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The chromenopyrazole scaffold in the modulation of the endocannabinoid system: a broad therapeutic prospect

Title in Spanish: *Aproximación terapéutica a la contribución del esqueleto de cromenopirazol en la modulación del sistema endocannabinoide*

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ABSTRACT: The endogenous cannabinoid system (ECS) has been recognized as one of the most important neuromodulatory systems. This system plays a crucial role in the regulation of numerous pathophysiological conditions such as pain, cancer, or neurodegeneration. Despite the vast effort focused on the development of drugs targeting the ECS, thus far, the clinical use of synthetic and phytogenic cannabinoids has been limited to pain, emesis and appetite due to their undesirable psychoactive properties. Therefore, novel strategies to therapeutically exploit the cannabinoids need to be developed to overcome these side-effects. Moreover, novel chemical tools to study the role of possible additional cannabinoid missing receptors, such as GPR55, need to be addressed to fully unravel the pharmacology of this complex system. In this scenario, the chromenopyrazole scaffold was recently discovered as a privileged structure in drug discovery targeting the ECS. In this review, the development of novel modulators of the ECS based on the chromenopyrazole scaffold will be thoroughly discussed. Pharmacological avenues for this novel chemotype, as well as future perspectives will be analyzed.

RESUMEN: El sistema endocannabinoide (SEC) ha sido reconocido por su gran relevancia a nivel neuromodulador. Este sistema juega un importante rol en la regulación de numerosos procesos fisiopatológicos tales como el cáncer, el dolor o la neurodegeneración. A pesar del amplio potencial terapéutico de los ligandos cannabinoides, su actual uso clínico se limita al tratamiento del dolor, la emesis y la mejora del apetito. El problema fundamental asociado al tratamiento con cannabinoides radica en la imposibilidad actual de separar los efectos terapéuticos de la acción psicoactiva. Por tanto, es de gran interés la identificación de nuevos cannabinoides sintéticos con efectos secundarios reducidos. Otros receptores acoplados a proteínas G, como GPR55, también se han propuesto como posibles miembros del sistema endocannabinoide. Sin embargo, esta categorización aún no ha sido confirmada debido a la falta de herramientas farmacológicas que permitan caracterizar apropiadamente las funciones biológicas de GPR55 y su relación con el SEC. En este contexto, se identificó el esqueleto de cromenopirazol como estructura privilegiada para el desarrollo de moléculas capaces de modular el sistema endocannabinoide. En esta revisión, se van a analizar la farmacología y las oportunidades terapéuticas ofrecidas por los diversos derivados de cromenopirazol descritos hasta la fecha. Posibles perspectivas y aplicaciones futuras así como nuevas aproximaciones serán también consideradas.

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1. INTRODUCTION

Over the centuries, the pharmacological effects of preparations from the plant *Cannabis sativa* have been utilized for recreational and medicinal purposes. Due to its psychoactive effects and despite its traditional medicinal use, cannabis did not gain a wide and lasting acceptance as a valuable drug. Different active substances isolated from *Cannabis sativa* were identified and characterized in the mid-last-century (1). Modern research studies on cannabis started with the structural characterization of tetrahydrocannabinol (Δ^9-THC, figure **1**) by Mechoulam in 1964 (2). More than 100 phytocannabinoids have been isolated from the plant. They all have in common typical C21 terpenophenolic skeleton o derivative chemical

structures that can be classified in 11 families: Δ^9-THC, Δ^8-THC, cannabigerol (CBG), cannabichromene (CBC), cannabidiol (CBD), cannabinodiol (CBND), cannabielsoin (CBE), cannabicyclol (CBL), cannabinol (CBN), and cannabitriol (CBT), miscellaneous-type cannabinoids. Whereas Δ^9-THC is the most abundant and main psychoactive constituent of cannabis, CBD, the second most abundant, is a non-psychotropic substance (3). However, Δ^9-THC has attracted much attention since its discovery. Thus, a considerable amount of pharmacological studies was done on its activity supporting its value as therapeutic agent and that, before the discovery of the endocannabinoid system (ECS).

Over the past three decades, the ECS has emerged as a promising therapeutic target. Two G-protein coupled receptors (GPCRs), the cannabinoid receptors type 1 (CB_1R) and type 2 (CB_2R), and their endogenous lipid ligands were identified in the 1990s as the main constituents of the ECS. CB_1R is highly expressed in the central nervous system (CNS) modulating numerous physiological processes such as cognition, emotion or pain control. This receptor is also localized in the peripheral tissue (liver, kidney, or lung among others) where it modulates energy balance and metabolism (4). On the other hand, CB_2R is mainly expressed in the immune system, the gastrointestinal tract and in certain neuronal subpopulations. Interestingly, the expression of CB_2R in the CNS is upregulated upon neuroinflammatory stimuli, what confers to this receptor an important role in the treatment of neurodegenerative disorders (5).

The orphan receptors GPR18 and GPR55 have also been proposed as potential members of the ECS (6). Unfortunately, the current lack of pharmacological tools to study these receptors is delaying the understanding of their relation with the cannabinoids.

It is widely demonstrated that compounds targeting the ECS have therapeutic potential for the clinical management of an ever growing number of disorders (7). These include inflammatory and neuropathic pain, neurological pathologies, metabolic syndrome, or cancer among others (8–10). The only cannabinoids on clinical use today are the phytocannabinoids Δ^9-THC and CBD, and the Δ^9-THC synthetic derivative nabilone, which are approved for pain, emesis and appetite disorders. Taking into account the fact that these compounds lack of CB_1R/CB_2R selectivity, identifying new synthetic selective cannabinoids is of great interest. These novel entities should be exempt of the undesirable psychotropic effects related to the activation of brain CB_1R to have greater opportunity to be explored as cannabinoid-based medicines.

Numerous cannabinergic ligands have been described thus far. Besides the phytocannabinoids previously detailed, other modulators of the cannabinoid receptors proceeding from endogenous or synthetic sources have been identified. These compounds present very different chemical structures and pharmacological profiles.

Endogenous cannabinoids, endocannabinoids, comprise a family of polyunsaturated fatty acids that structurally differ from phytocannabinoids. These molecules, are lipid neurotransmitters that mediate retrograde signal from postsynaptic neurons to presynaptic ones targeting CBRs (11). Among the most abundant endocannabinoids identified so far are anandamide [*N*-arachidonoylethanolamine (AEA), figure **1**], and 2-arachidonoylglycerol (2-AG, figure **1**).

In addition, the interest gained by the ECS as valuable target for drug discovery led to the synthesis of numerous cannabinoid ligands in the last years. The development of synthetic endocannabinoid and phytocannabinoid analogues, as well as chemically diverse scaffolds, offered novel pharmacological opportunities in this field. Among the most remarkable synthetic cannabimimetics discovered are the well-known aminoalkyindole *R*-(+)-WIN55,212-2 (figure **1**), and the synthetic phytocannabinoid CP55,940 (figure **1**). Both of them are very potent CB_1R/CB_2R agonists that have been extensively used to investigate the endocannabinoid system. Another compound that has significantly contributed to the understanding of the cannabinoid receptors is the arylpyrazole SR141716A (Rimonabant, figure **1**) (12). This compound is a potent CB_1R antagonist/inverse agonist which therapeutic potential was confirmed for the management of obesity (13), however, it also triggers undesired side effects.

Other classes of ligands that exhibit interesting cannabinoid activity have also been developed by pharmaceutical companies and academic research groups. For instance, indole-2-carboxamides, such as the CB_1R allosteric modulator ORG27569 (14), or CB_2R ligands derived from the 1,8-naphthyridine-3-carboxamide scaffold (15,16), were developped.

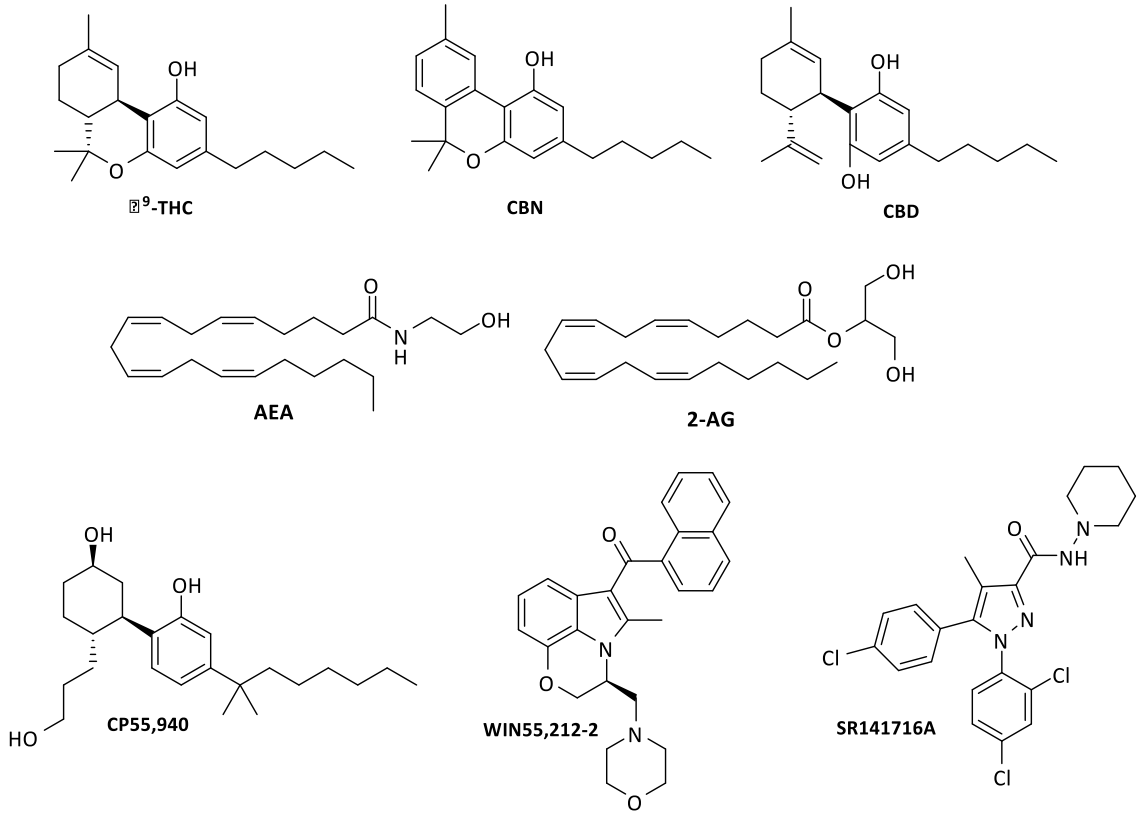
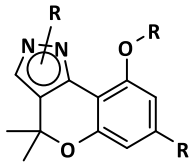


Figure 1. Structure of selected cannabinoid ligands: phytocannabinoids (Δ^9-THC, CBN, and CBD); endocannabinoids (AEA and 2-AG); synthetic derivatives (CP55,940, WIN55,212-2 and SR141716A).

The chromenopyrazole appears in the field of cannabinoid analogues in 1985, before the discovery of the cannabinoid receptors (17). The authors described them as heterocyclic-fused benzopyrans, reported their synthesis, and concluded that these compounds had no interesting levels of activity in the CNS. In 2012, the chromenopyrazole scaffold was re-explored as a cannabinoid chemotype taking advantage of the progress realized in the understanding of the ECS (figure **2**). This tricyclic structure was designed in analogy to the classical cannabinoid cannabinol (CBN), bearing a benzopyran moiety but exploring for the first time the contribution of a pyrazole ring in place of the CBN´s phenyl group.



Chromenopyrazole Scaffold

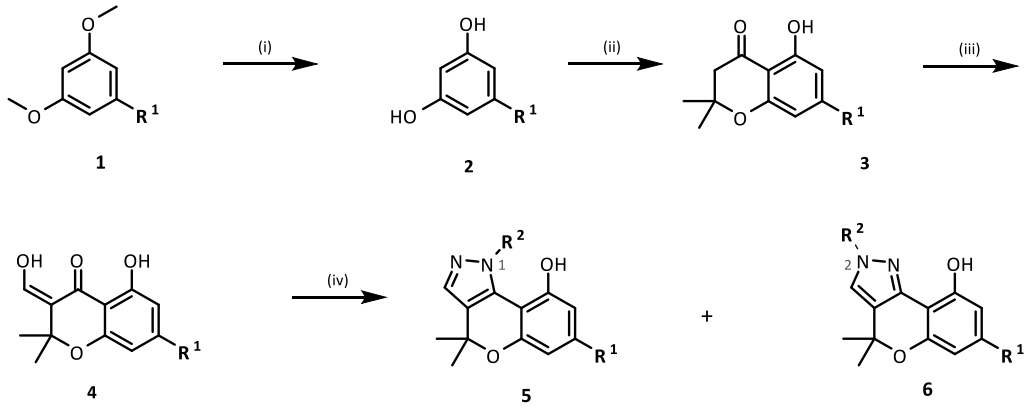
Figure 2. General structural features of the chromenopyrazole scaffold.

Since then, different strategies to target the ECS under diverse pathological conditions have driven the exploration of structural modifications of this tricyclic core. Structure-activity relationships as well as the rational understanding of ligand-receptor interactions helped fine-tuning the potential of the chromenopyrazole scaffold targeting diverse pathological conditions. This work, critically reviews the findings obtained so far offering future perspectives for the use of this versatile scaffold to continue unraveling the complex pharmacology of the ECS.

2. CB_1R SELECTIVE LIGANDS: APPLICATION IN PAIN

As previously mentioned, the psychotropic side-effects of cannabinoid receptor agonists have limited their exploitation as medications. These unwanted properties are mediated by the CB_1Rs located in the CNS, therefore, a strategy to selectively target CB_1Rs located outside the brain is the development of peripherally restricted ligands.

In an effort to identify novel cannabinoids, the chromenopyrazole scaffold was discovered by introducing a pyrazole ring to the benzopyran core of classical phytocannabinoids (18). The synthesis of these reported molecules is summarized in scheme **1**.



Scheme 1. Synthesis of chromenopyrazoles. Reaction conditions: **(i)** BBr_3, CH_2Cl_2, overnight, 0 ºC-r.t.; **(ii)** 3,3-dimethylacrylic acid, methanesulfonic acid, P_2O_5, 8 h, 70 ºC, **(iii)** a) NaH, THF, MW, 25 min, 45 ºC; b) ethyl formate, MW, 25 min, 45 ºC; **(iv)** corresponding hydrazine, EtOH, 1-4 h, 40 ºC. R^1: pentyl or 1,1-dimethylheltyl; R^2: hydrogen, methyl, ethyl, or dichlorophenyl (18).

Briefly, these compounds were prepared from the resorcinol (**2**) previously obtained after demethylation of the corresponding 1,3-dimethoxybenzene (**1**) with boron tribromide. Chromanone **3** was obtained by treatment of the resorcinol with 3,3-dimethylacrylic acid in presence of phosphorous pentoxide (19). Subsequent α–formylation of the chromanones under microwave conditions using sodium hydride followed by the addition of ethyl formate yielded **4**. Finally, condensation of the β-ketoaldehyde (**4**) with the appropriate hydrazine gave the *N*1- and *N*2-substituted chromenopyrazoles regioisomers (**5** and **6**) with different relative ratios.

The synthesized compounds were evaluated *in vitro* for their ability to displace the radioligand [^{3}H]CP55,940 from human CB_1R and CB_2R (18). These affinity binding assays revealed that among the lipophilic alkyl chains tested at R^1, aliphatic pharmacophoric position in phytocannabinoids, the 1,1-dimethylheptyl group is clearly preferred. Pentyl alkyl side chains lead to weaker binders or compounds that do not have affinity at all for these receptors. However, the 1,1-dimethylheptyl analogues display significant to high affinity and selectivity for CB_1R (CB_1R K_i: 4.5–28.5 nM; CB_2R K_i: >40000 nM). In what concerns the substitution on the pyrazole ring or the nature of the R^2 substituents, the affinity values remained similar among 1,1-dimethylheptyl analogues (18). Figure **3** summarizes the structural features of this series of compounds in relation to their cannabinoid receptor affinity.

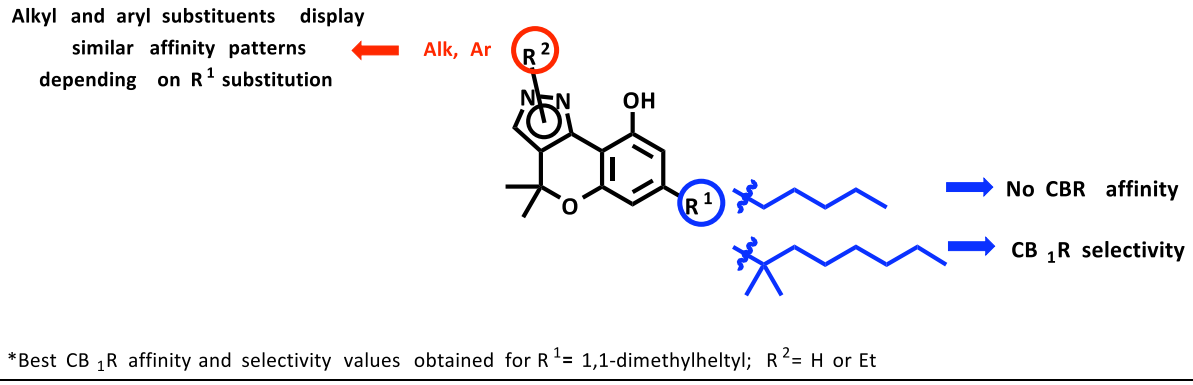


Figure 3. Summary of chromenopyrazole structural features related to CBR affinity.

Mouse vas deferens functional assays were performed with the compounds that exhibit higher binding affinities (**7**, **8a**, and **8b**, figure **4**). These chromenopyrazoles inhibited the electrically evoked contractile response of this tissue confirming their agonistic properties (18). The most potent and effective agonist (**8a**) was then tested in the cannabinoid mouse tetrad. This *in vivo* behavioral test indicates if a compound has CNS-mediated effects. Compound **8a** did not induce modifications in any of the tetrad parameters at doses up to 10 mg/kg which indicates that it is not acting in the brain, and thus, not readily crossing the blood–brain barrier.

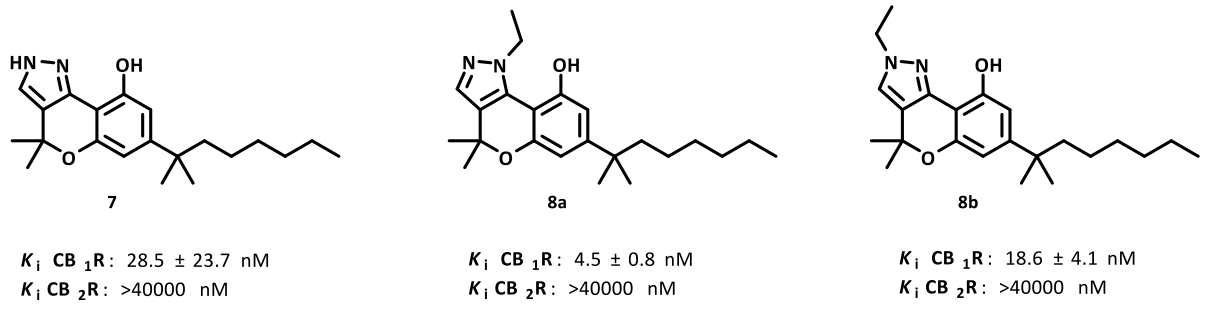


Figure 4. Structure and affinity of the three more potent and selective CB_1R ligands of the first chromenopyrazole series (18).

In a further approach to evaluate the therapeutic potential of the peripheral CB_1R agonist **8a**, this chromenopyrazole was tested in a rat model of orofacial pain. Interestingly, compound **8a** showed a remarkable antinociceptive response probably mediated by peripheral mechanisms.

To sum up, in this study, the chromenopyrazole scaffold was reported for the first time opening new avenues in the cannabinoid chemistry scenario. Among this first series of chromenopyrazoles, non-psychoactive and selective CB_1R agonists with peripheral antinociceptive properties were identified (18).

3. TOWARDS CB_2R SELECTIVITY: APPLICATION IN NEURODEGENERATIVE DISEASES

A second approach to pursue the separation of the therapeutic effects of cannabinoids from their psychotropic effects is the search of CB_2R selective ligands. Although CB_1R is the main receptor of the CNS, the presence of CB_2R in microglia and neuronal cells as well as its role in the immune system (20–22), suggest the possibility to use CB_2R agonists to treat certain neurological conditions without psychotropic unwanted effects (23,24). The role of the CB_2R in the CNS is closely related to neuronal damage, particularly inflammatory. Therefore, as suggested by numerous studies, CB_2R represents a promising target for alleviating the neuronal deterioration and neuroinflammation triggered by neurodegenerative diseases (5,23).

In an attempt to target the CB_2R, different structural modifications on the chromenopyrazole scaffold were accomplished while retaining the 1,1-dimethylheptyl aliphatic chain that provided better results in the previous study. In this case, the conversion of the phenolic hydroxyl, a pharmacophoric moiety of classical cannabinoids, to different alkoxy groups was explored. Moreover, different pyrazole substituents as well as bioisosteric replacement of the pyrazole by an isoxazole were also intended for further fine-tuning of CBRs affinity and selectivity (25).

The synthesis of these chromenopyrazoles has been reported to be achieved by alkylation of the phenolic oxygen of the chromenopyrazoles described in the previous section with the corresponding alkyl halides. The bioisosteric replacement by an isoxazole moiety, was obtained upon condensation of the β-ketoaldehyde (**4**) with hydroxylamine hydrochloride (25).

Figure **5** provides an overview of the affinity trends that can be featured from the radioligand binding assays of these chromenopyrazoles at the cannabinoid receptors CB_1R and CB_2R (25). As detailed in the previous section, chromenopyrazoles bearing a free phenolic hydroxyl group display CB_1R affinity and selectivity. Phenolic alkylation of these compounds causes a drastic loss of CB_1R affinity, whereas high CB_2R selectivity was achieved with alkoxychromenopyrazoles derivatives. These results suggest that the phenolic hydroxyl may play a crucial role in CBR selectivity.

Another relevant conclusion extracted from reported structure-activity relationship (SAR) is that the nature of the pyrazole substituent influences the affinity for the cannabinoid receptors. In general, alkyl groups showed better affinity for CB_2R compared to aryl substituents. Bioisosteric replacement of the pyrazole by an isoxazole results in cannabinoid ligands with affinity in the nanomolar range. Upon phenolic alkylation, chromenoisoxazoles follow the same affinity pattern observed for the chromenopyrazoles.

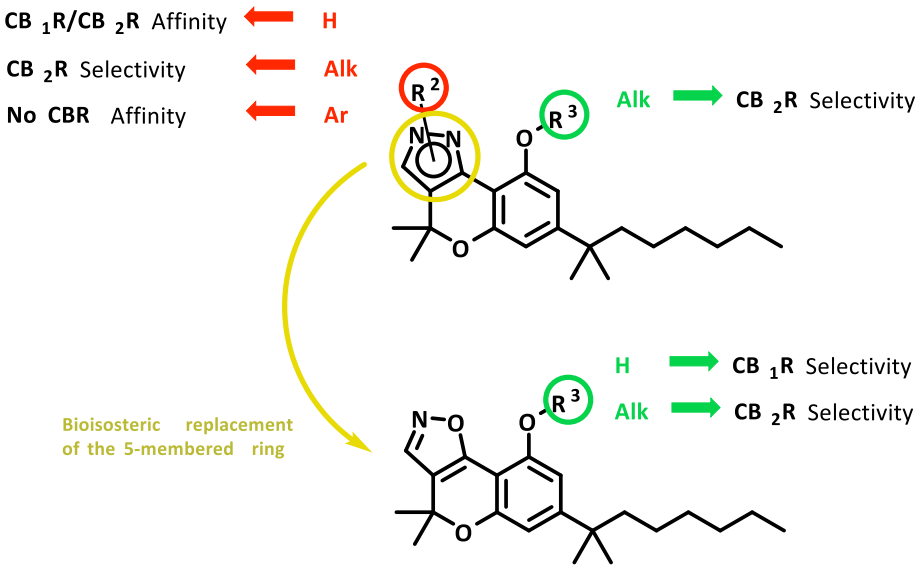
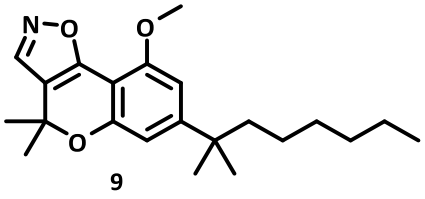


Figure 5. Summary of alkoxychromenopyrazole and chromenoisoxazoles structural features related to CBR affinity.

Among these novel compounds, CB_2R selective ligands with the best affinity values (K_i < 100 nM) were selected for functional appraisal. Most of these ligands showed CB_2R full agonism in forskolin-stimulated cAMP accumulation experiments and GTPγS binding assays exhibiting potency values in the nanomolar range. The most potent and efficacious ligand of this series is the chromenoisoxazole **9** (figure **6**), which displays higher CB_2R selectivity and potency than well-known cannabinoid agonists such as HU308 or JWH133 (25).



9

K_i CB 1R: >40000 nM
K_i CB 2R: 12.8 ± 2.4 nM

cAMP assays : EC_{50}: 4.2 ± 1.5 nM; E_{max}: 101%
GTP γS assays : EC_{50}: 38.6 ± 6.7 nM; E_{max}: 98%

CB 2R Selective Agonist

Figure 6. Cannabinoid receptor affinity and functional data of the most potent and efficacious CB_2R ligand of this series (chromenoisoxazole **9**).

Selected compounds of this series were investigated from a molecular modeling perspective in order to understand the governing ligand-receptor interactions that trigger CBR affinity and selectivity. Docking studies revealed that the presence of the pyrazole or isoxazole moiety as well as the phenolic oxygen play a crucial role in the binding mode of these compounds to the active state models of CB_1R and CB_2R. For CB_1R affinity, the phenolic lone pair need to be accessible to hydrogen bond with K3.28(192), as in phenols **7**, **8a** or **8b**. *O*-alkylation of the initial phenolic hydroxyl of the scaffold leads to selective CB_2R ligands (figure **5**). This might be due to the different orientation and low accessibility of the lone pair of electrons of the phenolic oxygen, essential for CB_1R activation. The steric hindrance generated by the *O*-substituent in the CB_1R binding site impacts their affinity towards this receptor; however, it clearly enables CB_2R activity. Substituted phenols align in the proper orientation in the CB_2R binding site leading to an interaction of S6.58(268) with the ligand pyran oxygen (25). These structural studies might lead to further fine-tuning of this versatile scaffold.

In order to evaluate the therapeutic potential of the promising CB_2R selective agonist, chromenoisoxazole **9**, additional *in vitro* and *in vivo* assays were performed. To evaluate its neuroprotective profile, **9** was tested in an *in vitro* model of neuronal death determining the cell viability of a neuronal cell line (M213-2O) upon neuroinflammatory stimuli (26). Interestingly, compound **9** showed a dose-dependent neuroprotective effect, and therefore, its potential was evaluated in an *in vivo* model based on mitochondrial damage and inflammation. This model, which is reminiscent of Huntington's disease (HD), is generated by intrastriatal application of malonate toxicity in rats. The administration of this chromenoisoxazole clearly decreased the volume of the striatal lesion as confirmed by histopathological studies (26). Moreover, it was demonstrated that these neuroprotective effects were mediated by the ability of **9** to activate CB_2R (effect reversed by administration of CB_2R

antagonists).

Additionally, chromenoisoxazole **9** was tested in a murine model of multiple sclerosis (MS) induced by TMEV. The Theiler's murine encephalomyelitis virus (TMEV) triggers a late-onset demyelinating disease which presents similar neuroinflammatory processes to human MS. During the acute inflammatory phase, compound **9** was intraperitoneally administered at a dose of 5 mg/kg during 7 days. Treatment with **9** led to a remarkable reduction of inflammatory events in TMEV-infected mice (25). All these studies demonstrate that chromenoisoxazole **9** has an outstanding neuroprotective profile due to its capacity to selectively activate CB_2R.

In summary, novel fully selective CB_2R agonists were identified through optimization of the chromenopyrazole scaffold. The lead compound of this series, chromenoisoxazole **9**, has shown antiinflammatory and neuroprotective properties *in vitro* and *in vivo* in various neurological disorders (murine and rat models of MS and HD) (25,26). This molecule presents a promising potential for further drug development, in particular, for the treatment of neurodegenerative pathologies.

4. A DUAL APPROACH: TARGETING CANCER WITH CANNABINOID-QUINONES

The development of new effective and safe antitumor treatments that improve the aggressive current chemotherapies remains an unmet clinical need. Cancer is one of the most prevalent diseases and its incidence is dramatically increasing (27). Within this pathological panorama, the endocannabinoid system emerges as a promising anticancer target involved in the modulation of the main hallmarks of this disease.

Δ^9-THC and its synthetic derivatives have long been known for their palliative effects in cancer patients. In the middle 1980´s, dronabinol (Marinol®) and nabilone (Cesamet®) were approved for the management of chemotherapy-induced nausea and emesis (28). Their orexigenic properties and their ability to alleviate pain associated with cancer have also been widely evidenced (29–34). However, nowadays, they are only prescribed in some countries after conventional anti-emetics fail (35,36).

Besides the aforementioned palliative potential of cannabinoids, more recent research revealed that these molecules exhibit antitumor effects in numerous *in vitro* and *in vivo* experimental models of cancer (37–39). The activation of cannabinoid receptors on cancer cells modulates signaling pathways implicated in cell proliferation and survival. Even though the underlying mechanisms are not fully unraveled, there is significant evidence for the involvement of at least four mechanisms: direct inhibition of transformed-cell growth through the suppression of mitogenic signal, induction of apoptosis, inhibition of tumor angiogenesis and metastasis (40).

The biological role of the ECS in cancer physiopathology is quite complex and far from being completely understood. In fact, this endogenous system is upregulated in neoplasms compared with non-tumor tissue (38,40–42). However, these observations are tumor type-specific and therefore, further research is needed to understand the regulation of cannabinoid receptor expression in each type of cancer (37,41).

With regard to the clinical translation of the antitumor properties of cannabinoids, a pilot clinical trial has been reported so far (43), whereas a few more are presently in progress. In this phase I pilot trial, the effects of intratumoral administration of Δ^9-THC were studied in nine patients with glioblastoma multiforme, who had failed surgical therapy and radiotherapy and exhibited clear evidence of tumor progression. The results obtained in this study suggested that cannabinoid treatment reduced tumor growth rate (43). Nonetheless, more extensive clinical studies are needed to extract significant conclusions that reinforce the potential utility of cannabinoids as anticancer therapeutics.

Among other pharmacological approaches for cancer treatment, cytotoxic quinones represent an important group of antineoplastic drugs. Antitumor properties of quinones have been widely reported and are still the focus of much research (44,45). The cytotoxic activity of quinoid derivatives can be accounted for their fast redox cycling potential and Michael acceptor properties (46–48). Even though their mechanism of action is not completely understood, several mechanisms have been suggested. Most investigations propose different combinations of DNA intercalation (49), topoisomerase inhibition (50), DNA alkylation (51,52), and induction of reactive oxygen species (ROS) (53) depending on the compound structural features.

Many of the drugs clinically approved or still in clinical trials against cancer are quinone-related compounds (figure **7**). The quinoid moiety is present in anthracyclines which are among the most used anticancer drugs ever developed (44). Daunorubicin (Cerubidine®) and doxorubicin (Adriamycin®), the most prominent members of this class of antitumor agents, are clinically used in the therapy of solid cancers as well as hematological malignancies (54). Likewise, mitoxantrone (Novantrone®), a dihydroxyanthracenedione, is approved for the treatment of certain types of neoplasms such as metastatic breast cancer, acute myeloid leukemia, and non-Hodgkin's lymphoma (55). Bioreductive alkylating agents such as mitomycin C and its derivatives also display remarkable antitumor effects. Mitomycin C, a potent DNA crosslinker, is a FDA approved drug for the treatment of solid tumors (56,57). Additionally, β-lapachone, an *ortho*-naphthoquinone, originally isolated from a tree, is also being evaluated for its cancer growth inhibitory properties in diverse tumors (44). Even though in many cases the antitumor mechanisms remain uncertain, it is unquestionable that the presence of the quinone moiety is exceptionally remarkable in the development of new anticancer drugs.

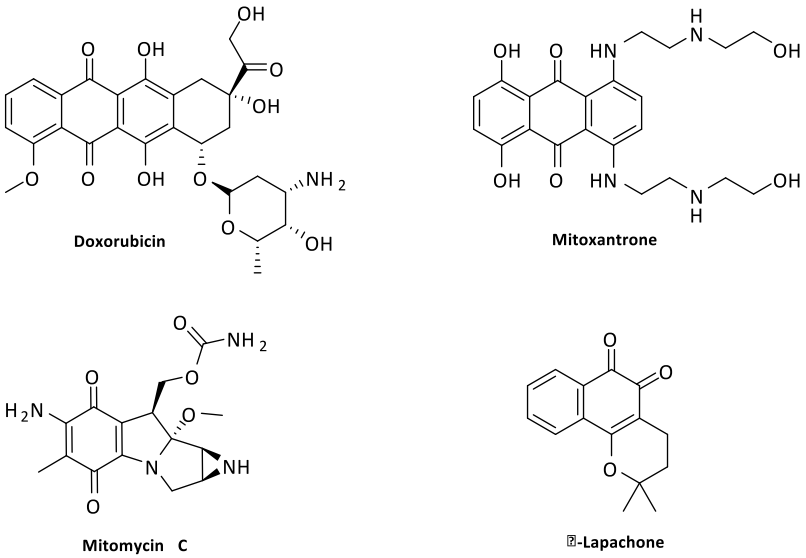


Figure 7. Some examples of quinoid compounds with antitumor activity.

Since cancer is a complex multifactorial disease, successful treatment of neoplasms often requires pharmaceutical intervention at multiple pathways. This is generally accomplished by using a combination of different drugs. However, a very attractive and promising approach is to target different anticancer modes of action in a single molecule (58–60). In this context, *ortho* and *para* cannabinoid-quinones were recently reported in the literature (61–64). They were designed using the chromenopyrazole scaffold as structural core (chromenopyrazolediones, figure **8**).

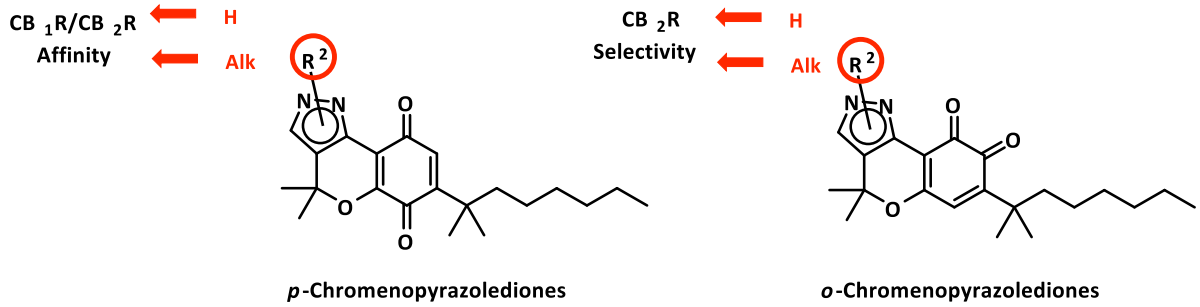


Figure 8. General structure of *ortho* and *para*-chromenopyrazolediones and their related CBR affinity.

The synthesis of these novel quinone derivatives was achieved by regio-controlled oxidation of the phenolic chromenopyrazoles (**5** and **6**, scheme **1**) to the corresponding 1,2- or 1,4-quinones using with hypervalent iodine reagents (63–65). The redox potential of selected chromenopyrazolediones was confirmed by cyclic voltammetry and electron spin resonance (63). In addition, as reported for previous chromenopyrazole derivatives (sections 2 and 3), their binding affinity to human CB_1R and CB_2R was tested. As summarized in figure **8**, *para*-chromenopyrazolediones display affinity for both receptors, CB_1R and CB_2R, in the low micromolar range. Interestingly, the unsubstituted pyrazole derivative (compound **11**, figure **10**) stands out showing better affinity values at both receptors. On the other hand, *ortho*-chromenopyrazolediones are fully selective towards CB_2R with affinity in the submicromolar range (63,64). Their lack of affinity for the CB_1R (higher than 40 µM) eliminates any psychotropic side effect that could be derived from activation of central CB_1R.

It is noteworthy that chromenopyrazolediones were the first cannabinoid structure-related quinones able to bind to the cannabinoid receptors. The only quinones related to cannabinoid structures (quinones of CBD, THC, and CBN) reported previous to the chromenopyrazole derivatives, did not display affinity for the cannabinoid receptors (66).

The biological activity of these phytocannabinoid quinones, was attributed to their quinoid structure (67,68) independently of their cannabinoid character, since they do not bind to CB_1R or CB_2R.

The antitumor potential and the mechanism of action of the reported chromenopyrazolediones derivatives were evaluated *in vitro* and *in vivo* in different cancer models. In particular, their antiproliferative activity was explored in two highly prevalent cancers: Triple Negative Breast Cancer (TNBC) and Prostate Cancer (PC). In both cases, the endocannabinoid system has been proved to be upregulated under these neoplastic conditions, and therefore, it represents an appropriate target.

4.1 Chromenopyrazolediones for Triple Negative Breast Cancer (TNBC)

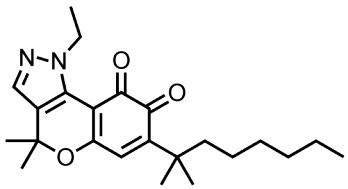
According to the World Health Organization, breast cancer is among the most common malignant diseases and the second leading cause of cancer death among Western women (69). Despite recent advances in earlier detection and adjuvant systemic therapies, mortality rates remain very high due to the emergence of refractory tumors associated with multidrug resistance.

From an immunopathological perspective, there are three main breast cancer subtypes: hormone receptor-positive, HER2-positive (human epidermal growth factor receptor 2) and triple-negative tumors. Triple-negative breast cancers (TNBC) are defined by the absence of immunohistochemical expression of estrogen, progesterone, and HER2 receptors. Although this molecular subtype of breast cancer accounts for a low percentage of all breast tumors, it represents a vast number of deaths (70). TNBC show aggressive clinical behavior, this fact, along with the lack of available targeted therapies, leave these patients with a bad prognosis (70–73). Chemotherapy with its well-known side effects is currently used as systemic treatment for this cancer (74). For that reason, the discovery of new targets and drugs for the treatment of this disease is an urgent and essential clinical challenge.

Recent evidence suggests that cannabinoid receptors are overexpressed in human breast cancer biopsies (42,75,76). Further insights into the endocannabinoid upregulation have demonstrated a correlation between CB_2R expression and tumor aggressiveness in triple-negative breast cancer cells (42). The putative novel cannabinoid receptor GPR55 is also highly expressed in these carcinomas (77). Consequently, the ECS represents a promising target for the treatment of TNBC (78,79).

In this context, the antiproliferative potential of the recently reported cannabinoid-quinones derived from the chromenopyrazole scaffold was explored in models of this highly aggressive breast cancer.

Cell viability, using a human derived triple-negative breast cancer cell line, MDA-MB-231, was evaluated after treatment with increasing doses of the novel *para* and *ortho*-chromenopyrazolediones. All the tested cannabinoid-quinones displayed growth inhibitory effects on triple-negative MDA-MB-231 breast cancer cells, displaying low micromolar IC_{50} values. The *para* and *ortho*-chromenopyrazolediones bearing an ethyl R substituent in the pyrazole (figure **8**) are the most potent inhibitors of cell proliferation with IC_{50} values of 2.5 and 2.8 μM respectively. Because of its antiproliferative capacity and its CB_2R selective profile, compound **10** (figure **9**) was selected for additional mechanistic and *in vivo* investigations (64).



10

K_i CB_1R: >40000 nM
K_i CB_2R: 529 ± 26 nM

Cell viability MDA-MB-231: IC_{50}: 2.8 ± 0.5 μM

Cell viability HMEC: IC_{50} >30 μM

Figure 9. Cannabinoid receptor affinity and half-maximum inhibitory concentrations (IC_{50}) in MDA-MB-231 of *ortho*-chromenopyrazoledione **10**.

Compound **10** was further tested in normal Human Mammary Epithelial Cells (HMEC). Interestingly, at doses up to 30 μM, this cannabinoid-quinone did not exhibit cytotoxicity in HMEC. This selective toxicity towards cancer cells versus non-transformed mammary cells is essential for the development of safer chemotherapies for TNBC.

Deeper studies into the antitumor mechanism of action of this compound were accomplished in MDA-MB-231 cells (64). According to these experiments, CB_2R activation and the generation of reactive oxygen species (ROS) are tightly involved in the antiproliferative action of compound **10**. Conversely, as expected, neither CB_1R nor GPR55 mechanisms seem to be involved in the cytotoxic effects of chromenopyrazoledione **10** (64).

Further evaluation of the cellular mechanism underlying the antiproliferative effect of compound **10** led to examine the involvement of caspase-3 in MDA-MB-231 cells. Western immunoblotting studies showed the procaspase-3 cleavage into caspase-3 confirming the proapoptotic effect of cannabinoid quinone **10** on this triple-negative breast cancer cell line (62,64).

Chromenopyrazoledione **10** was then evaluated in a murine model of TNBC. Tumor xenografts were generated in nude mice by subcutaneous inoculation of MDA-MB-231 human breast adenocarcinoma cells. After four weeks of intraperitoneal treatment with 2 mg/kg, compound **10** showed to effectively reduce the growth of triple-negative xenografts in this animal model. Histopathological analysis of treated mice revealed that compound **10** did not generate signs of toxicity in organs such as liver, spleen, lung, heart, or colon. Volume and weight of final tumors was significantly lower in all cases (64).

Summarizing, through this approach, the chromenopyrazole scaffold has been optimized following a dual target anticancer strategy focused on triple-negative breast cancer. Cannabinoid quinone **10** was discovered as a selective CB_2R agonist with potent antiproliferative

effects *in vitro* and *in vivo*. Its ability to selectively decrease viability in cancer *versus* normal cells, along with its striking antitumor capacity, open novel therapeutic avenues for the exploitation of the chromenopyrazole moiety in cancer physiopathology. In view of these results, chromenopyrazoledione **10** can be considered a new anticancer drug candidate offering hope for the treatment of TNBC.

4.2. Chromenopyrazolediones for Prostate cancer (PC)

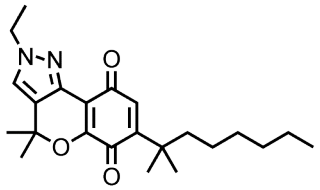
Prostate cancer (PC) is the second most common cancer worldwide for Western men. The rates are increasing in recent years since life expectancy is longer (80). Even though most prostate cancers grow slowly, aggressive cases are also diagnosed. These oncogenic cells may metastasize to other parts of the body such as bones and lymph nodes (81). Therefore, there are extensive ongoing efforts to develop new therapeutic strategies to treat prostate cancer (82).

The basis of medical treatment for advanced prostate cancer is androgen deprivation therapy (ADT), intended to lower testosterone levels. However, the reduction of clinical symptoms and tumor growth is accompanied by systemic consequences of testosterone deficiency such as osteoporosis, gynecomastia, anemia and insulin resistance among others (83,84). Androgen deprivation is associated with a gradual transition of prostate cancer cells through a spectrum of androgen dependence, androgen sensitivity, and ultimately androgen independence. Too often the appearance of hormone refractory cancer cells eventually leads to the recurrence of cancer which turns to a hormone-independent state. This type of prostate cancer has a more aggressive phenotype and is unresponsive to further hormonal therapy whereby prognosis is very poor. Therefore, to find a treatment which could reduce or block both types of prostate cancer would be a very good challenge to move forward.

The ECS is also deregulated on the course of this pathology. The expression of cannabinoid receptors has been studied in prostate cancer tissue. It was demonstrated that CB_1R expression is upregulated in these neoplasms (85). Indeed, high CB_1R immunoreactivity score in prostate cancer tissue is associated with prostate cancer severity and outcome (86). Moreover, expression of FAAH (87) and GPR55 (88) is demonstrated in some prostate carcinoma cell lines. In line with these observations, different endocannabinoids or cannabis-like compounds were evaluated exhibiting their ability to inhibit prostate cancer cell proliferation and produce apoptosis through cannabinoid receptor mechanisms (89). The dysregulation of this system correlates with prostate cancer grade and progression. Therefore, modulation of the ECS may offer novel therapeutic avenues for prostate cancer as well (90,91).

Because of the aforementioned expression patterns in prostate cancer, CB_1R agonists should provide a new therapeutic approach for this type of cancer. Therefore, among the series of chromenopyrazolediones previously described (figure **8**), the *para*-quinone derivatives, which bind to CB_1R, were selected for their evaluation in prostate cancer-derived cell lines (63).

Cell viability assays in androgen-dependent (LNCaP) and androgen-refractory (PC-3) prostate cancer cell lines revealed that these cannabinoid-quinones exhibit antiproliferative effects with IC_{50}s in the micromolar range. Compound **11** (figure **10**), which is the most potent among the derivatives tested, displayed an IC_{50} of 15 μM in both LNCaP and PC-3 prostate cancer cells.



11

K_i CB_1R: 324 ± 235 nM
K_i CB_2R: 134 ± 21 nM

Cell viability LNCaP: IC_{50}: 15 μM

Cell viability PC3: IC_{50}: 15 μM

Figure 10. Cannabinoid receptor affinity and half-maximum inhibitory concentrations (IC_{50}) in LNCaP and PC3 cancer cell lines of *para*-chromenopyrazoledione **11**.

Further analysis of chromenopyrazoledione **11** indicated that it induces cell death through apoptosis, being more efficient in the androgen-sensitive LNCaP cell line. At a molecular mechanistic level, the cytotoxicity of **11** was shown to be mediated through CB_1R, oxidative stress and modulation of the nuclear receptors PPARγ (peroxisome proliferator-activated receptors) receptors (63).

In vivo studies of *para*-quinone **11** confirmed the results obtained *in vitro*. Treatment with **11** at a dose of 2 mg/kg totally inhibited the growth of androgen-dependent prostate tumor xenografts in mice.

The results obtained for *para*-chromenopyrazoledione **11** validated the antiproliferative capacity of chromenopyrazole-derived cannabinoid quinones, and therefore, the cannabinoid/ROS antitumor proof-of-concept.

4.2a *Porphyrin conjugate strategy*

In the field of cancer therapy, strategies have been explored during these last years in which porphyrins are conjugated to molecules showing preferential accumulation for tumor tissues or having affinity for receptors expressed in tumors (92).

Porphyrin derivatives constitute the central element of

an effective and minimally invasive cancer therapy called photodynamic therapy (PDT) (93,94). PDT uses a photosensitizing drug, generally a porphyrin derivative, in combination with visible light irradiation. In presence of the oxygen accumulated in tumors, the photoactive sensitizer triggers a series of photochemical processes that lead to direct cancer cell death and tumor microvascular damage (95,96). PDT is clinically used for the treatment of various types of malignant disorders such as bladder, lung or esophageal cancer (97). A particular interest of the use of these photosensitizers is their preferential accumulation by malignant cells due to the presence of high amount of collagen and lipids.

Strategies targeting photosensitizers covalently attached to molecules have been reported lately with different therapeutic approaches. The photosensitizer can be combined with a carrier showing affinity for neoplasia or to receptors expressed on specific tumors such as monoclonal antibodies, antibody fragments, peptides, proteins such as transferrin, epidermal growth factor and insulin, low-density lipoproteins, various carbohydrates, somatostatin, folic acid among others (98). Another strategy will be the conjugation of a therapeutic agent to a photosensitizer in view to obtain dual antitumor activity and/or to use the photosensitizer as carrier due to their ability to preferentially accumulate in cancer tissues (99). This strategy has been reported with conjugates such as temoporfin/ibuprofen as a non-steroidal anti-inflammatory drug (100), tetraphenylporphyrin/trilobolide as a cytotoxic agent (101), porphyrazine/doxorubicin (102).

Two studies on porphyrin/cannabinoid conjugates have been reported so far (103,104). The first conjugate involved a phthalocyanine named IR700DX-mbc94 with the CB_2R antagonist SR144528 (103). Phototherapy treatment using this complex greatly inhibited the growth of expressed CB_2R tumors but not tumors that were not expressing CB_2R. Considering that the CB_2R antagonist does not have antitumor properties, the strategy used its affinity for receptors expressed in tumors to increase the accumulation of photosensitizer in the tumor.

Photosensitizer/chromenopyrazoledione conjugates have been proposed recently (figure **11**) in which meso-tetraphenylporphyrin (TPP) was elected as photosensitizer and chromenopyrazoledione as antitumor agent (104).

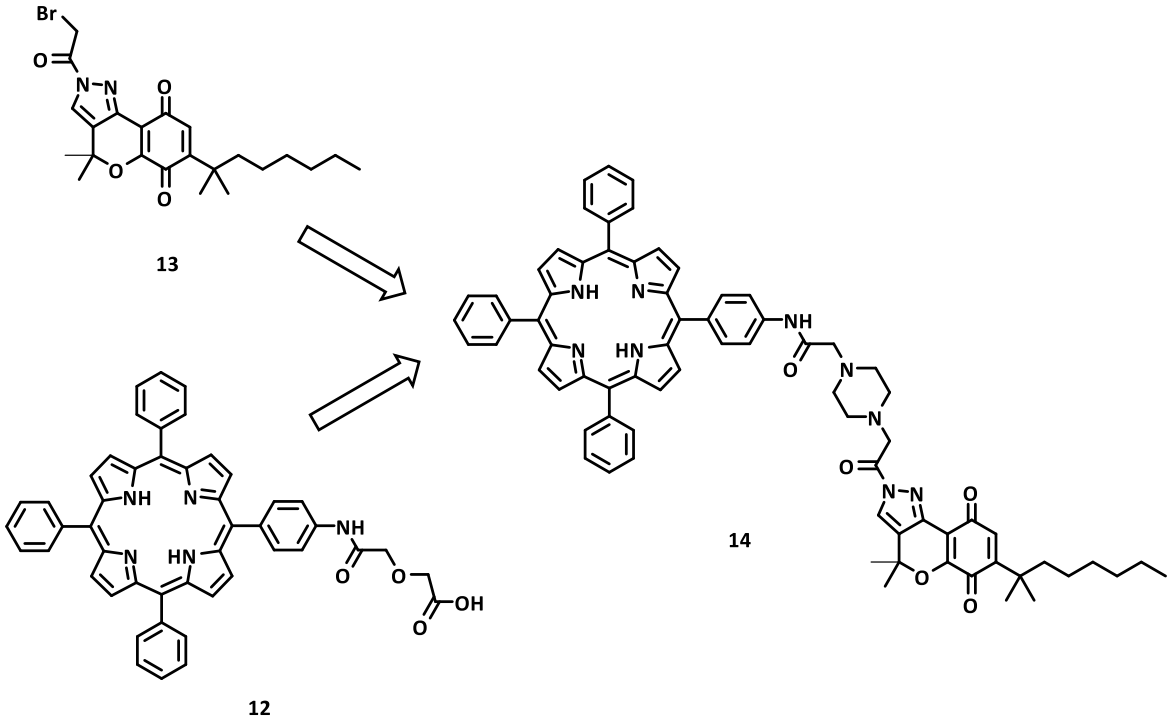


Figure 11. Photosensitizer/chromenopyrazoledione conjugate **14**, and the chromenopyrazole **13** and the TPP derivative **12** involved in its synthesis.

The synthesis of the porphyrin-chromenopyrazodione conjugate **14** was achieved from the porphyrin **12** and the chromenopyrazole **13**. TPP was regioselectively *para*-mononitrated with sodium nitrate to 5-(*p*-nitrophenyl)-10,15,20-triphenylporphyrin that was then reduced with tin (II) chloride, and finally converted under diglycolic anhydride treatment to the carboxylic porphyrin **12**. The *NH*-chromenopyrazoledione was first acylated using bromoacetyl bromide, and then was allowed to react with the carboxylic porphyrin **12** affording the conjugate **14**. A complete conformational analysis of this chromenopyrazole conjugate performed using *ab initio* calculations indicated that the global minimum energy conformer adopts an expanded spatial conformation whereas folded conformers, where the chromenopyrazole and the porphyrin core lay paralleled, exert higher relative

energy values. Photophysical properties of the chromenopyrazole conjugate **14** showed stronger absorption intensity for both Soret (420 nm) and Q-bands than the free TPP (500-700 nm). These data are suitable for a photosensitizer considering that an ideal photosensitizer should have high absorption with wavelengths between 600 and 800 nm, no shorter than 600 nm for tissue penetration and no longer than 800 nm to provide enough energy to excite oxygen to its singlet state. In what refers to the cannabinoid properties of this porphyrin/chromenopyrazole conjugate, it has been described to bind weakly but selectively to CB_2R.

In summary, a porphyrin-chromenopyrazole conjugate has been designed and synthesized even though the syntheses of porphyrin derivatives are known to be tedious. The reported photophysical and pharmacological properties are suitable for a potential activity as antitumor agent.

5. CHROMENOPYRAZOLES AS GPR55 MODULATORS

The orphan G protein-coupled receptor GPR55 (105) has been proposed as one of the missing cannabinoid receptor types even though it shares low identity with CB_1R and CB_2R (106). GPR55 has been related to the endocannabinoid system since CB_1R and CB_2R ligands from diverse origins, endogenous, natural, and synthetic, can modulate this receptor. Over these last years, its complex cellular signaling pathways and biological functions have been the focus of numerous studies (107) that identified GPR55 as a promising target for the treatment of various pathologies such as inflammation, neuropathic pain, bone physiology, diabetes and cancer. However, GPR55 validation as a therapeutic target is far from being confirmed due to the complexity of GPR55 downstream signaling and the lack of potent and selective GPR55 agonists and antagonists. Effectively, GPR55 couples to different G-proteins, $G\alpha_{13}$, $G\alpha_{q/11}$, $G\alpha_q$ /$G\alpha_{12}$ or $G\alpha_{12/13}$ depending on the cell type and GPR55 modulator. For instance, stimulation of the $G\alpha_q$ subunit involves the phospholipase C with intracellular calcium release with possible activation of the MAPK/ERK signaling, whereas the $G\alpha_{12/13}$ subunit preferably influences the RhoA/ROCK signaling pathway.

L-α-lysophosphatidylinositol (LPI) has been suggested to be a GPR55 endogenous ligand (108). GPR55 modulators from different origins have been reported in the literature. A structural update of these GPR55 ligands have been recently described in a review (107). GPR55 ligands include endogenous molecules such as 2-arachidonoyl-L-α-lysophosphatidylinositol (2-AGPI), *N*-arachidonyl glycine (NAGly) (109), and anandamide (AEA). The regulation of GPR55 activity by a range of phytocannabinoids and synthetic derivatives has been described but in some cases it is still controversial (107). For instance, Δ^9-THC was found to be effective activating GPR55 in [^{35}S]GTPγS binding, RhoA assays and intracellular calcium mobilization in transiently transfected *h*GPR55-HEK293 cells, whereas it was unable to stimulate ERK1/2 phosphorylation nor β-arrestin recruitment. Cannabidiol, reported as GPR55 antagonist in [^{35}S]GTPγS binding and Rho activation assays, resulted to be inactive in Ca^{2+} mobilization and β-arrestin recruitment experiments.

Among the large number of synthetic cannabinoids reported so far, some of them showed to have relevant activity at GPR55. Curiously, despite their structural diversity, in each family it has been possible to find GPR55 modulators (107). The CB_1R/CB_2 R agonist CP-55,940, a cyclohexylphenoxy derivative, displayed GPR55 agonism in [^{35}S]GTPγS evaluation and GPR55 antagonist in β-arrestin, ERK phosphorylation and calcium mobilization assays. Several diarylpyrazoles such as the CB_1R antagonist rimonabant, or aminoalkylindoles exemplified by WIN55,212-2 interact with GPR55. Coumarin and magnolol cannabinoids have also been proposed as GPR55 scaffold.

Few non-CB_1R/CB_2R-related GPR55 ligands have been reported so far (107). High throughput screening of a library of compounds from the Sandford-Burnham screening center of the Molecular Libraries Probe Production Centers Network (MLPCN) allowed the identification of different GPR55 scaffolds (110) exemplified by triazoloquinoline CID1172084, thienopyrimidine CID1434953, and pyrrolopyrazolone CID16020046 (figure **12**). Data resulting from this screening realized with β-arrestin assays in U2OS cells permanently expressing HA-GPR55E and βarr2-GFP, were confirmed by other biochemical tests from independent studies.

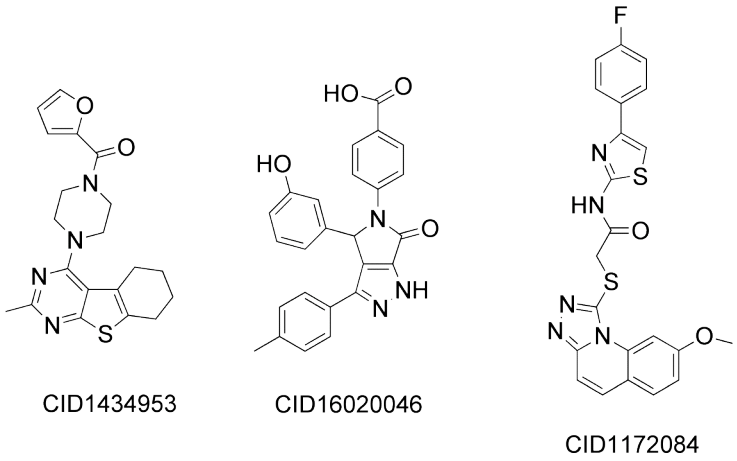


Figure 12. Some GPR55 ligands identified from a high throughput screening of a library of compounds from MLPCN.

Following the identification of the CID series as GPR55 modulators, *N*-(4-sulfamoylphenyl)thiourea-based GPR55 ligands have been described (111). Interestingly, the chromenopyrazole scaffold has also been developed as new GPR55 ligands (112). The design of these new molecules considered structural features of the GPR55 activity modulators described in figure **12**. Thus, two series of chromenopyrazoles (figure **13**) were synthesized from the 7-methoxy-*NH*-chromenopyrazole parent that was alkylated by the suitable phenylpiperazine in the first series, and that was alkylated by 1,3-dibromoethane then by the corresponding acylpiperazine in the second series. Preparation of the first series has been described with low yield due a tedious separation of both *N*-substituted isomers, whereas in the second series, the *N*-substituted isomers were separated at the stage of the *N*-bromoethyl substituted derivative.

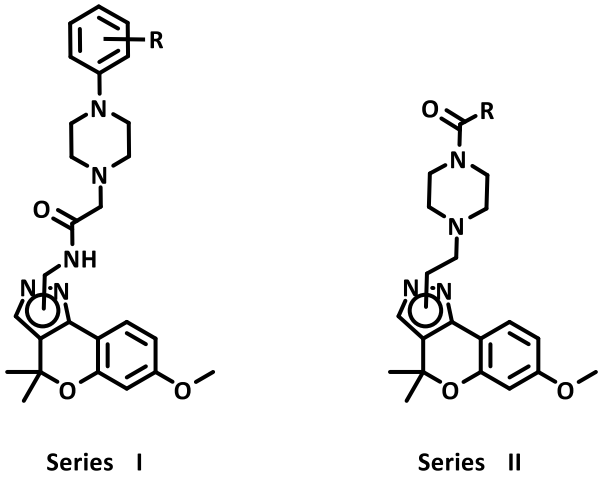


Figure 13. Two series of chromenopyrazoles as GPR55 ligands.

The novelty of the reported GPR55 chromenopyrazoles comes not only from new structures but also from the assay employed for the GPR55 evaluation. So far, the potential GPR55 compounds have been explored through different functional assays showing in some cases discrepancies in the resulting outcomes. These assays include β–arrestin recruitment, GTPγS binding, analysis of intracellular calcium levels, phosphorylation of ERK1/2, and the activation of the small GTPase proteins Rac1, RhoA and Cdc42. To overcome the complex signaling pathways related to GPR55 activation and the lack of GPR55 radioligand, the pharmacological evaluation of the chromenopyrazoles was accomplished in a cell-impedance-based assay. These label-free xCELLigence experiments detect cellular morphological changes triggered by ligand-dependent GPCR activation and coupling to downstream signaling pathways thus providing an integrative cellular response. The real-time cellular impedance response was monitored in a HEK293 cells stably expressing recombinant human GPR55 (*h*GPR55-HEK293). Most chromenopyrazoles of series II exhibited agonistic GPR55 profile whereas only one compound was active in series I. Compared to LPI, active compounds have been reported to display partial agonism in *h*GPR55-HEK293 cells with good potency showing EC_{50} values in the nanomolar range. One of these potent GPR55 partial agonists was selective *versus* classical CB_1R and CB_2R. The capacity of the chromenopyrazoles to antagonize LPI-

mediated GPR55 stimulation was also assessed and reported. Upon antagonist treatment, three of the chromenopyrazoles of series II inhibited LPI effect (1 μM), being one of them fully selective versus CB_1R and CB_2R. These studies allowed a tuning of compound properties that was achieved by small modifications of the substitution pattern.

Additional xCELLigence experiments performed in HEK293 cells with active chromenopyrazoles from both series allowed to confirm that the cellular responses observed in *h*GPR55-HEK293 cells were mediated through GPR55.

Administration, distribution, and metabolism (ADME) properties of the active chromenopyrazoles were predicted *in silico* using a set of 34 physicochemical descriptors computed by QikProp. According to this first approach, the predicted parameters suggest pharmacokinetic improvements compared to LPI that is very insoluble in water, air and light sensitive.

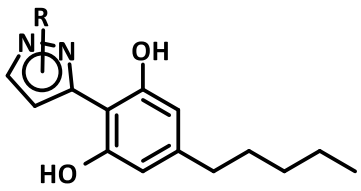
In summary, among the new proposed GPR55 scaffolds, chromenopyrazole was proposed to constitute a versatile scaffold for obtaining potent GPR55 modulators. Moreover, evaluation of these chromanopyrazoles at GPR55 was described in a cell-impedance-based assay integrating complex signaling pathways involved in GPR55 activation.

6. NOVEL CANNABIDIOL DERIVATIVES

Cannabidiol (CBD) (113) and Δ^9-THC are the two major phytocannabinoids isolated from *Cannabis Sativa*. CBD and Δ^9-THC are biosynthesized through the same metabolic pathway from cannabigerolic acid at the exception of the last step catalyzed by different enzymes, CBDA and THCA synthase respectively. Whereas there is a clear structural relationship between both structures, their pharmacology differs considerably. For instance, CBD does not induce the psychotropic effects associated to Δ^9-THC. Moreover, the complex pharmacology of CBD has not been fully elucidated yet. CBD shows only low affinity for the cannabinoid receptors CB_1R and CB_2R, but it modulates indirectly the endocannabinoid system through fatty acid-binding proteins (FABPs), transient receptor potential vanilloid type 1 (TRPV1), 5-hydroxytryptamine subtype 1A receptor ($5\text{-}HT_{1A}$), peroxisome proliferator-activated receptor γ (PPAR-γ), and the A_{1A} adenosine receptor (114).

The therapeutic potential of CBD for neurodegenerative diseases, inflammation-related diseases, epilepsy, anxiety disorders, and schizophrenia is highlighted by numerous preclinical studies (115). Clinical trials are already on going for treatment-resistant seizure disorders such as Lennox-Gastaut and Dravet syndromes and for the treatment of inflammatory bowel diseases among others (116).

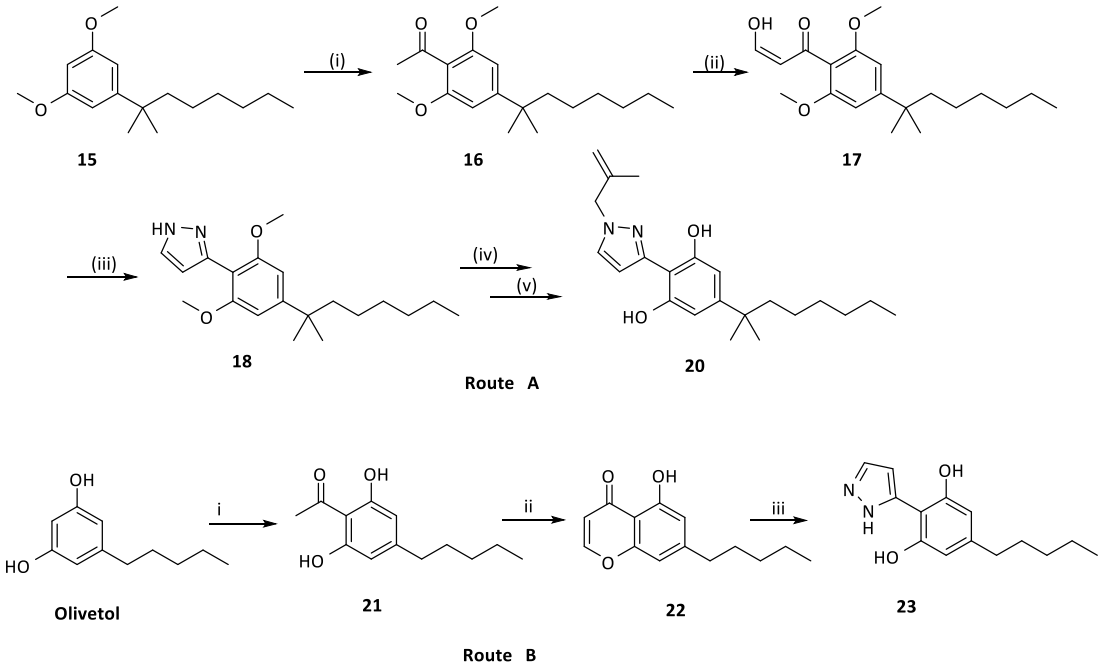
Despite the therapeutic interest of CBD, only few CBD derivatives have been reported (117). The structural modifications of CBD have been realized on the pentyl chain introducing heteroatoms or modifying the length of the alkyl chain, on the substituents of the cyclohexene, and on the cyclohexene itself using bioisosterism with cyclopentane or cyclohexane for instance. Replacement of the cyclohexene by a nitrogenated-heterocycle has been reported in 1983 with the synthesis of 5-pentyl-2-(pyrrolidin-2-yl)benzene-1,3-diol (118), and more recently with the preparation of 5-alkyl-2-(1*H*-pyrazol-5-yl)benzene-1,3-diols which are related to the chromenopyrazole scaffold (figure **14**) (117).



CBD derivatives

Figure 14. CBD derivatives based on chromenopyrazole scaffold.

Two synthetic route have been reported for the synthesis of these 5-alkyl-2-(1*H*-pyrazol-5-yl)benzene-1,3-diols illustrated in scheme 2. A Friedel-Crafts acylation of the appropriate 3,5-dimethoxyresorcinol followed by α–formylation and reaction with hydrazine constitutes the first synthetic route (scheme 2: Route A). In the second synthetic route, 5-dimethylheptyl-2-(1*H*-pyrazol-5-yl)benzene-1,3-diol **20** is prepared by reacting hydrazine with a chromone previously synthesized from the resorcinol olivetol by a succession of acylation and hydrolysis to 5-pentyl-2-(1*H*-pyrazol-5-yl)benzene-1,3-diol **23** (scheme 2: Route B). These two synthetic approaches led to new CBD derivatives which pharmacological evaluation has not been reported so far.



Scheme 2. Synthesis of 5-alkyl-2-(1*H*-pyrazol-5-yl)benzene-1,3-diols. Reagents and solvents: Route A: (i) $AlCl_3$, acyl chloride, CH_2Cl_2; (ii) NaH, ethyl formate, MW; (iii) Hydrazine, ethanol; (iv) NaH, 3- bromo-2-methylpropene; (v) Br_3B, CH_2Cl_2. Route B: i) $AlCl_3$, acyl chloride, CH_2Cl_2; ii) Perchloric acid, triethyl orthoformate ; iii) Hydrazine, ethanol.

This work shows that the chromenopyrazole experience inspired the design of 5-alkyl-2-(1*H*-pyrazol-5-yl)benzene-1,3-diols. So far, two synthetic routes have been proposed. With these compounds, the possibility of overcoming the psychotropic side effects related to Δ^9-THC still need to be confirmed before pharmacological prospection.

7. SUMMARY AND FUTURE PERSPECTIVES

The potential of cannabinoid receptor ligands has been preclinically explored in the treatment of diverse symptoms and diseases such as pain, inflammation, metabolic syndromes, cancer, hypertension, bone-related disorders or neurodegenerative processes. However, just a few of these diseases can be treated with cannabinoid-based medicines nowadays. Marinol® (dronabinol, synthetic Δ^9-THC) and Cesamet® (nabilone, a THC synthetic analogue) can be prescribed in several countries as antiemetic drugs for chemotherapy-induced nausea and vomiting (35,36), and for anorexia (119) treatment in patients with AIDS. Sativex® (nabiximols, a combination of Δ^9-THC and CBD, 1:1 ratio) is used for the symptomatic relief of neuropathic pain in adults suffering multiple sclerosis, and as an adjunctive analgesic treatment for adult cancer patients. Rimonabant (SR141716A), a CB_1R antagonist/inverse agonist, was commercialized in Europe in 2006 as Acomplia® for the management of obesity (13). Unfortunately, the beneficial effects were accompanied by a significantly increase of depression, anxiety, headache, and suicidal thoughts which forced its withdrawal from the market few years later.

Even though CB_1R/CB_2R agonists are currently in the forefront of clinical research (120) for different applications such as epilepsy (Epidiolex®), cancer and neuroprotection, there is an increasing interest in exploiting novel pharmacological strategies (121). For instance, CB_2R selective agonists or peripherally restricted CB_1R/CB_2R agonists may exhibit therapeutic potential for treating various pathologies while avoiding the adverse psychotropic effects related to the modulation of CB_1R in the brain (122). In addition, CB_1R and/or CB_2R antagonists or inverse agonist as well as allosteric cannabinoid ligands which are coming on the scene, may be useful in the treatment of certain diseases (121,123). Nonetheless, more preclinical and specially clinical research needs to be done in this field.

In this context, the chromenopyrazole scaffold emerges as a privileged structure in drug discovery targeting the endocannabinoid system. Several papers have been published describing the synthesis, the pharmacological and biological properties of chromenopyrazoles and derivatives. The first chromenopyrazoles, described in 1985, did not show significant activity in a neuroleptic evaluation. The discovery of the endocannabinoid system and specially the cannabinoid receptors, allowed exploring this scaffold as cannabinoid ligand. Structural modifications on the chromenopyrazole core allowed the development of cannabinoid drugs with a broad therapeutic prospect.

Strategies pursued so far in this field have been summarized in figure **15**. Phenolic chromenopyrazole derivatives were claimed in 2012 as non-psychoactive and selective CB_1R agonists with peripheral

antinociceptive properties (18). Structural modifications of the chromenopyrazole core allowed fine-tuning of cannabinoid receptor affinity and activity. Structural features required for CB_1R/CB_2R affinity and selectivity were determined using molecular modeling. These studies led to the identification of a potent and selective CB_2R agonist with confirmed neuroprotective properties in murine models of neurodegenerative disorders (25,26).

Further exploration of this scaffold allowed the design of multifunctional chromenopyrazoles. This strategy involved targeting different anticancer modes of action in a single molecule. The antitumor properties of cannabinoids and the redox properties characterizing quinones were fused in chromenopyrazolediones (figura **15**). The antiproliferative activity of these new compounds was successfully explored *in vitro* and *in vivo* in breast and prostate cancer models (63,64).

The chromenopyrazole scaffold was also exploited for the modulation of the putative cannabinoid receptor GPR55. Two series of compounds have been reported (112). Their ability to activate GPR55 was measured through an innovative label-free cell impedance assay allowing the discovery of novel chromenopyrazole GPR55 partial agonists and antagonists.

The last approach pursued in the exploration of this scaffold is the development of CBD-related chromenopyrazoles (117). Even though their therapeutic potential has not been reported yet, the nature of its resorcinol core may provide interesting antioxidant properties. Possible activity at cannabinoid related targets such as GPR3, GPR6, GPR12, GPR55 or GPR18 should be considered when evaluating this compound since CBD modulates all of them with moderate potencies (124–127).

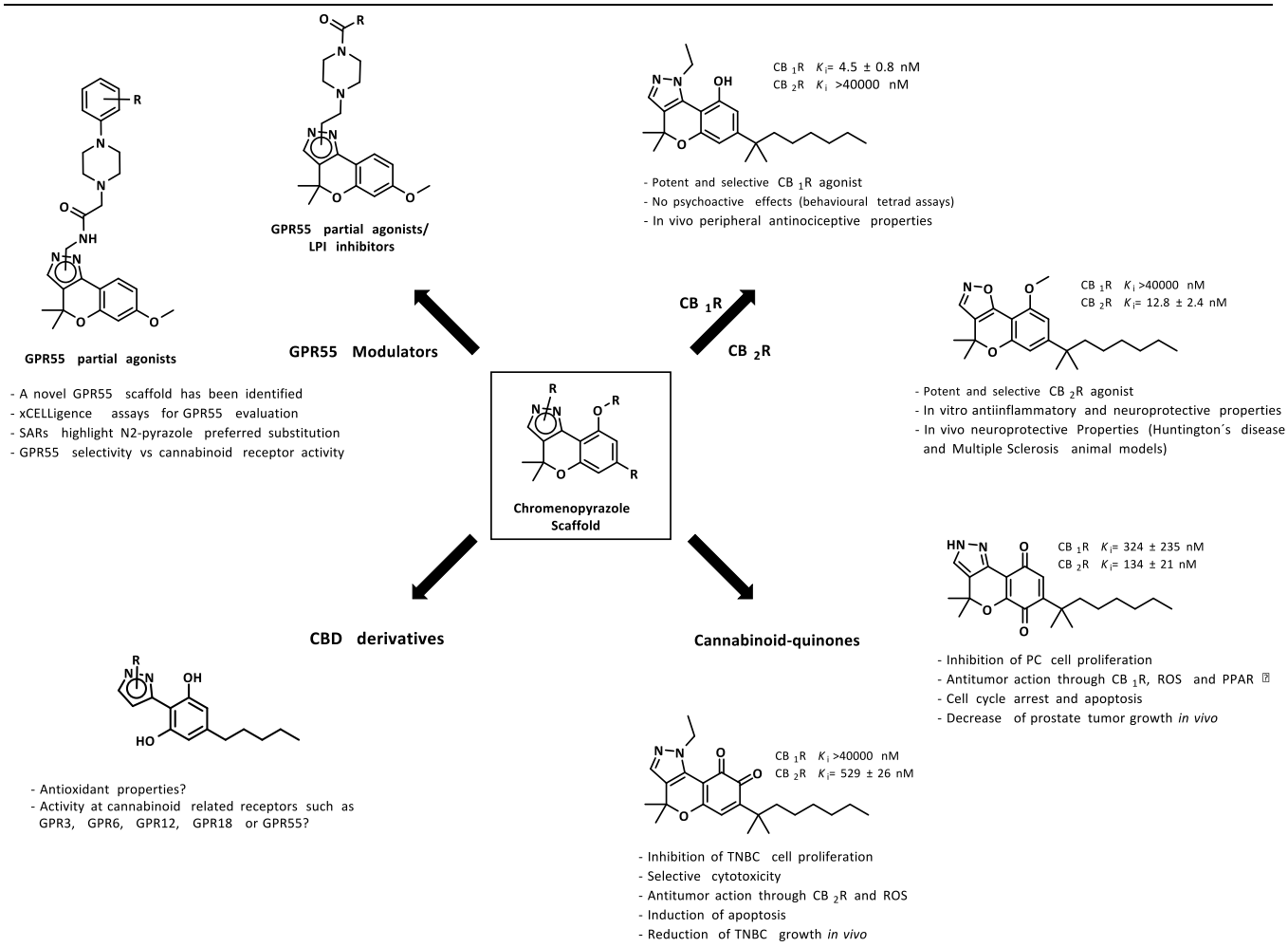


Figure 15. Summary of the chromenopyrazole derivatives reported so far and their pharmacological profile.

New chromenopyrazoles could be synthesized and screened against other relevant biological targets of the endocannabinoid system or related targets. Apart from the potential of chromenopyrazoles as therapeutic agents, they also should be considered tools to validate new biological targets.

Activation of the CB_1R and CB_2R has been shown to induce different cellular signaling cascades through coupling to different effector proteins: G-protein ($G\alpha_{i/o}$) and β-arrestins (1/2) (128–130). The search for a ligand that can induce specific receptor activation profiles resulting in specific subsets of signaling pathways (biased

signaling) has recently received a special attention in the field of GPCRs research (129,131–133). This is due to the possibility of attaining different therapeutic effects and/or avoiding untoward effects while targeting the same receptor protein (134). In this direction, a deeper functional analysis of the chromepyrazoles reviewed herein should also be pursued by evaluation of diverse signaling pathways using different readouts. Functionally profiling these compounds may lead to fine-tuning the chromenopyrazole chemotype in order to identify novel biased agonists of the cannabinoid receptors.

An important step to move forward in the drug discovery process is to evaluate the pharmacokinetic profile of the candidates. *In silico* ADME predictions suggested that chromenopyrazoles have a favorable druggability profile, however, *in vitro* and *in vivo* data of the most promising lead compounds is needed to confirm the pharmacokinetic properties of these novel derivatives.

Albeit more research is clearly needed to continue towards more preclinical evaluation, the efforts done for the development of modulators of the ECS based on the chromenopyrazole heterocycle open new avenues in the cannabinoid field. Novel pharmacological tools to study orphan receptors, as well as potential candidates for the treatment of diverse pathologies can be optimized using the outstanding starting point provided by the versatile chromenopyrazole chemotype.

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8. REFERENCIAS

1 Mechoulam R, Hanus LO, Pertwee R, Howlett AC. Early phytocannabinoid chemistry to endocannabinoids and beyond. Nat Rev Neurosci 2014; 15: 757–64.

2 Gaoni Y, Mechoulam R. Isolation, Structure, and Partial Synthesis of an Active Constituent of Hashish. J Am Chem Soc 1964; 86: 1646–47.

3 Pertwee RG. Handbook of Cannabis. 1st ed. New York: Oxford University Press 2014.

4 Silvestri C, Di Marzo V. The Endocannabinoid System in Energy Homeostasis and the Etiopathology of Metabolic Disorders. Cell Metab 2013; 17: 475–90.

5 Navarro G, Morales P, Rodríguez-Cueto C, Fernández-Ruiz J, Jagerovic N, Franco R. Targeting Cannabinoid CB2 Receptors in the Central Nervous System. Medicinal Chemistry Approaches with Focus on Neurodegenerative Disorders. Front Neurosci 2016; 10: 1–11.

6 Pertwee RG, Howlett AC, Abood ME, *et al*. International Union of Basic and Clinical Pharmacology. LXXIX. Cannabinoid Receptors and Their Ligands: Beyond CB1 and CB2. Pharmacol Rev 2010; 62: 588–631.

7 Pertwee RG. Emerging strategies for exploiting cannabinoid receptor agonists as medicines. Br J Pharmacol 2009; 156: 397–411.

8 Patel S, Hill MN, Cheer JF, Wotjak CT, Holmes A. The endocannabinoid system as a target for novel anxiolytic drugs. Neurosci Biobehav Rev 2017; 76: 56–66.

9 Velayudhan L, Van Diepen E, Marudkar M, *et al*. Therapeutic potential of cannabinoids in neurodegenerative disorders: a selective review. Curr Pharm Des 2014; 20: 2218–30.

10 Watt G, Karl T. In vivo Evidence for Therapeutic Properties of Cannabidiol (CBD) for Alzheimer's Disease. 2017; 8: 1–7.

11 Wilson RI, Nicoll R. Endogenous cannabinoids mediate retrograde signalling at hippocampal synapses. Nature 2001; 410: 588–92.

12 Rinaldi-Carmona M, Barth F, Héaulme M, *et al*. SR141716A, a potent and selective antagonist of the brain cannabinoid receptor. FEBS Lett 1994; 350: 240–4.

13 Després J-P, Lemieux I, Alméras N. Contribution of CB1 blockade to the management of high-risk abdominal obesity. Int J Obes 2006; 30: 44-52.

14 Price MR, Baillie GL, Thomas A, *et al*. Allosteric Modulation of the Cannabinoid CB1 Receptor. Mol Pharmacol 2005; 68: 1484–95.

15 Lucchesi V, Hurst DP, Shore DM, *et al*. CB2-Selective Cannabinoid Receptor Ligands: Synthesis, Pharmacological Evaluation, and Molecular Modeling Investigation of 1,8-Naphthyridin-2(1H)-one-3-carboxamides. J Med Chem 2014; 57: 8777–91.

16 Manera C, Benetti V, Castelli MP, *et al*. Design , Synthesis , and Biological Evaluation of New 1, 8-Naphthyridin-4(1H)-on-3-carboxamide and Quinolin-4 (1H)-on-3-carboxamide Derivatives as CB 2 Selective Agonists. Society 2006; 4: 5947–57.

17 Press JB, Birnberg GH. Heterocyclic-fused benzopyrans as cannabinoid analogues. J Heterocycl Chem 1985; 22: 561.

18 Cumella J, Hernández-Folgado L, Girón R, *et al*. Chromenopyrazoles: non-psychoactive and selective CB1 cannabinoid agonists with peripheral antinociceptive properties. ChemMedChem 2012; 7: 452–63.

19 Morales P, Azofra LM, Cumella J, *et al*. Preparation of 2 , 2-dimethylchroman-4-ones from 5-alkyl-substituted resorcinols : microwave-assisted synthesis and theoretical calculations. Arkivoc 2014; 2: 319–32.

20 Gong J-P, Onaivi ES, Ishiguro H, *et al*. Cannabinoid CB2 receptors: immunohistochemical localization in rat brain. Brain Res 2006; 1071: 10–23.

21 Van Sickle MD, Duncan M, Kingsley PJ, *et al*. Identification and functional characterization of

brainstem cannabinoid CB2 receptors. Science 2005; 310: 329–32.

22 Ashton JC, Friberg D, Darlington CL, Smith PF. Expression of the cannabinoid CB2 receptor in the rat cerebellum: an immunohistochemical study. Neurosci Lett 2006; 396: 113–6.

23 Onaivi ES, Ishiguro H, Liu Q-R. Future perspectives: Cannabinoid CB2 receptor ligands and their therapeutic potential in mental diseases. In: Fattore L, Ed. Cannabinoids in Neurologic and Mental Disease. New York: Elsevier, 2015, pp 425–44.

24 Chung ES, Bok E, Chung YC, Baik HH, Jin BK. Cannabinoids prevent lipopolysaccharide-induced neurodegeneration in the rat substantia nigra in vivo through inhibition of microglial activation and NADPH oxidase. Brain Res 2012; 1451: 110–16.

25 Morales P, Gómez-Cañas M, Navarro G, *et al*. Chromenopyrazole, a Versatile Cannabinoid Scaffold with in Vivo Activity in a Model of Multiple Sclerosis. J Med Chem 2016; 59: 6753–71.

26 Gómez-Cañas M, Morales P, García-Toscano L, *et al*. Biological characterization of PM226, a chromenoisoxazole, as a selective CB2 receptor agonist with neuroprotective profile. Pharmacol Res 2016; 110: 205–15.

27 Jemal A, Bray F, Center MM, Ferlay J, Ward E, Forman D. Global cancer statistics. CA Cancer J Clin 2011; 61: 69–90.

28 Tramèr MR, Carroll D, Campbell F, Reynolds DJ, Moore R, McQuay HJ. Cannabinoids for control of chemotherapy induced nausea and vomiting: quantitative systematic review. Br Med J 2001; 323: 16–21.

29 Berry EM, Mechoulam R. Tetrahydrocannabinol and endocannabinoids in feeding and appetite. Pharmacol Ther 2002; 95: 185–90.

30 Jatoi A, Windschitl HE, Loprinzi CL, *et al*. Dronabinol versus megestrol acetate versus combination therapy for cancer-associated anorexia: a North Central Cancer Treatment Group study. J Clin Oncol 2002; 20: 567–73.

31 Walsh D, Nelson KA, Mahmoud FA. Established and potential therapeutic applications of cannabinoids in oncology. Support Care Cancer 2003; 11: 137–43.

32 Mantyh PW, Clohisy DR, Koltzenburg M, Hunt SP. Molecular mechanisms of cancer pain. Nat Rev Cancer 2002; 2: 201–9.

33 Campbell FA, Tramèr MR, Carroll D, Reynolds DJ, Moore RA, McQuay HJ. Are cannabinoids an effective and safe treatment option in the management of pain? A qualitative systematic review. BMJ 2001; 323: 13–6.

34 Iversen L, Chapman V. Cannabinoids: a real prospect for pain relief? Curr Opin Pharmacol 2002; 2: 50–5.

35 Sharkey K, Darmani N, Parker L. Regulation of nausea and vomiting by cannabinoids and the endocannabinoid system. Eur J Pharmacol 2014; 722: 134–46.

36 Abrams DI, Guzman M. Cannabis in cancer care. Clin Pharmacol Ther 2015; 97: 575–86.

37 Velasco G, Hernández-Tiedra S, Dávila D, Lorente M. The use of cannabinoids as anticancer agents. Prog Neuropsychopharmacol Biol Psychiatry 2016; 64: 259–66.

38 Guzmán M. Cannabinoids: potential anticancer agents. Nat Rev Cancer 2003; 3: 745–55.

39 Chakravarti B, Ravi J, Ganju RK. Cannabinoids as therapeutic agents in cancer : current status and future implications. Oncotarget 2014; 5: 5852–72.

40 Velasco G, Sánchez C, Guzmán M. Towards the use of cannabinoids as antitumour agents. Nat Rev Cancer 2012; 12: 436–44.

41 Malfitano AM, Ciaglia E, Gangemi G, Gazzerro P, Laezza C, Bifulco M. Update on the endocannabinoid system as an anticancer target. 2011; 15: 297–308.

42 Caffarel MM, Sarrió D, Palacios J, Guzmán M, Sánchez C. Delta9-tetrahydrocannabinol inhibits cell cycle progression in human breast cancer cells through Cdc2 regulation. Cancer Res 2006; 66: 6615–21.

43 Guzmán M, Duarte MJ, Blázquez C, *et al*. A pilot clinical study of Delta9-tetrahydrocannabinol in patients with recurrent glioblastoma multiforme. Br J Cancer 2006; 95: 197–203.

44 Asche C. Antitumour Quinones. Mini-Reviews Med Chem 2005; 5: 449–467.

45 Lu J-J, Bao J-L, Wu G-S, *et al*. Quinones derived from plant secondary metabolites as anti-cancer agents. Anticancer Agents Med Chem 2013; 13: 456–63.

46 Alcaín FJ, Villalba JM. NQO1-directed antitumour quinones. Expert Opin Ther Patents 2007; 17: 649–65.

47 El-Najjar N, Gali-Muhtasib H, Ketola RA, Vuorela P, Urtti A, Vuorela H. The chemical and biological activities of quinones: overview and implications in analytical detection. Phytochem Rev 2011; 10: 353–70.

48 O'Brien PJ. Molecular mechanisms of quinone cytotoxicity. Chem Biol Interact 1991; 80: 1–41.

49 Vujčić MT, Tufegdžić S, Novaković I, Djikanović D, Gašić MJ, Sladić D. Studies on the interactions of bioactive quinone avarone and its methylamino derivatives with calf thymus DNA. Int J Biol Macromol 2013; 62: 405–410.

50 Wondrak GT. Redox-directed cancer therapeutics: molecular mechanisms and opportunities. Antioxid Redox Signal 2009; 11: 3013–69.

51 Cabello CM, Bair WB, Wondrak GT. Experimental therapeutics: targeting the redox Achilles heel of cancer. Curr Opin Investig Drugs 2007; 8: 1022–37.

52 Gehrmann M. Drug evaluation: STA-4783--enhancing taxane efficacy by induction of Hsp70. Curr Opin

Investig Drugs 2006; 7: 574–80.

53 Gorrini C, Harris IS, Mak TW. Modulation of oxidative stress as an anticancer strategy. Nat Rev Drug Discov 2013; 12: 931–47.

54 Tacar O, Sriamornsak P, Dass CR. Doxorubicin: an update on anticancer molecular action, toxicity and novel drug delivery systems. J Pharm Pharmacol 2013; 65: 157–70.

55 Dunn CJ, Goa KL. Mitoxantrone: a review of its pharmacological properties and use in acute nonlymphoblastic leukaemia. Drugs Aging 1996; 9: 122–47.

56 Siegel D, Gibson NW, Preusch PC, Ross D. Metabolism of mitomycin C by DT-diaphorase: role in mitomycin C-induced DNA damage and cytotoxicity in human colon carcinoma cells. Cancer Res 1990; 50: 7483–9.

57 Cummings J, Spanswick VJ, Tomasz M, Smyth JF. Enzymology of mitomycin C metabolic activation in tumour tissue: implications for enzyme-directed bioreductive drug development. Biochem Pharmacol 1998; 56: 405–14.

58 Anighoro A, Bajorath J, Rastelli G. Polypharmacology: Challenges and Opportunities in Drug Discovery. J Med Chem 2014; 57: 7874–87.

59 M. O'Boyle N, J. Meegan M. Designed Multiple Ligands for Cancer Therapy. Curr Med Chem 2011; 18: 4722–37.

60 Gediya LK, Njar VC. Promise and challenges in drug discovery and development of hybrid anticancer drugs. Expert Opin Drug Discov 2009; 4: 1099–111.

61 Jagerovic N, Morales P, Goya P, *et al*. Chromenopyrazolediones as cannabinoid quinone derivatives having anti-tumour activity. Patent WO2014013117. 2014 Jan.

62 Jagerovic N, Morales P, Goya P, *et al*. Nuevas cromenoquinonas moduladoras de receptores cannabinoides CB2 con actividad antitumoral. Patent WO2015140377. 2014 Mar.

63 Morales P, Vara D, Goméz-Cañas M, *et al*. Synthetic cannabinoid quinones: Preparation, in vitro antiproliferative effects and in vivo prostate antitumor activity. Eur J Med Chem 2013; 70: 111–19.

64 Morales P, Blasco-Benito S, Andradas C, *et al*. A selective, non-toxic CB2 cannabinoid o-quinone with in vivo activity against triple negative breast cancer. J Med Chem 2015; 58: 2256–64.

65 Wu A, Duan Y, Xu D, Penning TM, Harvey RG. Regiospecific oxidation of polycyclic aromatic phenols to quinones by hypervalent iodine reagents. Tetrahedron 2010; 66: 2111–18.

66 Kogan NM, Rabinowitz R, Levi P, *et al*. Synthesis and Antitumor Activity of Quinonoid Derivatives of Cannabinoids. J Med Chem 2004; 47: 3800–06.

67 Kogan NM, Bl C, Alvarez L, Gallily R, Schlesinger M, Guzman M *et al*. A Cannabinoid Quinone Inhibits Angiogenesis by Targeting Vascular Endothelial Cells. Mol Pharmacol 2006; 70: 51–9.

68 Kogan NM, Schlesinger M, Peters M, Marincheva G, Beeri R, Mechoulam R. A cannabinoid anticancer quinone, HU-331, is more potent and less cardiotoxic than doxorubicin: a comparative in vivo study. J Pharmacol Exp Ther 2007; 322: 646–53.

69 Siegel RL, Miller KD, Jemal A. Cancer statistics, 2015. CA Cancer J Clin 2015; 65: 5–29.

70 Abramson VG, Lehmann BD, Ballinger TJ, Pietenpol JA. Subtyping of triple-negative breast cancer: Implications for therapy. Cancer 2014; : 1–9.

71 Hoeferlin LA, E.Chalfant CE, Park MA. Challenges in the Treatment of Triple Negative and HER2-Overexpressing Breast Cancer. J Surg Sci 2014; 1: 3–7.

72 Foulkes WD, Smith IE, Reis-Filho JS. Triple-Negative Breast Cancer. N Engl J Med 2010; 363: 1938–48.

73 Hudis CA, Gianni L. Triple-negative breast cancer: an unmet medical need. Oncologist 2011; 16: 1–11.

74 Wahba HA, El-hadaad HA. Current approaches in treatment of triple-negative breast cancer Treatment modalities of TNBC. Cancer Biol Med 2015; 1: 106–16.

75 Caffarel MM, Andradas C, Mira E, *et al*. Cannabinoids reduce ErbB2-driven breast cancer progression through Akt inhibition. Mol Cancer 2010; 9: 196–206.

76 Perez-Gomez E, Andradas C, Blasco-Benito S, *et al*. Role of Cannabinoid Receptor CB2 in HER2 Pro-oncogenic Signaling in Breast Cancer. JNCI J Natl Cancer Inst 2015; 107: djv077.

77 Ford LA, Roelofs AJ, Anavi-Goffer S, *et al*. A role for L-alpha-lysophosphatidylinositol and GPR55 in the modulation of migration, orientation and polarization of human breast cancer cells. Br J Pharmacol 2010; 160: 762–71.

78 Shrivastava A, Kuzontkoski PM, Groopman JE, Prasad A. Cannabidiol induces programmed cell death in breast cancer cells by coordinating the cross-talk between apoptosis and autophagy. Mol Cancer Ther 2011; 10: 1161–72.

79 Caffarel MM, Andradas C, Pérez-Gómez E, Guzmán M, Sánchez C. Cannabinoids: a new hope for breast cancer therapy? Cancer Treat Rev 2012; 38: 911–28.

80 Gomella LG, Liu XS, Trabulsi EJ, *et al*. Screening for prostate cancer: the current evidence and guidelines controversy. Can J Urol 2011; 18: 5875–83.

81 Djulbegovic M, Beyth RJ, Neuberger MM, *et al*. Screening for prostate cancer: systematic review and meta-analysis of randomised controlled trials. BMJ 2010; 341: c4543.

82 Fu W, Madan E, Yee M, Zhang H. Progress of molecular targeted therapies for prostate cancers. Biochim Biophys Acta 2012; 1825: 140–52.

83 Darwish OM, Raj G V. Management of biochemical recurrence after primary localized therapy for prostate cancer. Front Oncol 2012; 2: 1–6.

84 Grossmann M, Zajac JD. Androgen deprivation therapy in men with prostate cancer: how should the side effects be monitored and treated? Clin Endocrinol 2011; 74: 289–93.

85 Czifra G, Varga A, Nyeste K, *et al*. Increased expressions of cannabinoid receptor-1 and transient receptor potential vanilloid-1 in human prostate carcinoma. J Cancer Res Clin Oncol 2009; 135: 507–14.

86 Chung SC, Hammarsten P, Josefsson A, *et al*. A high cannabinoid CB(1) receptor immunoreactivity is associated with disease severity and outcome in prostate cancer. Eur J Cancer 2009; 45: 174–82.

87 Thors L, Bergh A, Persson E, *et al*. Fatty acid amide hydrolase in prostate cancer: association with disease severity and outcome, CB1 receptor expression and regulation by IL-4. PLoS One 2010; 5: e12275.

88 Piñeiro R, Maffucci T, Falasca M. The putative cannabinoid receptor GPR55 defines a novel autocrine loop in cancer cell proliferation. Oncogene 2011; 30: 142–52.

89 Hohmann AG, Guindon J. The endocannabinoid system and cancer: therapeutic implication. Br J Pharmacol 2011; 163: 1447–63.

90 Díaz-Laviada I. The endocannabinoid system in prostate cancer. Nat Rev Urol 2011; 8: 553–61.

91 Orellana-Serradell O, Poblete CE, Sanchez C, *et al*. Proapoptotic effect of endocannabinoids in prostate cancer cells. Oncol Rep 2015; 33: 1599–608.

92 Agostinis P, Berg K, Cengel K, *et al*. Photodynamic Therapy of Cancer: an Update. CA Cancer J Clin 2011; 61: 250–81.

93 Allison RR, Moghissi K. Oncologic photodynamic therapy: Clinical strategies that modulate mechanisms of action. Photodiagnosis Photodyn Ther 2013; 10: 331–41.

94 Benov L. Photodynamic Therapy: Current Status and Future Directions. Med Princ Pract 2015; 24: 14–28.

95 Wang W, Moriyama LT, Bagnato VS. Photodynamic therapy induced vascular damage: an overview of experimental PDT. Laser Phys Lett 2013; 10: 23001.

96 Kushibiki T, Hirasawa T, Okawa S, Ishihara M. Responses of cancer cells induced by photodynamic therapy. J Healthc Eng 2013; 4: 87–108.

97 Anand S, Ortel BJ, Pereira SP, Hasan T, Maytin E V. Biomodulatory approaches to photodynamic therapy for solid tumors. Cancer Lett 2012; 326: 8–16.

98 Weijer R, Broekgaarden M, Kos M, *et al*. Enhancing photodynamic therapy of refractory solid cancers: combining second-generation photosensitizers with multi-targeted liposomal delivery. J Photochem Photobiol C Photochem Rev 2015; 23: 103–31.

99 Lamberti MJ. Breast cancer as photodynamic therapy target: Enhanced therapeutic efficiency by overview of tumor complexity. World J Clin Oncol 2014; 5: 901.

100 Rogers L, Sergeeva NN, Paszko E, Vaz GMF, Senge MO. Lead Structures for Applications in Photodynamic Therapy. 6. Temoporfin Anti-Inflammatory Conjugates to Target the Tumor Microenvironment for In Vitro PDT. PLoS One 2015; 10: e0125372.

101 Tomanová P, Rimpelová S, Jurášek M, *et al*. Trilobolide–porphyrin conjugates: On synthesis and biological effects evaluation. Steroids 2015; 97: 8–12.

102 Trivedi ER, Blumenfeld CM, Wielgos T, *et al*. Multi-gram synthesis of a porphyrazine platform for cellular translocation, conjugation to Doxorubicin, and cellular uptake. Tetrahedron Lett 2012; 53: 5475–78.

103 Zhang S, Jia N, Shao P, Tong Q, Xie X-Q, Bai M. Target-selective phototherapy using a ligand-based photosensitizer for type 2 cannabinoid receptor. Chem Biol 2014; 21: 338–44.

104 Morales P, Moreno L, Fernández-ruiz J, Jagerovic N. Synthesis of a novel CB2 cannabinoid-porphyrin conjugate based on an antitumor chromenopyrazoledione. J Porphyr Phthalocyanines 2017; 21: 67–76.

105 Sawzdargo M, Nguyen T, Lee DK, *et al*. Identification and cloning of three novel human G protein-coupled receptor genes GPR52, PsiGPR53 and GPR55: GPR55 is extensively expressed in human brain. Brain Res Mol Brain Res 1999; 64: 193–8.

106 Ryberg E, Larsson N, Sjögren S, Hjorth S, Hermansson N-O, Leonova J *et al*. The orphan receptor GPR55 is a novel cannabinoid receptor. Br J Pharmacol 2007; 152: 1092–101.

107 Morales P, Jagerovic N. Advances towards the Discovery of GPR55 Ligands. Curr Med Chem 2016; 23: 2087–100.

108 Ross RA. The enigmatic pharmacology of GPR55. Trends Pharmacol Sci 2009; 30: 156–63.

109 Console-Bram L, Ciuciu SM, Zhao P, Zipkin RE, Brailoiu E, Abood ME. N-arachidonoyl glycine, another endogenous agonist of GPR55. Biochem Biophys Res Commun 2017; 490: 1389–93.

110 Heynen-genel S, Dahl R, Shi S, *et al*. Screening for Selective Ligands for GPR55-Antagonists. Probe Reports from the NIH Molecular Libraries Program; National Center for Biotechnology Information (U.S.): Bethesda, MD, 2010; http://www.ncbi.nlm.nih.gov/books/NBK66153/. nih. .

111 Yrjölä S, Parkkari T, Navia-Paldanius D, *et al*. Potent and selective N-(4-sulfamoylphenyl)thiourea-based GPR55 agonists. Eur J Med Chem 2016; 107: 119–32.

112 Morales P, Whyte LS, Chicharro R, *et al*. Identification of Novel GPR55 Modulators Using Cell-Impedance-Based Label-Free Technology. J Med Chem 2016; 58: 1840–53.

113 Mechoulam R, Shvo Y. Hashish-I. The structure of cannabidiol. Tetrahedron 1963; 19: 2073–78.

114 Ibeas Bih C, Chen T, Nunn AVW, Bazelot M, Dallas M, Whalley BJ. Molecular Targets of Cannabidiol in Neurological Disorders. Neurotherapeutics 2015; 12: 699–730.

115 Pisanti S, Malfitano AM, Ciaglia E, *et al*. Cannabidiol: State of the art and new challenges for therapeutic applications. Pharmacol Ther 2017; 175: 133–50.

116 Fasinu PS, Phillips S, Elsohly MA, Walker LA. Current Status and Prospects for Cannabidiol Preparations as New Therapeutic Agents. Pharmacotherapy 2016; 36: 781–96.

117 Lago-Fernandez A, Redondo V, Hernandez-Folgado L, Figuerola-Asencio L, Jagerovic N. New Methods for the Synthesis of Cannabidiol Derivatives. In: Reggio PH, Ed. Cannabinoids and Their Receptors. London: Elsevier, 2017, pp 237–57.

118 Arnoldi A, Bizzozero N, Merlini L, Ragg E, Rossi AC. Analogs of cannabinoids. Synthesis of N-heterocyclic derivatives of olivetol. J Chem Res Synopses 1983; 1: 107.

119 Scherma M, Satta V, Fratta W, Fadda P. The endocannabinoid system: Anorexia nervosa and binge eating disorder. In: Fattore L., Ed. Cannabinoids in Neurologic and Mental Disease. New York: Elsevier, 2015, pp 389–413.

120 Clinical Trials Cannabinoids. https://clinicaltrials.gov/ct2/results?term=cannabinoids&pg=1 (accessed 28 Sep2017).

121 Picone RP, Kendall D. Minireview: From the Bench, Toward the Clinic: Therapeutic Opportunities for Cannabinoid Receptor Modulation. Mol Endocrinol 2015; 29: 801–13.

122 Dhopeshwarkar A, Mackie K. CB2 cannabinoid receptors as a therapeutic target - What does the future hold? Mol Pharmacol 2014; 86: 430–37.

123 Vemuri VK, Makriyannis A. Medicinal Chemistry of Cannabinoids. Clin Pharmacol Ther 2015; 97: 553–58.

124 Morales P, Hurst DP, Reggio PH. Molecular Targets of the Phytocannabinoids: A Complex Picture. In: Kinghorn AD, Gibbons S, Eds. Phytocannabinoids Unraveling the Complex Chemistry and Pharmacology of *Cannabis sativa*. Cham: Springer International Publishing, 2017, pp 103–31.

125 Morales P, Reggio PH, Jagerovic N. An Overview on Medicinal Chemistry of Synthetic and Natural Derivatives of Cannabidiol. Front Pharmacol 2017; 8: 422.

126 Brown KJ, Laun AS, Song Z. Cannabidiol, a novel inverse agonist for GPR12. Biochem Biophys Res Commun 2017; 493: 451–54.

127 Laun AS, Song Z-H. GPR3 and GPR6, novel molecular targets for cannabidiol. Biochem Biophys Res Commun 2017; 490: 17–21.

128 Soethoudt M, Grether U, Fingerle J, *et al*. Cannabinoid CB2 receptor ligand profiling reveals biased signalling and off-target activity. Nat Commun 2017; 8: 13958.

129 Laprairie RB, Bagher AM, Denovan-Wright EM. Cannabinoid receptor ligand bias: implications in the central nervous system. Curr Opin Pharmacol 2017; 32: 32–43.

130 Laprairie RB, Bagher AM, Kelly MEM, Denovan-Wright EM. Biased Type 1 Cannabinoid Receptor Signaling Influences Neuronal Viability in a Cell Culture Model of Huntington Disease. Mol Pharmacol 2016; 89: 364–75.

131 Pupo AS, Duarte DA, Lima V, Teixeira LB, Parreiras-e-Silva LT, Costa-Neto CM. Recent updates on GPCR biased agonism. Pharmacol Res 2016; 112: 49–57.

132 Rankovic Z, Brust TF, Bohn LM. Biased agonism: An emerging paradigm in GPCR drug discovery. Bioorganic Med Chem Lett 2016; 26: 241–50.

133 Zhou XE, Melcher K, Xu HE. Understanding the GPCR biased signaling through G protein and arrestin complex structures. Curr Opin Struct Biol 2017; 45: 150–59.

134 Ibsen MS, Connor M, Glass M. Cannabinoid CB_1 and CB_2 Receptor Signaling and Bias. Cannabis Cannabinoid Res 2017; 2: 48–60.