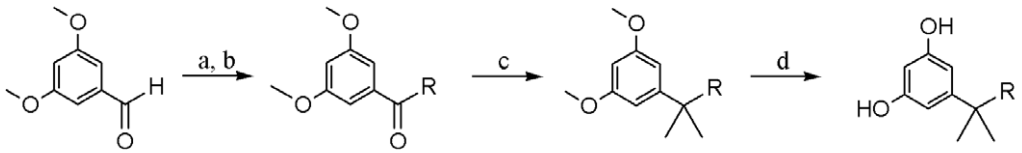


29 **46**

Scheme 3. Reagents and conditions: (a) $Pd_2(dba)_2$, DCPB, LiHMDS; (b) acetyl chloride, pyr; (c) BBr_3, −78 °C, 12 h.

2.2. Receptor-binding assays

Cell membranes from HEK293 EBNA cells transfected with the human CB1 receptor or the human CB2 receptors were used in the receptor-binding assays.[15] The binding affinities of compounds

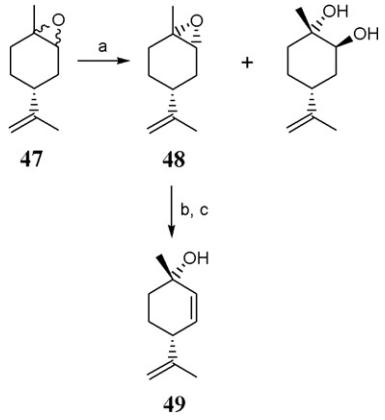


5, R = 4-methylphenyl
6, R = 3-methylphenyl
7, R = 2-methylphenyl
8, R = 3,5-dimethylphenyl
9, R = 4-ethylphenyl
10, R = 4-propylphenyl
11, R = 4-fluorophenyl
12, R = 3-fluorophenyl
13, R = 4-chlorophenyl
14 R = 3-chlorophenyl
15, R = 4-bromophenyl
16, R = 3-bromophenyl
17, R = thiophene

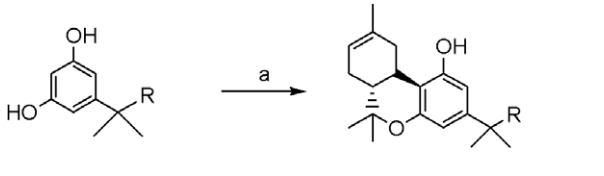
18, R = 4-methylphenyl
19, R = 3-methylphenyl
20, R = 2-methylphenyl
21, R = 3,5-dimethylphenyl
22, R = 4-ethylphenyl
23, R = 4-propylphenyl
24, R = 4-fluorophenyl
25, R = 3-fluorophenyl
26, R = 4-chlorophenyl
27, R = 3-chlorophenyl
28, R = 4-bromophenyl
29, R = 3-bromophenyl
30, R = thiophene

31, R = 4-methylphenyl
32, R = 3-methylphenyl
33, R = 2-methylphenyl
34, R = 3,5-dimethylphenyl
35, R = 4-ethylphenyl
36, R = 4-propylphenyl
37, R = 4-fluorophenyl
38, R = 3-fluorophenyl
39, R = 4-chlorophenyl
40, R = 3-chlorophenyl
41, R = 4-bromophenyl
42, R = 3-bromophenyl
43, R = thiophene

Scheme 1. Reagents and conditions: (a) 1—ArMgBr, THF, −20 °C, 6 h; 2—1 N HCl; (b) PCC, DCM, 12 h; (c) Me_2Zn, $TiCl_4$, DCM, −40 °C, 4 h; (d) BBr_3, −78 °C, 12 h.



Scheme 4. Reagents and conditions: (a) pyrazole, H_2O, reflux, 5 h; (b) $NaBH_4$, diphenyl diselenide, reflux, 2 h; (c) 1—H_2O_2, 20 °C 5 h; 2—reflux.



31, R = 4-methylphenyl
32, R = 3-methylphenyl
33, R = 2-methylphenyl
34, R = 3,5-dimethylphenyl
35, R = 4-ethylphenyl
36, R = 4-propylphenyl
37, R = 4-fluorophenyl
38, R = 3-fluorophenyl
39, R = 4-chlorophenyl
40, R = 3-chlorophenyl
41, R = 4-bromophenyl
42, R = 3-bromophenyl
43, R = 2-thiophene
44, R = 4-cyanophenyl
45, R = 3-cyanophenyl
46, R = 4-*N*-acetamidophenyl

50, R = 4-methylphenyl
51, R = 3-methylphenyl
52, R = 2-methylphenyl
53, R = 3,5-dimethylphenyl
54, R = 4-ethylphenyl
55, R = 4-propylphenyl
56, R = 4-fluorophenyl
57, R = 3-fluorophenyl
58, R = 4-chlorophenyl
59, R = 3-chlorophenyl
60, R = 4-bromophenyl
61, R = 3-bromophenyl
4, R = 2-thiophene
62, R = 4-cyanophenyl
63, R = 3-cyanophenyl
64, R = 4-*N*-acetamidophenyl

Scheme 5. Reagents and conditions: (a) $\Delta^{2,8}$-*p*-menthadien-1-ol or Δ^{2}-*p*-menthene-1,8-diol, *p*-TSA, C_6H_6, 80 °C.

4 and **50**–**64** were determined by measuring the displacement of [^{3}H]CP 55, 940 from the CB1 and CB2 receptor preparations by increasing concentrations of the Δ^8-THC analogs (Table 1). Nonspecific binding was determined using 10 μM WIN 55212-2.

The substituted phenyl analogs displayed a broad range of binding affinities for the CB1 and CB2 receptors (Table 1). The CB1-binding affinities ranged from 1.08 to 76.1 nM while the CB2 affinities ranged from 0.27 to 13.6 nM. The *para*- and the *meta*-substituted compounds showed significantly different binding profiles, with the *meta*-substituted analogs generally displaying enhanced binding affinities for both the receptor subtypes when compared to their *para*-substituted counterparts.

Introduction of halogen substituents fluoro, chloro, and bromo at either the *para* or *meta*-positions of the phenyl side chain resulted in considerable changes in CB1 and CB2 receptor-binding affinities when compared to compound **2** (CB1 K_i = 12.3 nM and CB2 K_i = 0.91 nM). In the para series, the CB1 affinities increased with increasing size and decreased electronegativity of the halogen substituent, thus following the order Br (**60**, K_i = 5.03 nM) > Cl (**58**, K_i = 18.8 nM) > F (**56**, K_i = 76.1 nM). As with the *para*-substituted series, analogs with a halogen substituent at the *meta*-position of the phenyl side chain, **61** (Br, K_i = 1.59 nM) > **59** (Cl, K_i = 2.8 nM) > **57** (F, K_i = 5.26 nM), showed increase in CB1 receptor-binding affinities with increasing size and decreased electronegativity of the halogen substituent. Interestingly, the *meta*-substituted analogs exhibited 3- to 14-fold improvements in CB1 receptor-binding affinity when compared to their *para*-substituted counterparts as well as the lead compound **2** (K_i = 12.3 nM). These data show that halogen substitution in the *meta*-position of the phenyl side chain is beneficial to enhancing CB1 receptor affinity.

Table 1
Binding affinities of the C1′-substituted aryl $^8\Delta$-THC analogs for the CB1 and CB2 receptors[a]

Compound	C1′-Substituent	CB1 K_i[a] (nM)	CB2 K_i[a] (nM)	Ratio CB1/CB2
Δ^8-THC		28.5 (±3.30)	25.0 (±4.80)	1.14
2	Phenyl	12.3 (±0.61)	0.91 (±0.08)	13.5
50	4-Methylphenyl	3.13 (±0.37)	0.88 (±0.05)	3.56
51	3-Methylphenyl	2.53 (±0.54)	1.13 (±0.02)	2.24
52	2-Methylphenyl	34.4 (±2.84)	10.65 (±1.27)	3.23
53	3,5-Dimethylphenyl	11.0 (±1.67)	7.45 (±0.38)	1.48
54	4-Ethylphenyl	1.85 (±0.16)	0.67 (±0.05)	2.76
55	4-Propylphenyl	1.77 (±0.20)	7.83 (±0.79)	0.23
56	4-Fluorophenyl	76.1 (±1.55)	12.4 (±0.24)	6.14
57	3-Fluorophenyl	5.26 (±0.94)	0.90 (±0.02)	5.84
58	4-Chlorophenyl	18.8 (±1.39)	1.68 (±0.20)	11.2
59	3-Chlorophenyl	2.80 (±0.05)	3.54 (±0.71)	0.79
60	4-Bromophenyl	5.03 (±0.39)	1.54 (±0.16)	3.26
61	3-Bromophenyl	1.59 (±0.16)	0.54 (±0.03)	2.94
4	2-Thiophene	1.08 (±0.04)	0.27 (±0.01)	4.0
62	4-Cyanophenyl	9.25 (±0.23)	2.53 (±0.23)	3.66
63	3-Cyanophenyl	2.72 (±0.29)	0.91 (±0.05)	2.98
64	*N*-Acetamidophenyl	13.7 (±1.88)	13.6 (±1.62)	1.01

[a] The K_i values for Δ^8-THC and the C1′ aryl analogs were obtained from three independent experiments run in triplicate showing the standard error of the mean in parentheses.

The CB2 receptor-binding affinities also increased with increasing size and decreased electronegativity of the halogen substituent in the *para*-position for compounds **60** (Br, K_i = 1.54 nM) > **58** (Cl, K_i = 1.68 nM) > **56** (F, K_i = 12.4 nM). These compounds, however, displayed a modest to high reduction in affinity for the CB2 receptor when compared to compound **2** (K_i = 0.91 M). On the other hand, introduction of halogens at the *meta*-position of the phenyl side chain resulted in analogs with CB2 receptor affinities comparable to or better than compound **2**. These results suggest that the presence of halogen substituents at the *meta*-position of the phenyl side chain is favorable for both CB1- and CB2-binding affinities.

Introduction of the nitrile and amide substituents also produced significant effects on both CB1 and CB2 receptor-binding affinities. The presence of a nitrile group at the *para*-position of the phenyl side chain resulted in a slight improvement in CB1 receptor-binding affinity (**63**, K_i = 9.25 nM), relative to the lead compound **2.** However, the *meta* substitution yielded almost a 5-fold improvement in CB1-binding affinity (**63**, K_i = 2.72) when compared to **2**. The improvement in CB1 receptor-binding affinity with the introduction of a nitrile group is in agreement with previously published reports on nitrile derivatives of Δ^8-THC.[17] A comparison of the CB2 receptor-binding affinities of the nitrile-substituted analogs shows that these compounds either displayed lower affinity (**62**, K_i = 2.53 nM) or comparable affinity (**63**, K_i = 0.91 nM) to the lead compound **2** (K_i = 0.91 nM). Introduction of the amide group into the phenyl ring (**64)** had no effect on the CB1 affinity (K_i = 13.7 nM) relative to the parent compound **2**, however, this substitution resulted in a 13-fold decrease in the CB2 receptor binding. The reduction in CB2-binding affinity for the *m*-amide analog may be attributed to the presence of a bulky substituent at the *meta*-position of the side chain, a conclusion

that is supported by the data obtained for the alkyl side chain analogs.

The substituted C1′-phenyl-Δ^8-THC analogs containing a halogen, nitrile, or amide provide insights into potential electrostatic and/or hydrogen bond interactions within the ligand-binding receptor pockets. For the CB1 receptor, introduction of electron withdrawing or hydrogen bond acceptors at either the *para* or *meta*-position causes a significant change in CB1 binding. The greatest impact on affinities is observed for *meta* substitution suggesting the presence of favorable electrostatic interactions and/or hydrogen-bond donors in the corresponding regions of the LBP of the CB1 receptor. In the case of the CB2 receptor, introduction of these substituents either resulted in decrease in CB2-binding affinity with halogens and nitrile at the *para*-position, or *meta* analogs with comparable or slightly higher CB2 affinity.

Introduction of a *para*-methyl phenyl side chain (**50**) resulted in almost 4-fold improvement in CB1-binding affinity (K_i = 3.13 nM) when compared to the lead compound **2** (K_i = 12.3 nM) while the presence of a *meta* methyl group (**51**, K_i = 2.53 nM) produced a 5-fold increase in CB1 affinity. The CB2-binding affinities of compounds **50** and **51** were comparable to that of compound **2**. On the other hand, introduction of the *ortho* methyl group (**52**) caused a significant decrease in both CB1 (K_i = 34.4 nM) and CB2 (K_i = 10.6 nM) binding affinities. The presence of the bulky methyl group at the *ortho*-position will produce unfavorable steric interactions with the dimethyl substituent at the C-1′ position which, in turn, would cause the side chain to adopt an unfavorable geometry for receptor binding. Elongation of the alkyl substituent resulted in analogs with enhanced CB1-binding affinities. The CB1-binding affinities of the *p*-alkyl-substituted analogs followed the order *p*-methyl (**50**, K_i = 3.13 nM) < *p*-ethyl (**54**, K_i = 1.85 nM) < *p*-propyl (**55**, K_i = 1.77 nM). The rank order indicates that the LBP of the CB1 receptor can tolerate bulky hydrophobic substituents, which is in concurrence with previous reports that CB1 affinity is directly linked to the length of the side chain. Specifically, the CB1 affinity increases with increasing carbons from 3 to 10, with a seven or eight carbon chain being optimal for activity.[22] In the case of CB2 affinity, elongation of the alkyl substituent to ethyl (**54**, K_i = 0.67 nM) resulted in increase in affinity while further elongation to a propyl group (**55**, K_i = 7.83 nM) led to an 8-fold decrease in affinity, when compared to **2** (K_i = 0.91 nM). These results in combination with the halogen and nitrile data suggest that the CB2 receptor has lower steric tolerance as compared to the CB1 receptor. Introduction of 3,5-dimethyl functionality in the phenyl side chain (**53**) decreased both CB1- and CB2-binding affinities (CB1, K_i = 11.0 nM; CB2, K_i = 7.45 nM) relative to the *meta*- and *para*-methyl analogs.

The introduction of the bioisosteric phenyl ring replacement thiophene (**62**) led to 11-fold improvement in CB1 affinity (K_i = 1.08 nM) and 3-fold improvement in CB2 affinity (K_i = 0.27 nM) when compared to the lead compound **2**. The enhanced affinity of the 2-thiophene ring may be related to reduced steric interactions with the LBP. Alternatively, a reduction in the rotational energy around the C1′–C2′ bond may allow the molecule to adopt a favorable geometry to bind with the LBP. The most significant finding is that compound **4** displays very high CB2 affinity that is comparable to some of the most potent CB2 analogs reported to date such as CP-55244 (CB2, K_i = 0.21 nM)[23] and *R*-(+)-WIN55212 (CB2, K_i = 0.28 nM).[24]

2.3. Cytotoxicity against human glioma cancer cells

Cannabinoids are gaining increasing attention as novel therapeutic agents for the treatment of a broad spectrum of cancer types. Cannabinoids have now been shown to induce apoptosis in prostate[25–27] and skin carcinomas,[28] glioma[29–31] and lymphoma/leukemia[32]; furthermore, a decrease in tumor size and/or cell growth inhibition has been reported in breast,[25,33,34] uterus,[35,36] and lung cancers,[37,38] and in thyroid epithelioma.[39] In addition, cannabinoids manifest collateral effects by decreasing the production of vascular epidermal growth factor and activation of the vascular epidermal growth factor receptor 2 in vitro and in vivo,[40] inhibiting bi-dimensional capillary-like tube formation and activity of matrix metalloprotease 2[41]; and down-regulation of the expression of tissue inhibitors of metalloproteinase 1 in vitro.[42]

Glioblastoma multiforme (GBM) is the most common and malignant of all the primary brain tumors with a median survival for GBM patients of 3–12 months.[43–45] Recent reports suggest that chemotherapeutic intervention with Δ^8-THC and synthetic cannabinoids may offer a novel strategy for GBM therapy.[30,31,46,47] The lead compound **2** was previously evaluated in the side flank model of human glioma using the U87 cell line, wherein the tumor load was decreased 75% relative to untreated controls.[48] In efforts to develop cannabinoid-based anti-neoplastic agents with unique properties, selected analogs of **2** were screened against a panel of human glioma cell lines U-87MG, T98G, LN-229, DBTRG-O5MG, and MT310 (a primary tumor cell line). Compounds were selected based on increasing ratios of CB1- to CB2-binding affinities. Within this set of compounds the K_is ranged from 1.08 to 76.1 nM for the CB1 and 0.27 to 12.4 nM for the CB2. Increasing concentrations of compounds **4**, **54**, **55**, **56**, and **58** were assayed compared to the lead compound **2** (Table 2). A quantitative analysis of the limited data set did not reveal a significant correlation between the compound K_i and the EC_{50}s. Qualitatively, compound **4** manifested broad-spectrum cytotoxicity across glioma cell lines (Fig. 2). The LN-229 glioma exhibited the highest sensitivity to the C1′ aryl analogs and the primary cell line MT310 manifested reduced cytotoxicity. The high mortality, short life-expectancy, and lack of efficacious drugs against GBM make our novel series of Δ^8-THC analogs attractive leads for anti-cancer drug development. Probably the most important property of these compounds is the high probability for blood brain barrier penetration. Unlike existing therapies, this property should allow the drug to bath the CNS and facilitate treatment of distant glioblastoma foci, the most common cause of reoccurrence and death in GBM patients.

3. Conclusions

In this study, we evaluated the functional group requirements of the LBPs of the CB1 and CB2 cannabinoid receptors by introducing aromatic/heteroaromatic side chains. The substituents methyl, ethyl, propyl, 3,5-dimethyl, fluoro, chloro, bromo, nitrile, and amide were introduced in the phenyl ring. A classical bioisosteric replacement of the phenyl ring, thiophene, was also introduced in the side chain. In general, the *meta*-substituted series of compounds exhibited higher CB1 and CB2 affinities than their *para*-substituted counterparts. Introduction of electron-withdrawing substituents at the *meta*-position of the phenyl side chain greatly enhanced CB1-binding affinities while the CB2 affinities were equivalent or slightly enhanced when compared to the lead compound **2**. The introduction of aliphatic chains on the phenyl side chain differentially affected CB1 and CB2 binding with respect to the size/steric bulk of the substituent. Analog **4** containing the thiophene ring displayed very high CB2 and CB1-binding affinities. The CB2-binding affinity of compound **4** is comparable to some of the most potent CB2 ligands reported so far, such as CP-55244 and *R*-(+)-WIN55212. A more thorough QSAR analysis of analogs **2**, **4**, and **50**–**64** is presented in an accompanying paper.

Table 2
The EC_{50} values of selected C1′ aryl Δ^8-THC analogs[a]

Compound	U-87MG EC_{50} (μM)	T98G EC_{50} (μM)	LN-229 EC_{50} (μM)	DBTRG-O5MG EC_{50} (μM)	MT310 EC_{50} (μM)
2	3.08 (±0.30)	4.68 (±0.45)	2.63 (±0.32)	2.27 (±0.18)	4.92 (±1.08)
54	2.52 (±0.26)	3.23 (±0.47)	1.67 (±0.21)	3.00 (±0.23)	4.11 (±0.18)
55	3.17 (±0.31)	3.63 (±0.51)	2.44 (±0.45)	3.58 (±0.22)	6.46 (±0.62)
56	2.55 (±0.43)	3.30 (±0.73)	2.02 (±0.29)	3.08 (±0.24)	8.78 (±0.80)
58	3.27 (±0.17)	3.40 (±0.32)	2.87 (±0.21)	4.61 (±0.17)	7.31 (±0.53)
4	1.83 (±0.06)	2.21 (±0.2)	2.02 (±0.33)	2.67 (±0.04)	4.10 (±0.24)

[a] The EC_{50} values for the Δ^8 -THC C1′ aryl analogs were obtained from three independent experiments run in triplicate showing the standard error of the mean in parentheses.

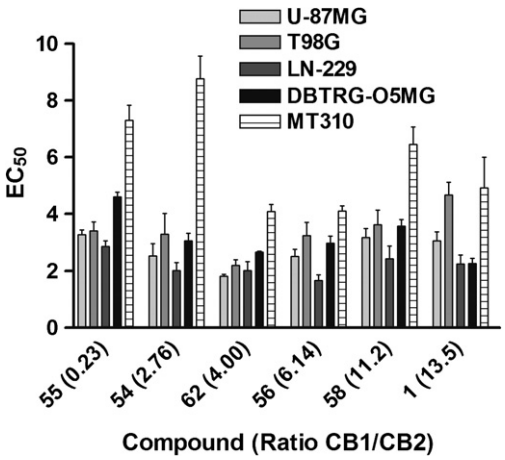


Figure 2. Comparison of the EC_{50} values as a function of the CB1/CB2K_i ratio in five human glioma cell lines.

Cannabinoids are gaining increasing attention as novel therapeutic agents for the treatment of a broad spectrum of cancer types. The ability of Δ^8-THC to cross the blood–brain barrier and the potential of related analogs to do the same make compounds **4** and **50**–**64** a unique platform from which to develop novel GBM therapies. To test the potential of the C1′ aryl-substituted analogs, compounds **2**, **4**, **54**, **55**, **56**, and **58** were screened for cytotoxicity against five human glioma cell lines. The compounds manifested broad-spectrum cytotoxicity against primary and standard human glioma cell lines. The EC_{50}s ranged from 1.67 to 8.78 μM with compound **4** exhibiting the highest efficacy. In conclusion, we believe that the continued development of the C1′ aryl analogs will provide novel insights into the LBP of cannabinoid receptors and generate novel leads for anti-cancer drug development.

4. Experimental

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. Reactions were carried out under dry conditions under an argon atmosphere. Silica Gel 60, 200–425 mesh was used for flash chromatography. Routine NMR spectra were obtained on a Varian Inova 500 MHz NMR and were consistent with the assigned structures. High-resolution 1D and 2D spectra were obtained on a Varian Inova 500 MHz NMR. All NMR were recorded in $CDCl_3$ unless otherwise specified. Routine mass spectra were determined on a Bruker ESQUIRE Ion Trap LC/MS(n) system. Exact mass was determined at the University of Michigan mass spectrometry facility. Thin-layer chromatography was performed on silica gel plates (Merck TLC plates, silica gel 60, F_{254}).

4.1. (*p*-Tolyl)-(3,5-dimethoxy-phenyl)-methanol

The starting material 3,5-dimethoxy benzaldehyde (3 g, 18.1 mmol) was dissolved in THF (30.5 mL) and the solution was cooled to −20 °C. To the solution was added *p*-tolyl magnesium bromide (21.7 mL, 1 M solution, 21.7 mmol) and the reaction mixture was allowed to stir at −20 °C for 6 h. The reaction mixture was then quenched with 1 N HCl, extracted with ether and the organic layer was then dried over sodium sulfate and evaporated. The crude mixture was then purified by column chromatography using ethyl acetate/hexane (20:80) yielding alcohol as a white solid (3.73 g, 79.9%). R_f = 0.24 (ethyl acetate/hexane 20:80); ^{1}H NMR (500 MHz, $CDCl_3$): δ (ppm) 2.17 (s, 1H), 2.33 (s, 3H), 3.76 (s, 6H), 5.73 (s, 1H), 6.35 (t, J = 2.5 Hz, 1H), 6.55 (d, J = 2.5 Hz, 2H), 7.14 (d, J = 7.5 Hz, 2H), 7.26 (d, J = 8 Hz, 2H); MS: (ESI, Pos) m/z 281.0 [(M+23)$^+$].

Utilizing the appropriately substituted Grignard reagents, the following alcohols were similarly prepared.

4.2. (*m*-Tolyl)-(3,5-dimethoxy-phenyl)-methanol

Colorless oil (1.45 g, 31.1%). R_f = 0.26 (ethyl acetate/hexane 20:80); ^{1}H NMR (500 MHz, $CDCl_3$): δ (ppm) 2.17 (d, J = 3.5 Hz, 1H), 2.33 (s, 3H), 3.77 (s, 6H), 5.73 (d, J = 3.5 Hz, 1H), 6.36 (t, J = 2.5 Hz, 1H), 6.56 (d, J = 2 Hz, 2H), 7.08 (d, J = 7.5 Hz, 1H), 7.19 (m, 3H); MS: (ESI, Pos) m/z 281.0 [(M+23)$^+$].

4.3. (*o*-Tolyl)-(3,5-dimethoxy-phenyl)-methanol

Colorless oil (4.47 g, 95.9%). R_f = 0.3 (ethyl acetate/hexane 20:80); ^{1}H NMR (500 MHz, $CDCl_3$): δ (ppm) 2.13 (d, J = 3.5 Hz, 1H), 2.29 (s, 3H), 3.75 (s, 6H), 5.93 (d, J = 3.5 Hz, 1H), 6.36 (t, J = 2.5 Hz, 1H), 6.49 (d, J = 2 Hz, 2H), 7.14 (m, 2H), 7.21 (m, 2H), 7.46 (m, 1H); MS: (ESI, Pos) m/z 281.0 [(M+23)$^+$].

4.4. (4-Fluoro-phenyl)-(3,5-dimethoxy-phenyl)-methanol

White solid (4.17 g, 88.1%). R_f = 0.18 (ethyl acetate/hexane 20:80); ^{1}H NMR (500 MHz, $CDCl_3$): δ (ppm) 2.27 (d, J = 3 Hz, 1H), 3.76 (s, 6H), 5.74 (d, J = 2 Hz, 1H), 6.37 (t, J = 2.5 Hz, 1H), 6.51 (d, J = 2.5 Hz, 2H), 7.01 (m, 2H), 7.34 (m, 2H); MS: (ESI, Pos) m/z 285.2 [(M+23)$^+$].

4.5. (3-Fluoro-phenyl)-(3,5-dimethoxy-phenyl)-methanol

White solid (3.95 g, 83.4%). R_f = 0.19 (ethyl acetate/hexane 20:80); ^{1}H NMR (500 MHz, $CDCl_3$): δ (ppm) 2.30 (d, J = 3.5 Hz, 1H), 3.77 (s, 6H), 5.73 (d, J = 3.5 Hz, 1H), 6.37 (t, J = 2 Hz, 1H), 6.52 (d, J = 2 Hz, 2H), 6.95 (m, 1H), 7.13 (m, 2H), 7.28 (m, 1H); MS: (ESI, Pos) m/z 285.2 [(M+23)$^+$].

4.6. (4-Chloro-phenyl)-(3,5-dimethoxy-phenyl)-methanol

White solid (4.23 g, 84.0%). R_f = 0.24 (ethyl acetate/hexane 20:80); ^{1}H NMR (500 MHz, $CDCl_3$): δ (ppm) 2.25 (d, J = 3.5 Hz, 1H),

3.76 (s, 6H), 5.72 (d, J = 3 Hz, 1H), 6.36 (t, J = 2.5 Hz, 1H), 6.50 (d, J = 2.5 Hz, 2H), 7.3 (m, 4H); MS: (ESI, Pos) m/z 301.0 [(M+23)$^+$].

4.7. (3-Chloro-phenyl)-(3,5-dimethoxy-phenyl)-methanol

White solid (3.59 g, 71.3%). R_f = 0.27 (ethyl acetate/hexane 20:80); ^{1}H NMR (500 MHz, $CDCl_3$): δ (ppm) 2.22 (d, J = 3.5 Hz, 1H), 3.77 (s, 6H), 5.73 (d, J = 3.5 Hz, 1H), 6.38 (t, J = 2.5 Hz, 1H), 6.52 (d, J = 2.5 Hz, 2H), 7.25 (m, 3H), 7.39 (m, 1H); MS: (ESI, Pos) m/z 301.0 [(M+23)$^+$].

4.8. (4-Bromo-phenyl)-(3,5-dimethoxy-phenyl)-methanol

The reaction of 1,4-dibromo-benzene (1 g, 4.24 mmol) with magnesium turnings (0.15 g, 6.35 mmol) in ether (6 mL) followed by reaction with 3,5-dimethoxy benzaldehyde (0.527 g, 3.18 mmol) in THF (3 mL) yielded the alcohol (0.63 g, 61.3%) as a colorless oil. R_f = 0.3 (ethyl acetate/hexane 20:80); ^{1}H NMR (500 MHz, $CDCl_3$): δ (ppm) 2.10 (br s, 1H), 3.69 (s, 6H), 5.64 (s, 1H), 6.30 (t, J = 2.5 Hz, 1H), 6.43 (d, J = 2.5 Hz, 2H), 7.19 (d, J = 8 Hz, 2H), 7.38 (d, J = 8.5 Hz, 2H); MS: (ESI, Pos) m/z 346.2 [(M+23)$^+$].

4.9. (3-Bromo-phenyl)-(3,5-dimethoxy-phenyl)-methanol

White solid (0.87 g, 56.5%). R_f = 0.32 (ethyl acetate/hexane 20:80); ^{1}H NMR (500 MHz, $CDCl_3$): δ (ppm) 2.25 (br s, 1H), 3.79 (s, 6H), 5.73 (s, 1H), 6.40 (t, J = 2.5 Hz, 1H), 6.53 (d, J = 2.5 Hz, 2H), 7.21 (t, J = 8.0 Hz, 1H), 7.32 (d, 8 Hz, 1H), 7.42 (dt, J = 4, 8 Hz, 1H), 7.58 (m, 1H); MS: (ESI, Pos) m/z 346.2 [(M+23)$^+$].

4.10. (3,5-Dimethoxy-phenyl)-(4-ethyl-phenyl)-methanol

4-Bromo-ethyl benzene (3.72 mL, d = 1.34, 27 mmol) and magnesium turnings (0.97 g, 40 mmol) were heated to reflux for 1 h in THF (40 mL) then cooled to room temperature. The solution was cooled to −20 °C and 3,5-dimethoxy benzaldehyde (3.32 g, 20 mmol) dissolved in THF (18 mL) cooled to −20 °C was added and followed by stirring at that temperature for 6 h. The reaction mixture was then quenched with 1 N HCl, extracted with ether, and the organic layer was then dried over sodium sulfate and evaporated. The crude mixture was then purified by column chromatography using ethyl acetate/hexane (20:80) to give the pure alcohol as a white solid (4.95 g, 67.2%). R_f = 0.3 (ethyl acetate/hexane 20:80); ^{1}H NMR (500 MHz, $CDCl_3$): δ (ppm) 1.143 (t, J = 7.5 Hz, 3H), 2.26 (br s, 1H), 2.55 (q, J = 7.5 Hz, 2H), 3.69 (s, 6H), 5.66 (s, 1H), 6.28 (t, J = 2.5 Hz, 1H), 6.48 (d, J = 2.5 Hz, 2H), 7.09 (d, J = 8.5 Hz, 2H), 7.21 (d, J = 9 Hz, 2H); MS: (ESI, Pos) m/z 295.1 [(M+23)$^+$].

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

4.11. (3,5-Dimethoxy-phenyl)-(4-propyl-phenyl)-methanol

White solid (4.44 g, 61.8%). R_f = 0.31 (ethyl acetate/hexane 20:80); ^{1}H NMR (500 MHz, $CDCl_3$): δ (ppm) 0.97 (t, J = 7.5 Hz, 3H), 1.69 (m, 2H), 2.28 (br s, 1H), 2.67 (t, J = 7.5 Hz, 2H), 3.72 (s, 6H), 5.68 (s, 1H), 6.30 (t, J = 2.5 Hz, 1H), 6.52 (d, J = 2.5 Hz, 2H), 7.1 (d, J = 8.5 Hz, 2H), 7.36 (d, J = 9 Hz, 2H); MS: (ESI, Pos) m/z 309.1 [(M+23)$^+$].

4.12. (3,5-Dimethoxy-phenyl)-(3,5-dimethyl-phenyl)-methanol

White solid (4.02 g, 54.6%). R_f = 0.33 (ethyl acetate/hexane 20:80); ^{1}H NMR (500 MHz, $CDCl_3$): δ (ppm) 2.25 (br s, 1H), 2.37 (s, 6H), 3.76 (s, 6H), 5.69 (s, 1H), 6.36 (t, J = 2.5 Hz, 1H), 6.61 (d, J = 2.5 Hz, 2H), 7.1 (s, 1H), 7.21 (s, 2H); MS: (ESI, Pos) m/z 295.1 [(M+23)$^+$].

4.13. (2-Thiophenyl)-(3,5-dimethoxy-phenyl)-methanol

Gray oil (2.52 g, 55.7%). R_f = 0.25 (ethyl acetate/hexane 20:80); ^{1}H NMR (500 MHz, $CDCl_3$): δ (ppm) 2.38 (m, 1H), 3.78 (s, 6H), 5.99 (d, J = 4 Hz, 1H), 6.40 (t, J = 2 Hz, 1H), 6.62 (d, J = 2 Hz, 2H), 6.93 (m, 2H), 7.26 (m, 1H); MS: (ESI, Pos) m/z 273.0 [(M+23)$^+$].

4.14. (*p*-Tolyl)-(3,5-dimethoxy-phenyl)-methanone (5)

(*p*-Tolyl)-(3,5-dimethoxy-phenyl)-methanol (3.02 g, 11.7 mmol) was dissolved in CH_2Cl_2 (46 mL) and to that Celite (5.04 g) and PCC (5.04 g, 23.4 mmol) were added. The reaction mixture was allowed to stir at room temperature overnight, then diluted with ether (100 mL), and filtered over a pad of silica. The filtrate was evaporated and purified by column chromatography using ethyl acetate/hexane (10:90) to afford the ketone as a white solid (1.71 g, 57.0%). R_f = 0.27 (ethyl acetate/hexane 10:90); ^{1}H NMR (500 MHz, $CDCl_3$): δ (ppm) 2.44 (s, 3H), 3.83 (s, 6H), 5.99 (d, J = 4 Hz, 1H), 6.40 (t, J = 2 Hz, 1H), 6.62 (d, J = 2 Hz, 2H), 6.93 (m, 2H), 7.26 (m, 2H); MS: (ESI, Pos) m/z 279.0 [(M+23)$^+$].

Utilizing the appropriately substituted alcohols, the following ketones were similarly prepared.

4.15. (*m*-Tolyl)-(3,5-dimethoxy-phenyl)-methanone (6)

White solid (0.77 g, 59.7%). R_f = 0.29 (ethyl acetate/hexane 10:90); ^{1}H NMR (500 MHz, $CDCl_3$): δ (ppm) 2.42 (s, 3H), 3.83 (s, 6H), 6.67 (t, J = 2.5 Hz, 1H), 6.92 (d, J = 2 Hz, 2H), 7.37 (m, 2H), 7.58 (d, J = 7.5 Hz, 1H), 7.64 (s, 1H); MS: (ESI, Pos) m/z 279.2 [(M+23)$^+$].

4.16. (*o*-Tolyl)-(3,5-dimethoxy-phenyl)-methanone (7)

White solid (2.08 g, 67.8%). R_f = 0.31 (ethyl acetate/hexane 10:90). Mp 65–67 °C; ^{1}H NMR (500 MHz, $CDCl_3$): δ (ppm) 2.34 (s, 3H), 3.81 (s, 6H), 6.67 (t, J = 2.5 Hz, 1H), 6.93 (d, J = 2 Hz, 2H), 7.24 (m, 1H), 7.30 (m, 2H), 7.38 (td, J = 1.5, 7.5 Hz, 1H); MS: (ESI, Pos) m/z 279.1 [(M+23)$^+$].

4.17. (3,5-Dimethyl-phenyl)-(3,5-dimethoxy-phenyl)-methanone (8)

White solid (3.5 g, 86.9%); R_f = 0.35 (ethyl acetate/hexane 10:90); ^{1}H NMR (500 MHz, $CDCl_3$): δ (ppm) 2.37 (s, 6H), 3.83 (s, 6H), 6.67 (t, J = 2.5 Hz, 1H), 6.91 (d, J = 2.5 Hz, 2H), 7.22 (br s, 1H), 7.41 (br s, 2H); MS: (ESI, Pos) m/z 293.2 [(M+23)$^+$].

4.18. (4-Ethyl-phenyl)-(3,5-dimethoxy-phenyl)-methanone (9)

White solid (3.67 g, 74.7%). R_f = 0.35 (ethyl acetate/hexane 10:90); ^{1}H NMR (500 MHz, $CDCl_3$): δ (ppm) 1.30 (t, J = 7.5 Hz, 3H), 2.75 (q, J = 7.5 Hz, 2H), 3.85 (s, 6H), 6.68 (t, J = 2.5 Hz, 1H), 6.93 (d, J = 2.5 Hz, 2H), 7.32 (d, J = 8.5 Hz, 2H), 7.78 (d, J = 9 Hz, 2H); MS: (ESI, Pos) m/z 293.2 [(M+23)$^+$].

4.19. (4-Propyl-phenyl)-(3,5-dimethoxy-phenyl)-methanone (10)

White solid (3.34 g, 75.9%). R_f = 0.36 (ethyl acetate/hexane 10:90); ^{1}H NMR (500 MHz, $CDCl_3$): δ (ppm) 0.97 (t, J = 7.5 Hz, 3H), 1.69 (m, 2H), 2.67 (t, J = 7.5 Hz, 2H), 3.83 (s, 6H), 6.67 (t, J = 2.5 Hz, 1H), 6.91 (d, J = 2.5 Hz, 2H), 7.28 (d, J = 8.5 Hz, 2H), 7.75 (d, J = 9 Hz, 2H); MS: (ESI, Pos) m/z 307.2 [(M+23)$^+$].

4.20. (4-Fluoro-phenyl)-(3,5-dimethoxy-phenyl)-methanone (11)

Colorless oil (2.71 g, 91.0%). R_f = 0.29 (ethyl acetate/hexane 10:90); ^{1}H NMR (500 MHz, $CDCl_3$): δ (ppm) 3.83 (s, 6H), 6.67 (t,

J = 2.5 Hz, 1H), 6.88 (d, J = 2.5 Hz, 2H), 7.15 (m, 2H), 7.86 (m, 2H); MS: (ESI, Pos) m/z 283.1 [(M+23)$^+$].

4.21. (3-Fluoro-phenyl)-(3,5-dimethoxy-phenyl)-methanone (12)

Colorless oil (2.81 g, 89.9%). R_f = 0.31 (ethyl acetate/hexane 10:90); ^{1}H NMR (500 MHz, $CDCl_3$): δ (ppm) 3.83 (s, 6H), 6.69 (t, J = 2 Hz, 1H), 6.90 (d, J = 2 Hz, 2H), 7.29 (m, 1H), 7.46 (m, 1H), 7.51 (m, 1H), 7.58 (m, 1H); MS: (ESI, Pos) m/z 283.2 [(M+23)$^+$].

4.22. (4-Chloro-phenyl)-(3,5-dimethoxy-phenyl)-methanone (13)

White solid (2.07 g, 74.8%). R_f = 0.29 (ethyl acetate/hexane 10:90); ^{1}H NMR (500 MHz, $CDCl_3$): δ (ppm) 3.83 (s, 6H), 6.68 (t, J = 2 Hz, 1H), 6.88 (d, J = 2.5 Hz, 2H), 7.46 (d, J = 8.5 Hz, 2H), 7.77 (d, J = 8.5 Hz, 2H); MS: (ESI, Pos) m/z 299.0 [(M+23)$^+$].

4.23. (3-Chloro-phenyl)-(3,5-dimethoxy-phenyl)-methanone (14)

Yellow oil (2.22 g, 76.6%). R_f = 0.3 (ethyl acetate/hexane 10:90); ^{1}H NMR (500 MHz, $CDCl_3$): δ (ppm) 3.83 (s, 6H), 6.69 (t, J = 2.5 Hz, 1H), 6.89 (d, J = 2.5 Hz, 2H), 7.41 (t, J = 8 Hz, 1H), 7.55 (m, 1H), 7.67 (m, 1H), 7.79 (t, J = 2.5 Hz, 1H); MS: (ESI, Pos) m/z 299.1 [(M+23)$^+$].

4.24. (4-Bromo-phenyl)-(3,5-dimethoxy-phenyl)-methanone (15)

White solid (3.3 g, 83.0%). R_f = 0.39 (ethyl acetate/hexane 10:90); ^{1}H NMR (500 MHz, $CDCl_3$): δ (ppm) 3.85 (s, 6H), 6.70 (t, J = 2 Hz, 1H), 6.90 (d, J = 2.5 Hz, 2H), 7.64 (d, J = 8.5 Hz, 2H), 7.71 (d, J = 8.5 Hz, 2H); MS: (ESI, Pos) m/z 344.1 [(M+23)$^+$].

4.25. (3-Bromo-phenyl)-(3,5-dimethoxy-phenyl)-methanone (16)

White solid (0.72 g, 63.6%). R_f = 0.39 (ethyl acetate/hexane 10:90); ^{1}H NMR (500 MHz, $CDCl_3$): δ (ppm) 3.85 (s, 6H), 6.71 (t, J = 2 Hz, 1H), 6.91 (d, J = 2.5 Hz, 2H), 7.38 (t, J = 8 Hz, 1H), 7.74 (m, 2H), 7.97 (br s, 1H); MS: (ESI, Pos) m/z 344.1 [(M+23)$^+$].

4.26. (2-Thiophenyl)-(3,5-dimethoxy-phenyl)-methanone (17)

Yellow solid (1.12 g, 49.6%). R_f = 0.21 (ethyl acetate/hexane 10:90); ^{1}H NMR (500 MHz, $CDCl_3$): δ (ppm) 3.84 (s, 6H), 6.67 (t, J = 2.5 Hz, 1H), 6.91 (d, J = 2.5 Hz, 2H), 7.16 (m, 1H), 7.70 (d, J = 4 Hz, 1H), 7.72 (d, J = 4 Hz, 1H); MS: (ESI, Pos) m/z 270.9 [(M+23)$^+$].

4.27. 1-[1-(*p*-Tolyl)-1-methyl-ethyl]-3,5-dimethoxy-benzene (18)

To methylene chloride (26.3 mL) in a flask cooled to −40 °C and held at this temperature and titanium(IV) chloride (37.3 mL, 1 M solution, 37.3 mmol) was added dropwise, followed by dimethyl zinc (18.5 mL, 2 M solution, 37.3 mmol). The reaction mixture was allowed to stir at that temperature for 15 min then a solution of ketone **5** (1.6 g, 6.25 mmol) in methylene chloride (10 mL) was added and the mixtusre was stirred at −40 °C for 2 h after which it was allowed to warm to −10 °C for 2 h. The mixture was then poured into ice and the aqueous layer was extracted with methylene chloride. The organic layer was then evaporated under reduced pressure and the crude product was purified by column chromatography using ethyl acetate/hexane (4:96) to afford **18** as a yellow oil (1.11 g, 65.8%). R_f = 0.36 (ethyl acetate/hexane 5:95); ^{1}H NMR (500 MHz, $CDCl_3$): δ (ppm) 1.63 (s, 6H), 2.30 (s, 3H), 3.73 (s, 6H), 6.29 (t, J = 2.5 Hz, 1H), 6.39 (d, J = 2.5 Hz, 2H), 7.06 (d, J = 8.5 Hz, 2H), 7.12 (d, J = 8.5 Hz, 1H); MS: (ESI, Pos) m/z 293.3 [(M+23)$^+$].

Utilizing appropriately substituted ketones, the following dimethyl intermediates were similarly synthesized.

4.28. 1-[1-(*m*-Tolyl)-1-methyl-ethyl]-3,5-dimethoxy-benzene (19)

Colorless oil (0.54 g, 75.3%). R_f = 0.35 (ethyl acetate/hexane 5:95); ^{1}H NMR (500 MHz, $CDCl_3$): δ (ppm) 1.55 (s, 6H), 2.22 (s, 3H), 3.65 (s, 6H), 6.22 (t, J = 2 Hz, 1H), 6.32 (d, J = 2.5 Hz, 2H), 6.89 (m, 1H), 6.96 (m, 2H), 7.06 (t, J = 7.5 Hz, 1H); MS: (ESI, Pos) m/z 293.2 [(M+23)$^+$].

4.29. 1-[1-(*o*-Tolyl)-1-methyl-ethyl]-3,5-dimethoxy-benzene (20)

Yellow oil (1.82 g, 82.9%). R_f = 0.35 (ethyl acetate/hexane 5:95); ^{1}H NMR (500 MHz, $CDCl_3$): δ (ppm) 1.67 (s, 6H), 1.88 (s, 3H), 3.74 (s, 6H), 6.30 (t, J = 2 Hz, 1H), 6.34 (d, J = 2.5 Hz, 2H), 7.06 (d, J = 8 Hz, 1H), 7.22 (m, 2H), 7.54 (dd, J = 2, 8 Hz, 1H); MS: (ESI, Pos) m/z 293.2 [(M+23)$^+$].

4.30. 1-[1-(3,5-Dimethyl-phenyl)-1-methyl-ethyl]-3,5-dimethoxy-benzene (21)

Yellow oil (2.17 g, 81.9%). R_f = 0.5 (ethyl acetate/hexane 6:94); ^{1}H NMR (500 MHz, $CDCl_3$): δ (ppm) 1.62 (s, 6H), 2.26 (s, 6H), 3.73 (s, 6H), 6.29 (t, J = 2.5 Hz, 1H), 6.39 (d, J = 2.5 Hz, 2H), 6.80 (br s, 1H), 6.84 (br s, 2H); MS: (ESI, Pos) m/z 307.3 [(M+23)$^+$].

4.31. 1-[1-(4-Ethyl-phenyl)-1-methyl-ethyl]-3,5-dimethoxy-benzene (22)

Yellow oil (1.01 g, 78.3%). R_f = 0.45 (ethyl acetate/hexane 6:94); ^{1}H NMR (500 MHz, $CDCl_3$): δ (ppm) 1.22 (t, J = 7.5 Hz, 3H), 1.64 (s, 6H), 2.60 (q, J = 7.5 Hz, 2H), 3.74 (s, 6H), 6.29 (t, J = 2.5 Hz, 1H), 6.40 (d, J = 2 Hz, 2H), 7.08 (d, J = 8 Hz, 2H), 7.14 (d, J = 8.5 Hz, 2H); MS: (ESI, Pos) m/z 307.4 [(M+23)$^+$].

4.32. 1-[1-(4-Propyl-phenyl)-1-methyl-ethyl]-3,5-dimethoxy-benzene (23)

Yellow oil (0.3 g, 74.5%). R_f = 0.47 (ethyl acetate/hexane 6:94); ^{1}H NMR (500 MHz, $CDCl_3$): δ (ppm) 0.93 (t, J = 7.5 Hz, 3H), 1.61 (m, 2H), 1.64 (s, 6H), 2.55 (t, J = 7.5 Hz, 2H), 3.73 (s, 6H), 6.29 (t, J = 2.5 Hz, 1H), 6.39 (d, J = 2.5 Hz, 2H), 7.06 (d, J = 8 Hz, 2H), 7.13 (d, J = 8 Hz, 2H); MS: (ESI, Pos) m/z 321.2 [(M+23)$^+$].

4.33. 1-[1-(4-Fluoro-phenyl)-1-methyl-ethyl]-3,5-dimethoxy-benzene (24)

Yellow oil (1.52 g, 76.7%). R_f = 0.37 (ethyl acetate/hexane 6:94); ^{1}H NMR (500 MHz, $CDCl_3$): δ (ppm) 1.63 (s, 6H), 3.73 (s, 6H), 6.30 (t, J = 2 Hz, 1H), 6.35 (d, J = 2.5 Hz, 2H), 6.93 (m, 2H), 7.19 (m, 2H); MS: (ESI, Pos) m/z 297.3 [(M+23)$^+$].

4.34. 1-[1-(3-Fluoro-phenyl)-1-methyl-ethyl]-3,5-dimethoxy-benzene (25)

Yellow oil (1.66 g, 87.0%). R_f = 0.37 (ethyl acetate/hexane 6:94); ^{1}H NMR (500 MHz, $CDCl_3$): δ (ppm) 1.68 (s, 6H), 3.77 (s, 6H), 6.35

(t, J = 2 Hz, 1H), 6.41 (d, J = 2.5 Hz, 2H), 6.87 (td, J = 3, 8.5 Hz, 1H), 6.97 (dt, J = 2, 11 Hz, 1H), 7.04 (d, J = 8 Hz, 1H), 7.24 (m, 1H); MS: (ESI, Pos) m/z 297.2 [$(M+23)^+$].

4.35. 1-[1-(4-Chloro-phenyl)-1-methyl-ethyl]-3,5-dimethoxy-benzene (26)

Yellow oil (1.63 g, 88.6%). R_f = 0.42 (ethyl acetate/hexane 6:94); ^{1}H NMR (500 MHz, $CDCl_3$): δ (ppm) 1.62 (s, 6H), 3.73 (s, 6H), 6.30 (t, J = 2 Hz, 1H), 6.35 (d, J = 2 Hz, 2H), 7.16 (d, J = 8.5 Hz, 2H), 7.21 (d, J = 9 Hz, 3H); MS: (ESI, Pos) m/z 313.1 [$(M+23)^+$].

4.36. 1-[1-(3-Chloro-phenyl)-1-methyl-ethyl]-3,5-dimethoxy-benzene (27)

Colorless oil (1.85 g, 88.0%). R_f = 0.42 (ethyl acetate/hexane 6:94); ^{1}H NMR (500 MHz, $CDCl_3$): δ (ppm) 1.63 (s, 6H), 3.73 (s, 6H), 6.30 (t, J = 2 Hz, 1H), 6.36 (d, J = 2 Hz, 2H), 7.08 (dt, J = 1.5, 7.5 Hz, 1H), 7.16 (m, 2H), 7.23 (t, J = 2 Hz, 1H); MS: (ESI, Pos) m/z 313.1 [$(M+23)^+$].

4.37. 1-[1-(4-Bromo-phenyl)-1-methyl-ethyl]-3,5-dimethoxy-benzene (28)

Colorless oil (2.37 g, 82.3%). R_f = 0.43 (ethyl acetate/hexane 6:94); ^{1}H NMR (500 MHz, $CDCl_3$): δ (ppm) 1.66 (s, 6H), 3.79 (s, 6H), 6.32 (t, J = 2 Hz, 1H), 6.38 (d, J = 2.5 Hz, 2H), 7.12 (d, J = 8.5 Hz, 2H), 7.40 (d, J = 8.5 Hz, 2H); MS: (ESI, Pos) m/z 358.2 [$(M+23)^+$].

4.38. 1-[1-(3-Bromo-phenyl)-1-methyl-ethyl]-3,5-dimethoxy-benzene (29)

Colorless oil (2.34 g, 89.7%). R_f = 0.39 (ethyl acetate/hexane 5:95); ^{1}H NMR (500 MHz, $CDCl_3$): δ (ppm) 1.68 (s, 6H), 3.77 (s, 6H), 6.33 (t, J = 2 Hz, 1H), 6.38 (d, J = 2.5 Hz, 2H), 7.15 (m, 2H), 7.33 (m, 1H), 7.42 (m, 1H); MS: (ESI, Pos) m/z 358.1 [$(M+23)^+$].

4.39. 2-[1-(3,5-Dimethoxy-phenyl)-1-methyl-ethyl]-thiophene (30)

Colorless oil (0.69 g, 65.3%). R_f = 0.31 (ethyl acetate/hexane 5:95); ^{1}H NMR (500 MHz, $CDCl_3$): δ (ppm) 1.74 (s, 6H), 3.74 (s, 6H), 6.31 (t, J = 2.5 Hz, 1H), 6.47 (d, J = 2 Hz, 2H), 6.83 (dd, J = 1.5, 3.5 Hz, 1H), 6.90 (m, 1H), 7.13 (dd, J = 1.5, 5 Hz, 1H); MS: (ESI, Pos) m/z 285.2 [$(M+23)^+$].

4.40. 4-[1-(3,5-Dimethoxy-phenyl)-1-methyl-ethyl]-benzonitrile (44)

Bromide **28** (0.57 g, 1.7 mmol) and copper (I) cyanide (0.40 g, 4.48 mmol) in DMF (4.1 mL) were heated to reflux for 6 h. The mixture was then poured into a solution of ethylene diamine (3 mL) in water (10 mL). The crude product was extracted by repeated washing with ethyl acetate. The organic layer was washed with water and brine and then dried over sodium sulfate. The solvent was removed under reduced pressure and purified via column chromatography using ethyl acetate/hexane (8:92) to yield a colorless oil (0.42 g, 87.8%). R_f = 0.21 (ethyl acetate/hexane 8:92); ^{1}H NMR (500 MHz, $CDCl_3$): δ (ppm) 1.67 (s, 6H), 3.76 (s, 6H), 6.32 (t, J = 2 Hz, 1H), 6.38 (d, J = 2.5 Hz, 2H), 7.35 (d, J = 8.5 Hz, 2H), 7.58 (d, J = 8.5 Hz, 2H); MS: (ESI, Pos) m/z 304.3 [$(M+23)^+$].

Utilizing appropriately substituted bromide, the following dimethyl intermediate was similarly synthesized.

4.41. 3-[1-(3,5-Dimethoxy-phenyl)-1-methyl-ethyl]-benzonitrile (45)

Colorless oil. R_f = 0.23 (ethyl acetate/hexane 8:92); ^{1}H NMR (500 MHz, $CDCl_3$): δ (ppm) 1.67 (s, 6H), 3.80 (s, 6H), 6.31 (t, J = 2 Hz, 1H), 6.35 (d, J = 2.5 Hz, 2H), 7.37 (t, J = 7.5 Hz, 1H), 7.48 (m, 2H), 7.56 (m, 1H); MS: (ESI, Pos) m/z 304.3 [$(M+23)^+$].

4.42. 3-[1-(3,5-Dimethoxy-phenyl)-1-methyl-ethyl]-phenyl amine

Into an oven-dried flask were added $Pd_2(dba)_3$ (0.016 g, 0.018 mmol) and 2-dicyclohexylphosphinobiphenyl (0.015 g, 0.043 mmol) and the flask was evacuated and filled with argon. To this were added bromide **29** (1.2 g, 3.58 mmol) and LiHMDS (4.3 mL, 1 M solution, 4.3 mmol) and the reaction mixture was stirred in a preheated oil bath at 65 °C for 15 h. The mixture was cooled to room temperature and then reacted with 1 M HCl (5 mL) for 5 min. The solution was then neutralized with 1 M NaOH and the aqueous phase was extracted with CH_2Cl_2, washed with water and brine, and then dried over sodium sulfate. The organic layer was evaporated and the product was obtained as a light brown oil (0.75 g, 77.2%) by column chromatography using ethyl acetate/hexane (15:85). R_f = 0.21 (ethyl acetate/hexane 15:85); ^{1}H NMR (500 MHz, $CDCl_3$): δ (ppm) 1.61 (s, 6H), 3.21 (br s, 1H), 3.73 (s, 6H), 6.29 (t, J = 2 Hz, 1H), 6.40 (d, J = 2.5 Hz, 2H), 6.51 (m, 1H), 6.54 (t, J = 2.5 Hz, 1H), 6.67 (m, 1H), 7.05 (t, J = 7.5 Hz, 1H); MS: (ESI, Pos) m/z 294.2 [$(M+23)^+$] and 272.2 [$(M+1)^+$].

4.43. *N*-{3-[1-(3,5-Dimethoxy-phenyl)-1-methyl-ethyl]-phenyl}acetamide (46)

Acetyl chloride (0.08 mL, d = 1.10, 1.21 mmol) and pyridine (0.15 mL) were added into a solution of 3-[1-(3,5-Dimethoxy-phenyl)-1-methyl-ethyl]-phenyl amine (0.3 g, 1.10 mmol) in CH_2Cl_2 (1.53 mL) cooled to 0 °C. The reaction mixture was then allowed to stir at 0 °C for 2 h. The mixture was washed with 2 N HCl, water, and brine and dried over sodium sulfate. The organic layer was then evaporated and the amide was obtained by column chromatography using ethyl acetate/hexane (30:70) as colorless oil (0.24 g, 69.3%). R_f = 0.39 (ethyl acetate/hexane 50:50); ^{1}H NMR (500 MHz, $CDCl_3$): δ (ppm) 1.63(s, 6H), 2.13 (s, 3H), 3.74 (s, 6H), 6.30 (t, J = 2 Hz, 1H), 6.38 (d, J = 2.5 Hz, 2H), 7.01 (d, J = 7.5 Hz, 1H), 7.12 (s, 1H), 7.13 (br s, 1H), 7.23 (t, J = 8 Hz, 1H), 7.53 (d, J = 7.5 Hz, 1H); MS: (ESI, Pos) m/z 336.2 [$(M+23)^+$].

4.44. 5-(1-Methyl-1-*p*-tolyl-ethyl)-benzene-1,3-diol (31)

A solution of compound **18** (0.85 g, 3.15 mmol) in methylene chloride (128 mL) was cooled to −78 °C to which was added BBr_3 (8.06 mL, 1 M solution, 8.06 mmol). The reaction mixture was allowed to stir at −78 °C for 3 h then warmed to room temperature and stirred overnight. The reaction was then quenched with methanol (20 mL) and extracted with ether. The ethereal layer was washed with bicarbonate, water, and brine and dried over sodium sulfate. The organic layer was evaporated and compound **31** was obtained by column chromatography using ethyl acetate/hexane (30:70) as a white solid (0.71 g, 93.2%). R_f = 0.25 (ethyl acetate/hexane 30:70); ^{1}H NMR (500 MHz, $CDCl_3$): δ (ppm) 1.60 (s, 6H), 2.31 (s, 3H), 4.69 (s, 2H), 6.16 (t, J = 2.5 Hz, 1H), 6.27 (d, J = 2.5 Hz, 2H), 7.07 (d, J = 8 Hz, 2H), 7.13 (d, J = 8.5 Hz, 2H); MS: (ESI, Neg) m/z 241.2 [$(M-1)^-$].

Utilizing the same procedure, the following resorcinols were synthesized.

4.45. 5-(1-Methyl-1-*m*-tolyl-ethyl)-benzene-1,3-diol (32)

Colorless oil (0.33 g, 94.4%). R_f = 0.28 (ethyl acetate/hexane 30:70); ^{1}H NMR (500 MHz, $CDCl_3$): δ (ppm) 1.61 (s, 6H), 2.31 (s, 3H), 4.80 (s, 2H), 6.17 (t, J = 2.5 Hz, 1H), 6.27 (d, J = 2.5 Hz, 2H), 6.99 (d, J = 7.5 Hz, 1H), 7.03 (m, 2H), 7.16 (t, J = 8 Hz, 1H); MS: (ESI, Neg) m/z 241.2 [(M−1)$^-$].

4.46. 5-(1-Methyl-1-*o*-tolyl-ethyl)-benzene-1,3-diol (33)

Colorless oil (0.32 g, 93.2%). R_f = 0.26 (ethyl acetate/hexane 30:70); ^{1}H NMR (500 MHz, $CDCl_3$): δ (ppm) 1.64 (s, 6H), 1.98 (s, 3H), 4.59 (s, 2H), 6.18 (t, J = 2 Hz, 1H), 6.23 (d, J = 2.5 Hz, 2H), 7.08 (d, J = 8 Hz, 1H), 7.20 (m, 2H), 7.55 (dd, J = 2, 8 Hz, 1H); MS: (ESI, Neg) m/z 241.2 [(M−1)$^-$].

4.47. 5-(1-(3,5-Dimethyl-phenyl)-1-methyl-ethyl)-benzene-1,3-diol (34)

White solid (1.3 g, 83.4%). R_f = 0.33 (ethyl acetate/hexane 30:70); ^{1}H NMR (500 MHz, $CDCl_3$): δ (ppm) 1.62 (s, 6H), 2.23 (s, 6H), 4.60 (s, 2H), 6.19 (t, J = 2 Hz, 1H), 6.29 (d, J = 2.5 Hz, 2H), 6.86 (m, 3H); MS: (ESI, Neg) m/z 255.3 [(M−1)$^-$].

4.48. 5-(1-(4-Ethyl-phenyl)-1-methyl-ethyl)-benzene-1,3-diol (35)

Colorless oil (1 g, 65.2%). R_f = 0.32 (ethyl acetate/hexane 30:70); ^{1}H NMR (500 MHz, $CDCl_3$): δ (ppm) 1.25 (t, J = 7.5 Hz, 3H), 1.63 (s, 6H), 2.65 (q, J = 7.5 Hz, 2H), 4.59 (s, 2H), 6.19 (t, J = 2.5 Hz, 1H), 6.30 (d, J = 2.5 Hz, 2H), 7.12 (d, J = 8.5 Hz, 2H), 7.17 (d, J = 8.5 Hz, 2H); MS: (ESI, Neg) m/z 255.3 [(M−1)$^-$].

4.49. 5-(1-(4-Propyl-phenyl)-1-methyl-ethyl)-benzene-1,3-diol (36)

Colorless oil (0.68 g, 68.2%). R_f = 0.34 (ethyl acetate/hexane 30:70); ^{1}H NMR (500 MHz, $CDCl_3$): δ (ppm) 0.97 (t, J = 7.5 Hz, 3H), 1.28 (t, J = 7.5 Hz, 2H), 1.63 (s, 6H), 2.57 (m, 2H), 4.64 (s, 2H), 6.19 (t, J = 2 Hz, 1H), 6.30 (d, J = 2.5 Hz, 2H), 7.09 (d, J = 8.5 Hz, 2H), 7.16 (d, J = 8.5 Hz, 2H); MS: (ESI, Neg) m/z 269.3 [(M−1)$^-$].

4.50. 5-(1-(4-Fluoro-phenyl)-1-methyl-ethyl)-benzene-1,3-diol (37)

Cream colored solid (0.89 g, 96.2%). R_f = 0.29 (ethyl acetate/hexane 30:70); ^{1}H NMR (500 MHz, $CDCl_3$): δ (ppm) 1.63 (s, 6H), 4.63 (s, 2H), 6.20 (t, J = 2.5 Hz, 1H), 6.274 (d, J = 2.5 Hz, 2H), 6.97 (m, 2H), 7.203 (m, 2H); MS: (ESI, Neg) m/z 245.1 [(M−1)$^-$].

4.51. 5-(1-(3-Fluoro-phenyl)-1-methyl-ethyl)-benzene-1,3-diol (38)

Cream colored solid (1.01 g, 94.5%). R_f = 0.28 (ethyl acetate/hexane 30:70); ^{1}H NMR (500 MHz, $CDCl_3$): δ (ppm) 1.63 (s, 6H), 4.60 (s, 2H), 6.20 (t, J = 2.5 Hz, 1H), 6.28 (d, J = 2.5 Hz, 2H), 6.94 (m, 3H), 7.23 (m, 1H); MS: (ESI, Neg) m/z 245.1 [(M−1)$^-$].

4.52. 5-(1-(4-Chloro-phenyl)-1-methyl-ethyl)-benzene-1,3-diol (39)

White solid (0.87 g, 95.3%). R_f = 0.3 (ethyl acetate/hexane 30:70); ^{1}H NMR (500 MHz, $CDCl_3$): δ (ppm) 1.60 (s, 6H), 4.65 (s, 2H), 6.18 (t, J = 2.5 Hz, 1H), 6.24 (d, J = 2.5 Hz, 2H), 7.15 (d, J = 8.5 Hz, 2H), 7.23 (d, J = 8.5 Hz, 2H); MS: (ESI, Neg) m/z 261.2 [(M−1)$^-$].

4.53. 5-(1-(3-Chloro-phenyl)-1-methyl-ethyl)-benzene-1,3-diol (40)

Cream colored solid (0.94 g, 93.7%). R_f = 0.31 (ethyl acetate/hexane 30:70); ^{1}H NMR (500 MHz, $CDCl_3$): δ (ppm) 1.60 (s, 6H), 4.89 (s, 2H), 6.19 (t, J = 2.5 Hz, 1H), 6.25 (d, J = 2.5 Hz, 2H), 7.08 (dt, J = 1.5, 7.5 Hz, 1H), 7.19 (m, 3H); MS: (ESI, Neg) m/z 261.2 [(M−1)$^-$].

4.54. 5-(1-(4-Bromo-phenyl)-1-methyl-ethyl)-benzene-1,3-diol (41)

White solid (0.39 g, 92.5%). R_f = 0.33 (ethyl acetate/hexane 30:70); ^{1}H NMR (500 MHz, $CDCl_3$): δ (ppm) 1.62 (s, 6H), 4.61 (s, 2H), 6.20 (t, J = 2.5 Hz, 1H), 6.26 (d, J = 2.5 Hz, 2H), 7.12 (d, J = 8.5 Hz, 2H), 7.39 (d, J = 8.5 Hz, 2H); MS: (ESI, Neg) m/z 306.2 [(M−1)$^-$].

4.55. 5-(1-(3-Bromo-phenyl)-1-methyl-ethyl)-benzene-1,3-diol (42)

Yellow oil (0.49 g, 92.5%). R_f = 0.34 (ethyl acetate/hexane 30:70); ^{1}H NMR (500 MHz, $CDCl_3$): δ (ppm) 1.63 (s, 6H), 4.62 (s, 2H), 6.20 (t, J = 2.5 Hz, 1H), 6.27 (d, J = 2.5 Hz, 2H), 7.16 (d, J = 4 Hz, 2H), 7.33 (m, 1H), 7.40 (m, 1H); MS: (ESI, Neg) m/z 306.2 [(M−1)$^-$].

4.56. 5-(1-Methyl-1-thiophen-2-yl-ethyl)-benzene-1,3-diol (43)

White solid (0.87 g, 95.3%). R_f = 0.3 (ethyl acetate/hexane 30:70); ^{1}H NMR (500 MHz, $CDCl_3$): δ (ppm) 1.60 (s, 6H), 4.65 (s, 2H), 6.18 (t, J = 2 Hz, 1H), 6.24 (d, J = 2.5 Hz, 2H), 7.15 (d, J = 8.5 Hz, 2H), 7.23 (d, J = 8.5 Hz, 2H); MS: (ESI, Neg) m/z 233.1 [(M−1)$^-$].

4.57. 4-[1-(3,5-Dihydroxy-phenyl)-1-methyl-ethyl]-benzonitrile (44)

Colorless oil (0.19 g, 54.1%). R_f = 0.21 (ethyl acetate/hexane 30:70); ^{1}H NMR (500 MHz, $CDCl_3$): δ (ppm) 1.65 (s, 6H), 4.73 (s, 2H), 6.22 (t, J = 2.5 Hz, 1H), 6.26 (d, J = 2.5 Hz, 2H), 7.35 (d, J = 8.5 Hz, 2H), 7.59 (d, J = 8.5 Hz, 2H); MS: (ESI, Neg) m/z 252.3 [(M−1)$^-$].

4.58. 3-[1-(3,5-Dihydroxy-phenyl)-1-methyl-ethyl]-benzonitrile (45)

Colorless oil (0.28 g, 97.2%). R_f = 0.22 (ethyl acetate/hexane 30:70); ^{1}H NMR (500 MHz, $CDCl_3$): δ (ppm) 1.65 (s, 6H), 4.68 (s, 2H), 6.22 (t, J = 2 Hz, 1H), 6.26 (d, J = 2.5 Hz, 2H), 7.38 (t, J = 7.5 Hz, 2H), 7.49 (tt, J = 1.5, 7.5 Hz, 2H), 7.56 (t, J = 1.5 Hz, 1H); MS: (ESI, Neg) m/z 252.3 [(M−1)$^-$].

4.59. N-{3-[1-(3,5-dihydroxy-phenyl)-1-methyl-ethyl]-phenyl}-acetamide (46)

Light brown oil (0.18 g, 79.1%). R_f = 0.15 (ethyl acetate/hexane 50:50); ^{1}H NMR (500 MHz, DMSO): δ (ppm) 1.51 (s, 6H), 1.99 (s, 3H), 5.99 (t, J = 2 Hz, 1H), 6.04 (d, J = 2.5 Hz, 2H), 6.89 (d, J = 7.5 Hz, 1H), 7.18 (t, J = 8 Hz, 1H), 7.29 (s, 1H), 7.53 (d, J = 7.5 Hz, 1H), 9.01 (s, 2H), 9.83 (s, 1H); MS: (ESI, Pos) m/z 308.2 [(M+23)$^+$].

4.60. *trans*-(*R*)-(+)-Limonene oxide (48)

The kinetic separation of *cis*/*trans*(+)-limonene oxide (53.8 mL, d = 0.93, 328 mmol) was carried out as described by Steiner et al.,[21] using pyrazole (3.72 g, 54.7 mmol) and de-ionized water (177 mL). Yield 21.2 g, (84.8%); ^{1}H NMR (500 MHz, $CDCl_3$): δ

(ppm) 1.31 (s, 3H), 1.39 (m, 2H), 1.69 (s, 3H), 1.71 (m, 2H), 1.86 (m, 2H), 2.06 (m, 1H), 2.98 (m, 1H), 4.66 (s, 2H); MS: (ESI, Pos) *m/z* 175.2 [(M+23)$^+$].

4.61. (+)-(1*S*,4*R*)-*p*-Mentha-2,8-dien-1-ol (49)

trans-(*R*)-(+)-Limonene oxide (**48**, 5.1 g, 33.5 mmol) was converted to (+)-(1*S*,4*R*)-*p*-mentha-2,8-dien-1-ol, utilizing the method of Richards and Watson.[20] Yield 1.58 g (31.6%) as a colorless oil. α_D^{23} +53.8° ($CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ (ppm) 1.22 (s, 3H), 1.46 (br s, 1H), 1.59 (m, 2H), 1.67 (s, 3H), 1.76 (m, 2H), 2.67 (m, 1H), 4.59 (m, 1H), 4.71 (t, *J* = 1.5 Hz, 1H), 5.54 (dd, *J* = 3, 7 Hz, 1H), 5.64 (dd, *J* = 1.5, 7 Hz, 1H); MS: (ESI, Pos) *m/z* 151.2 [(M−1)$^-$].

4.62. 6,6,9-Trimethyl-3-(1-methyl-1-*p*-tolyl-ethyl)-6a,7,10,10a-tetrahydro-6*H*-benzo[*c*]chromen-1-ol (50)

Resorcinol **31** (0.63 g, 2.60 mmol), (+)-Δ^2-*p*-menthene-1,8-diol (0.44 g, 2.60 mmol), and *p*-toluene sulfonic acid (0.02 g, 0.11 mmol) were dissolved in benzene (21.7 mL) and the reaction mixture was placed in a preheated oil bath at 80 °C and allowed to stir at that temperature for 6 h. The mixture was then cooled, diluted with ether, and washed with bicarbonate, water, and brine. The ethereal layer was then separated, dried over sodium sulfate, and evaporated. The product was purified by column chromatography using methylene chloride/hexane (40:60) yielding a white foam (0.545 g, 55.6%). R_f = 0.33 (methylene chloride/hexane, 1:1); ^{1}H NMR (500 MHz, $CDCl_3$): δ (ppm) 1.11 (s, C*H*$_3$, 3H), 1.37 (s, C*H*$_3$, 3H), 1.59 (m, C*H*$_3$, 6H), 1.69 (s, C*H*$_3$, 3H), 1.81 (m, C*H*$_2$ and C*H*, 3H), 2.14 (m, C*H*$_2$, 1H), 2.31 (s, C*H*$_3$, 3H), 2.67 (m, C*H*, 1H), 3.16 (dd, *J* = 4.5, 15 Hz, C*H*$_2$, 1H), 4.53 (s, O*H*, 1H), 5.42 (d, *J* = 4.5 Hz, =C*H*, 1H), 5.98 (d, *J* = 2 Hz, Ar*H*, 1H), 6.43 (d, *J* = 2 Hz, Ar*H*, 1H), 7.07 (d, *J* = 8 Hz, Ar*H*, 2H), 7.143 (d, *J* = 8 Hz, Ar*H*, 2H); MS: (ESI, Neg) *m/z* 375.4 [(M−1)$^-$]. HR-EI-MS *m/z* calcd for $C_{26}H_{32}O_2$: 376.2402, experimental 376.2391.

Utilizing the same procedure, the following analogs were synthesized using the appropriately substituted resorcinols.

4.63. 6,6,9-Trimethyl-3-(1-methyl-1-*m*-tolyl-ethyl)-6a,7,10,10a-tetrahydro-6*H*-benzo[*c*]chromen-1-ol (51)

Yellow oil (0.139 g, 28.8%). R_f = 0.34 (methylene chloride/hexane, 1:1); ^{1}H NMR (500 MHz, $CDCl_3$): δ (ppm) 1.12 (s, C*H*$_3$, 3H), 1.38 (s, C*H*$_3$, 3H), 1.59 (m, C*H*$_3$, 6H), 1.69 (s, C*H*$_3$, 3H), 1.82 (m, C*H*$_2$ and C*H*, 3H), 2.14 (m, C*H*$_2$, 1H), 2.31 (s, C*H*$_3$, 3H), 2.68 (m, C*H*, 1H), 3.16 (dd, *J* = 4.5, 15 Hz, C*H*$_2$, 1H), 4.53 (s, O*H*, 1H), 5.42 (d, *J* = 4.5 Hz, =C*H*, 1H), 5.98 (d, *J* = 2 Hz, Ar*H*, 1H), 6.43 (d, *J* = 2 Hz, Ar*H*, 1H), 6.98 (d, *J* = 7 Hz, Ar*H*, 1H), 7.07 (m, Ar*H*, 2H), 7.15 (t, *J* = 7.5 Hz, Ar*H*, 1H); MS: (ESI, Neg) *m/z* 375.5 [(M−1)$^-$]. HR-EI-MS *m/z* calcd for $C_{26}H_{32}O_2$: 376.2402, experimental 376.2387.

4.64. 6,6,9-Trimethyl-3-(1-methyl-1-*o*-tolyl-ethyl)-6a,7,10,10a-tetrahydro-6*H*-benzo[*c*]chromen-1-ol (52)

White foam (0.13 g, 27.9%). R_f = 0.33 (methylene chloride/hexane, 1:1); ^{1}H NMR (500 MHz, $CDCl_3$): δ (ppm) 1.11 (s, C*H*$_3$, 3H), 1.37 (s, C*H*$_3$, 3H), 1.61 (m, C*H*$_3$, 6H), 1.69 (s, C*H*$_3$, 3H), 1.82 (m, C*H*$_2$ and C*H*, 3H), 1.88 (s, C*H*$_3$, 3H), 2.12 (m, C*H*$_2$, 1H), 2.67 (m, C*H*, 1H), 3.16 (dd, *J* = 4.5 Hz, 15 Hz, C*H*$_2$, 1H), 4.52 (s, O*H*, 1H), 5.42 (d, *J* = 4.5 Hz, =C*H*, 1H), 5.90 (d, *J* = 2 Hz, Ar*H*, 1H), 6.38 (d, *J* = 2 Hz, Ar*H*, 1H), 7.05 (d, *J* = 7.5 Hz, Ar*H*, 1H), 7.19 (m, Ar*H*, 2H), 7.50 (dd, *J* = 1, 7.5 Hz, Ar*H*, 1H); MS: (ESI, Neg) *m/z* 375.4 [(M−1)$^-$]. HR-EI-MS *m/z* calcd for $C_{26}H_{32}O_2$: 376.2402, experimental 376.2413.

4.65. 3-[1-(4-Fluoro-phenyl)-1-methyl-ethyl]-6,6,9-trimethyl-6a,7,10,10a-tetrahydro-6*H*-benzo[*c*]chromen-1-ol (56)

Light pink foam (0.59 g, 45.5%). R_f = 0.37 (methylene chloride/hexane, 1:1); ^{1}H NMR (500 MHz, $CDCl_3$): δ (ppm) 1.04 (s, C*H*$_3$, 3H), 1.30 (s, C*H*$_3$, 3H), 1.51 (m, C*H*$_3$, 6H), 1.62 (s, C*H*$_3$, 3H), 1.75 (m, C*H*$_2$ and C*H*, 3H), 2.07 (m, C*H*$_2$, 1H), 2.61 (m, C*H*, 1H), 3.09 (dd, *J* = 4.5, 15 Hz, C*H*$_2$, 1H), 4.49 (s, O*H*, 1H), 5.35 (d, *J* = 4.5 Hz, =C*H*, 1H), 5.90 (d, *J* = 2 Hz, Ar*H*, 1H), 6.32 (d, *J* = 2 Hz, Ar*H*, 1H), 6.86 (m, Ar*H*, 2H), 7.12 (m, Ar*H*, 2H); MS: (ESI, Neg) *m/z* 379.5 [(M−1)$^-$]. HR-EI-MS *m/z* calcd for $C_{25}H_{29}FO_2$: 380.2152, experimental 380.2152.

4.66. 3-[1-(3-Fluoro-phenyl)-1-methyl-ethyl]-6,6,9-trimethyl-6a,7,10,10a-tetrahydro-6*H*-benzo[*c*]chromen-1-ol (57)

White foam (0.36 g, 24.4%). R_f = 0.37 (methylene chloride/hexane, 1:1); ^{1}H NMR (500 MHz, $CDCl_3$): δ (ppm) 1.06 (s, C*H*$_3$, 3H), 1.21 (s, C*H*$_3$, 3H), 1.53 (m, C*H*$_3$, 6H), 1.66 (s, C*H*$_3$, 3H), 1.76 (m, C*H*$_2$ and C*H*, 3H), 2.17 (m, C*H*$_2$, 1H), 2.52 (m, C*H*, 1H), 3.08 (dd, *J* = 4.5, 15 Hz, C*H*$_2$, 1H), 4.36 (s, O*H*, 1H), 5.41 (d, *J* = 4.5 Hz, =C*H*, 1H), 5.82 (d, *J* = 2 Hz, Ar*H*, 1H), 6.22 (d, *J* = 2 Hz, Ar*H*, 1H), 6.67 (td, *J* = 2.5, 8.5 Hz, Ar*H*, 1H), 6.88 (m, Ar*H*, 1H), 7.02 (m, Ar*H*, 1H), 7.12 (m, Ar*H*, 1H); MS: (ESI, Neg) *m/z* 379.5 [(M−1)$^-$]. HR-EI-MS *m/z* calcd for $C_{25}H_{29}FO_2$: 380.2152, experimental 380.2152.

4.67. 3-[1-(4-Chloro-phenyl)-1-methyl-ethyl]-6,6,9-trimethyl-6a,7,10,10a-tetrahydro-6*H*-benzo[*c*]chromen-1-ol (58)

White foam (0.63 g, 50.4%). R_f = 0.41 (methylene chloride/hexane, 1:1); ^{1}H NMR (500 MHz, $CDCl_3$): δ (ppm) 1.04 (s, C*H*$_3$, 3H), 1.30 (s, C*H*$_3$, 3H), 1.51 (m, C*H*$_3$, 6H), 1.62 (s, C*H*$_3$, 3H), 1.75 (m, C*H*$_2$ and C*H*, 3H), 2.07 (m, C*H*$_2$, 1H), 2.61 (m, C*H*, 1H), 3.09 (dd, *J* = 4.5, 15 Hz, C*H*$_2$, 1H), 4.49 (s, O*H*, 1H), 5.35 (d, *J* = 5 Hz, =C*H*, 1H), 5.89 (d, *J* = 2 Hz, Ar*H*, 1H), 6.30 (d, *J* = 2 Hz, Ar*H*, 1H), 7.09 (d, *J* = 8.5 Hz, Ar*H*, 2H), 7.15 (d, *J* = 9 Hz, Ar*H*, 2H); MS: (ESI, Neg) *m/z* 395.9 [(M−1)$^-$]. HR-EI-MS *m/z* calcd for $C_{25}H_{29}ClO_2$: 396.1856, experimental 396.1856.

4.68. 3-[1-(3-Chloro-phenyl)-1-methyl-ethyl]-6,6,9-trimethyl-6a,7,10,10a-tetrahydro-6*H*-benzo[*c*]chromen-1-ol (59)

White foam (0.66 g, 50.6%). R_f = 0.37 (methylene chloride/hexane, 1:1); ^{1}H NMR (500 MHz, $CDCl_3$): δ (ppm) 1.04 (s, C*H*$_3$, 3H), 1.30 (s, C*H*$_3$, 3H), 1.51 (m, C*H*$_3$, 6H), 1.62 (s, C*H*$_3$, 3H), 1.75 (m, C*H*$_2$ and C*H*, 3H), 2.07 (m, C*H*$_2$, 1H), 2.61 (m, C*H*, 1H), 3.09 (m, C*H*$_2$, 1H), 4.51 (s, O*H*, 1H), 5.35 (d, *J* = 4.5, 15 Hz, =C*H*, 1H), 5.89 (d, *J* = 1.5 Hz, Ar*H*, 1H), 6.31 (d, *J* = 1.5 Hz, Ar*H*, 1H), 7.03 (dt, *J* = 2, 7.5 Hz, Ar*H*, 1H), 7.07 (m, Ar*H*, 2H), 7.17 (t, *J* = 2 Hz, Ar*H*, 1H); MS: (ESI, Neg) *m/z* 395.9 [(M−1)$^-$]. HR-EI-MS *m/z* calcd for $C_{25}H_{29}ClO_2$: 396.1856, experimental 396.1869.

4.69. 3-[1-(4-Bromo-phenyl)-1-methyl-ethyl]-6,6,9-trimethyl-6a,7,10,10a-tetrahydro-6*H*-benzo[*c*]chromen-1-ol (60)

White foam (0.13 g, 25.2%). R_f = 0.34 (methylene chloride/hexane, 40:60); ^{1}H NMR (500 MHz, $CDCl_3$): δ (ppm) 1.11 (s, C*H*$_3$, 3H), 1.37 (s, C*H*$_3$, 3H), 1.58 (m, C*H*$_3$, 6H), 1.69 (s, C*H*$_3$, 3H), 1.82 (m, C*H*$_2$ and C*H*, 3H), 2.15 (m, C*H*$_2$, 1H), 2.68 (m, C*H*, 1H), 3.17 (dd, *J* = 4.5, 15 Hz, C*H*$_2$, 1H), 4.57 (s, O*H*, 1H), 5.42 (d, *J* = 4.5 Hz, =C*H*, 1H), 5.97 (d, *J* = 2 Hz, Ar*H*, 1H), 6.37 (d, *J* = 2 Hz, Ar*H*, 1H), 7.12 (d, *J* = 8.5 Hz, Ar*H*, 2H), 7.36 (d, *J* = 8.5 Hz, Ar*H*, 2H); MS: (ESI, Neg) *m/z* 440.4 [(M−1)$^-$]. HR-EI-MS *m/z* calcd for $C_{25}H_{29}BrO_2$: 440.1351, experimental 440.1358.

4.70. 3-[1-(3-Bromo-phenyl)-1-methyl-ethyl]-6,6,9-trimethyl-6a,7,10,10a-tetrahydro-6*H*-benzo[*c*]chromen-1-ol (61)

White foam (0.14 g, 21.3%). R_f = 0.34 (methylene chloride/hexane, 40:60); ^{1}H NMR (500 MHz, $CDCl_3$): δ (ppm) 1.12 (s, C*H*$_3$, 3H), 1.38 (s, C*H*$_3$, 3H), 1.58 (m, C*H*$_3$, 6H), 1.69 (s, C*H*$_3$, 3H), 1.83(m, C*H*$_2$ and C*H*, 3H), 2.15 (m, C*H*$_2$, 1H), 2.68 (m, C*H*, 1H), 3.16 (dd, *J* = 4.5, 15 Hz, C*H*$_2$, 1H), 4.59 (s, O*H*, 1H), 5.42 (d, *J* = 4.5 Hz, =C*H*, 1H), 5.96 (d, *J* = 2 Hz, Ar*H*, 1H), 6.38 (d, *J* = 2 Hz, Ar*H*, 1H), 7.14 (m, Ar*H*, 2H), 7.30 (m, Ar*H*, 1H), 7.4 (m, Ar*H*, 1H); MS: (ESI, Neg) *m/z* 440.4 [(M−1)$^-$]. HR-EI-MS *m/z* calcd for $C_{25}H_{29}BrO_2$: 440.1351, experimental 440.1367.

4.71. 6,6,9-Trimethyl-3-(1-methyl-1-thiophen-2-yl-ethyl)-6a,7,10,10a-tetrahydro-6*H*-benzo[*c*]chromen-1-ol (4)

White foam (0.12 g, 21.9%). R_f = 0.37 (methylene chloride/hexane, 1:1); ^{1}H NMR (500 MHz, $CDCl_3$): δ (ppm) 1.04 (s, C*H*$_3$, 3H), 1.30 (s, C*H*$_3$, 3H), 1.63 (m, C*H*$_3$, 9H), 1.74 (m, C*H*$_2$ and C*H*, 3H), 2.07 (m, C*H*$_2$, 1H), 2.61 (m, C*H*, 1H), 3.10 (dd, *J* = 4.5, 15 Hz, C*H*$_2$, 1H), 4.50 (s, O*H*, 1H), 5.35 (d, *J* = 4.5 Hz, =C*H*, 1H), 6.04 (d, *J* = 2 Hz, Ar*H*, 1H), 6.38 (d, *J* = 2 Hz, Ar*H*, 1H), 6.76 (dd, *J* = 1.5 Hz, 3.5 Hz, Ar*H*, 1H), 6.84 (m, Ar*H*, 1H), 7.07 (dd, *J* = 1 Hz, 5 Hz, Ar*H*, 1H); MS: (ESI, Neg) *m/z* 367.4 [(M−1)$^-$]. HR-EI-MS *m/z* calcd for $C_{23}H_{28}O_2S$: 368.1810, experimental 368.1818.

4.72. 3-[1-(4-Ethyl-phenyl)-1-methyl-ethyl]-6,6,9-trimethyl-6a,7,10,10a-tetrahydro-6*H*-benzo[*c*]chromen-1-ol (54)

Resorcinol **35** (0.4 g, 1.56 mmol), (+)-$\Delta^{2,8}$-*p*-mentha-2,8-dien-1-ol (**49**, 0.29 g, 1.95 mmol) and *p*-toluene sulfonic acid (0.055 g, 0.29 mmol) in benzene (13 mL) were dissolved in benzene (18.3 mL) and the reaction mixture was placed in a preheated oil bath at 80 °C and allowed to stir for 6 h. The mixture was then cooled, diluted with ether, and washed with bicarbonate, water, and brine. The ethereal layer was then separated, dried over sodium sulfate, and evaporated. The product was purified by column chromatography using methylene chloride/hexane (40:60) yielding a white foam (0.13 g, 25.2%). R_f = 0.29 (methylene chloride/hexane, 40:60); ^{1}H NMR (500 MHz, $CDCl_3$): δ (ppm) 1.11 (s, C*H*$_3$, 3H), 1.22 (t, *J* = 7.5 Hz, C*H*$_3$, 3H), 1.37(s, C*H*$_3$, 3H), 1.59 (m, C*H*$_3$, 6H), 1.69 (s, C*H*$_3$, 3H), 1.82(m, C*H*$_2$ and C*H*, 3H), 2.15 (m, C*H*$_2$, 1H), 2.62 (q, *J* = 7.5 Hz, C*H*$_2$, 2H), 2.68 (m, C*H*, 1H), 3.17 (dd, *J* = 4.5, 15 Hz, C*H*$_2$, 1H), 4.55 (s, O*H*, 1H), 5.42 (d, *J* = 4.5 Hz, =C*H*, 1H), 5.98 (d, *J* = 2 Hz, Ar*H*, 1H), 6.43 (d, *J* = 2 Hz, Ar*H*, 1H), 7.09 (d, *J* = 8.0 Hz, Ar*H*, 2H), 7.16 (d, *J* = 8.5 Hz, Ar*H*, 2H); MS: (ESI, Neg) *m/z* 389.4 [(M−1)$^-$]. HR-EI-MS *m/z* calcd for $C_{27}H_{34}O_2$: 390.2559, experimental 390.2570.

4.73. 3-[1-(3,5-Dimethyl-phenyl)-1-methyl-ethyl]-6,6,9-trimethyl-6a,7,10,10a-tetrahydro-6*H*-benzo[*c*]chromen-1-ol (53)

Cream colored foam (0.20 g, 33.1%). R_f = 0.34 (methylene chloride/hexane, 40:60); ^{1}H NMR (500 MHz, $CDCl_3$): δ (ppm) 1.12 (s, C*H*$_3$, 3H), 1.38(s, C*H*$_3$, 3H), 1.58 (m, C*H*$_3$, 6H), 1.69 (s, C*H*$_3$, 3H), 1.80 (m, C*H*$_2$ and C*H*, 3H), 2.14 (m, C*H*$_2$, 1H), 2.27 (s, C*H*$_3$, 6H), 2.67 (m, C*H*, 1H), 3.18 (dd, *J* = 4.5, 15 Hz, C*H*$_2$, 1H), 4.54 (s, O*H* 1H), 5.41 (d, *J* = 4.5 Hz, =C*H*, 1H), 5.97 (d, *J* = 2 Hz, Ar*H*, 1H), 6.42 (d, *J* = 2 Hz, Ar*H*, 1H), 6.81 (s, Ar*H*, 1H), 6.86 (s, Ar*H*, 2H); MS: (ESI, Neg) *m/z* 389.3 [(M−1)$^-$]. HR-EI-MS *m/z* calcd for $C_{27}H_{34}O_2$, 390.2559: experimental 390.2560.

4.74. 3-[1-(4-Propyl-phenyl)-1-methyl-ethyl]-6,6,9-trimethyl-6a,7,10,10a-tetrahydro-6*H*-benzo[*c*]chromen-1-ol (55)

Cream colored foam (0.30 g, 50.1%). R_f = 0.35 (methylene chloride/hexane, 40:60); ^{1}H NMR (500 MHz, $CDCl_3$): δ (ppm) 0.94 (t, *J* = 7.5 Hz, C*H*$_3$, 3H), 1.11 (s, C*H*$_3$, 3H), 1.37(s, C*H*$_3$, 3H), 1.59 (m, C*H*$_3$, 6H), 1.63 (m, C*H*$_2$, 2H), 1.69 (s, C*H*$_3$, 3H), 1.83 (m, C*H*$_2$ and C*H*, 3H), 2.14 (m, C*H*$_2$, 1H), 2.54 (t, *J* = 7.5 Hz, C*H*$_2$, 2H), 2.68 (m, C*H*, 1H), 3.16 (dd, *J* = 4.5, 15 Hz, C*H*$_2$, 1H), 4.54 (s, O*H*, 1H), 5.42 (d, *J* = 4.5 Hz, =C*H*, 1H), 5.98 (d, *J* = 2 Hz, Ar*H*, 1H), 6.44 (d, *J* = 2 Hz, Ar*H*, 1H), 7.06 (d, *J* = 8.0 Hz, Ar*H*, 2H), 7.15(d, *J* = 8.5 Hz, Ar*H*, 2H); MS: (ESI, Neg) *m/z* 403.2 [(M−1)$^-$]. HR-EI-MS *m/z* calcd for $C_{28}H_{36}O_2$: 404.2715, experimental 404.2734.

4.75. 3-[1-(4-Cyano-phenyl)-1-methyl-ethyl]-6,6,9-trimethyl-6a,7,10,10a-tetrahydro-6*H*-benzo[*c*]chromen-1-ol (62)

Cream colored foam (0.08 g, 40.2%). R_f = 0.2 (methylene chloride/hexane, 50:50); ^{1}H NMR (500 MHz, $CDCl_3$): δ (ppm) 1.11 (s, C*H*$_3$, 3H), 1.37 (s, C*H*$_3$, 3H), 1.61 (m, C*H*$_3$, 6H), 1.69 (s, C*H*$_3$, 3H), 1.83 (m, C*H*$_2$ and C*H*, 3H), 2.14 (m, C*H*$_2$, 1H), 2.68 (m, C*H*, 1H), 3.16 (dd, *J* = 4.5, 15 Hz, C*H*$_2$, 1H), 4.54 (s, O*H*, 1H), 5.42 (d, *J* = 4.5 Hz, =C*H*, 1H), 5.98 (d, *J* = 2 Hz, Ar*H*, 1H), 6.34 (d, *J* = 2 Hz, Ar*H*, 1H), 7.35 (d, *J* = 8.0 Hz, Ar*H*, 2H), 7.55(d, *J* = 8.5 Hz, Ar*H*, 2H); MS: (ESI, Neg) *m/z* 386.2 [(M−1)$^-$]. HR-EI-MS *m/z* calcd for $C_{26}H_{29}NO_2$: 387.2198, experimental 387.2202.

4.76. 3-[1-(3-Cyano-phenyl)-1-methyl-ethyl]-6,6,9-trimethyl-6a,7,10,10a-tetrahydro-6*H*-benzo[*c*]chromen-1-ol (63)

Cream colored foam (0.04 g, 12.1%). R_f = 0.21(methylene chloride/hexane, 50:50); ^{1}H NMR (500 MHz, $CDCl_3$): δ (ppm) 1.11 (s, C*H*$_3$, 3H), 1.37(s, C*H*$_3$, 3H), 1.61 (m, C*H*$_3$, 6H), 1.69 (s, C*H*$_3$, 3H), 1.80 (m, C*H*$_2$ and C*H*, 3H), 2.16 (m, C*H*$_2$, 1H), 2.68 (m, C*H*, 1H), 3.16 (dd, *J* = 4.5, 15 Hz, C*H*$_2$, 1H), 4.63 (s, O*H*, 1H), 5.42 (d, *J* = 4.5 Hz, =C*H*, 1H), 5.96 (d, *J* = 2 Hz, Ar*H*, 1H), 6.33 (d, *J* = 2 Hz, Ar*H*, 1H), 7.35 (t, *J* = 7.5 Hz, Ar*H*, 1H), 7.46 (m, Ar*H*, 2H), 7.54 (m, Ar*H*, 1H); MS: (ESI, Neg) *m/z* 386.2 [(M−1)$^-$]. HR-EI-MS *m/z* calcd for $C_{26}H_{29}NO_2$: 387.2198, experimental 387.2203.

4.77. *N*-{3-[1-(1-hydroxy-6,6,9-trimethyl)-6a,7,10,10a-tetrahydro-6*H*-benzo[*c*]chromen-3-yl-1-methyl-ethyl]-phenyl}acetamide (64)

Yellow oil (0.1 g, 24.6%). R_f = 0.35 (ether/methylene chloride, 8:92); ^{1}H NMR (500 MHz, $CDCl_3$): δ (ppm) 1.11 (s, C*H*$_3$, 3H), 1.37(s, C*H*$_3$, 3H), 1.56 (m, C*H*$_3$, 6H), 1.67 (s, C*H*$_3$, 3H), 1.84 (m, C*H*$_2$ and C*H*, 3H), 2.03 (s, –NHCOC*H*$_3$, 3H), 2.16 (m, C*H*$_2$, 1H), 2.67 (m, C*H*, 1H), 3.21 (dd, *J* = 4.5, 15 Hz, C*H*$_2$, 1H), 5.41 (d, *J* = 4.5 Hz, =C*H*, 1H), 5.91 (d, *J* = 2 Hz, 1H), 6.40 (d, *J* = 2 Hz, 1H), 7.01 (d, 8 Hz, Ar*H*, 1H), 7.11 (s, Ar*H*, 1H). 7.18 (t, *J* = 8 Hz, Ar*H*, 1H), 7.32 (br s, O*H*, 1H), 7.41 (d, *J* = 8 Hz, Ar*H*, 1H), 8.09 (s, -N*H*COCH$_3$, 1H); MS: (ESI, Neg) *m/z* 418.5 [(M−1)$^-$]. HPLC (retention time: 2.75 min).

4.78. Receptor-binding assays

Cell membranes from HEK293 cells transfected with the human CB1 receptor (B_{max}: 1.7 pmol/mg protein, K_d for [^{3}H]CP 55,940 binding: 186 pM) and membranes from CHO-K1 cells transfected with the human CB2 receptor (B_{max}: 3.3 pmol/mg protein, K_d for [^{3}H]CP 55,940 binding: 0.12 nM) were purchased from Perkin-Elmer Life Sciences, Inc. [^{3}H]CP 55,940 having a specific activity of 120 Ci/mmol was obtained from Perkin-Elmer Life Sciences, Inc. All other chemicals and reagents were obtained from Sigma–Aldrich. The assays were carried out in 96-well plates obtained from Millipore, Inc. fitted with glass fiber filters (hydrophilic, GFC filters) having a pore size of 1.2 μm. The filters were soaked with 0.05% polyethyleneimine solution and washed five times with deionized water prior to carrying out the assays. The filtrations were carried out on a 96-well vacuum manifold (Millipore Inc.), the filters punched out with a pipette tip directly into scintillation vials at

the end of the experiment, and vials filled with 5 mL scintillation cocktail Ecolite (+) (Fisher Scientific). Counting was carried out on a Beckmann Scintillation Counter model LS6500. Drug solutions were prepared in DMSO and the radioligand was dissolved in ethanol. Incubation buffer: 50 mM Tris–HCl, 5 mM $MgCl_2$, 2.5 mM EDTA, 0.5 mg/mL fatty acid-free bovine serum albumin, pH 7.4.

Binding protocol for the CB1 receptor: 8 μg of membranes (20 μL of a 1:8 dilution in incubation buffer) was incubated with 5 μL of drug solution (10^{-4}–10^{-12} M) and 5 μL of 5.4 nM [^{3}H]CP 55,940 in a total volume of 200 μL for 90 min at 30 °C. Non-specific binding was determined using 10 μM WIN55, 212-2 (K_i = 20 nM). The membranes were filtered and the filters washed seven times with 0.2 mL ice-cold incubation buffer, and allowed to air-dry under vacuum.

Binding protocol for the CB2 receptor: 15.3 μg of membranes (20 μL of a 1:20 dilution in incubation buffer) was incubated with 5 μL of drug solution (10^{-4}–10^{-12} M) and 5 μL of 10 nM [^{3}H]CP 55,940 in a total volume of 200 μL for 90 min at 30 °C. Non-specific binding was determined using 10 μM WIN55,212-2 (K_i = 4.4 nM). The membranes were filtered and the filters washed seven times with 0.2 mL ice-cold incubation buffer were allowed to air-dry under vacuum.

Data accumulation and statistical analysis: varying concentrations of drug ranging from 10^{-4}–10^{-12} M were added in triplicate for each experiment and the individual molar IC_{50} values were determined using GraphPad Prism. The corresponding K_i values for each drug were determined utilizing the Cheng and Prusoff equation[49] and final data are presented as K_i ± standard error of mean of n = 3 experiments.

4.79. Glioma cytotoxicity assays

Human cancer cells U-87MG, T98G, LN-229, DBTRG-O5MG (American Type Culture Collection), and MT310 (primary GBM cell line, grade IV, a gift from Dr. Valery Kukekov, University of Tennessee Health Science Center, Department of Neurosurgery) were cultured in supplemented media according to the recommendations of the supplier. Cell lines were plated in 96-well flat-bottomed plates at 70% confluency in a 100 μl total volume of supplemented media as indicated, and incubated overnight at 37 °C to allow for adherence. The cultures were inoculated with escalating amounts of drug and cell death was analyzed at 18 h, using the BioTek Synergy 2 Multidetection Microplate Reader. The percentage of viable cells present in the culture at each time point was calculated by comparing the absorbance value at 450 nm from the CCK-8 assay (Dojindo Molecular Technologies) for each condition with untreated control cells. All assays were conducted per manufacturer's protocol. All described values represent the average of three data points per determination and three independent determinations.

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