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1,2,3-Triazole derivatives as highly selective cannabinoid receptor type 2 (CB2) agonists

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Abstract The cannabinoid receptor 2 (CB2 receptor) has attracted considerable interest, mainly due to its potential as a target for therapeutics for treating various diseases that have a neuroinflammatory or neurodegenerative component while avoiding the adverse psychotropic effects that accompany CB1 receptor-based therapies. With the appreciation that CB2-selective ligands show marked functional selectivity, there is a renewed opportunity to explore this promising area of research from both a mechanistic as well as a therapeutic perspective. In this research, we are interested in the discovery of new chemotypes as highly selective CB2 modulators, which may serve as good starting points for further optimization towards the development of CB2 therapeutics. In search of new chemotypes as CB2 selective agents, we screened a series of triazole derivatives with interesting bioactive scaffolds, which led to the discovery of two novel and highly selective ligands for CB2 receptors. Compounds **6** and **11** produced a concentration-dependent inhibition of specific [^{3}H]-CP55,940 (CB2) binding with K_i ± SEM values of 105.3 ± 22.6 and 116.4 ± 19.5 nM, respectively, while no binding affinity towards CB1 receptors or opioid receptors was observed. The CB2 functional activity of **6** and **11**, as measured by a GPCR Tango assay (G-protein independent β-arrestin translocation assay), revealed that these compounds act as CB2 agonists with EC_{50} values ± SEM of 1.83 ± 0.16 and 1.14 ± 0.52 μM, respectively. Molecular modeling results showed

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that both compounds fit well into the active site of the CB2 receptor and showed strong hydrophobic interactions with key residues. In conclusion, the new triazole derivatives (**6** and **11**) showed promising activity towards CB2 receptors and have great potential to be developed into therapeutically useful CB2 agonists through hit-to-lead optimization.

1. Introduction

The endocannabinoid system (ECS) consists of cannabinoid receptors (CB1 and CB2), endogenous cannabinoids (endocannabinoids), and the enzymes that synthesize and degrade the natural endocannabinoid ligands (Dhopeshwarkar and Mackie, 2014). The endocannabinoid system is involved in a variety of physiological processes in the human body including pain, appetite, mood, memory formation, and the immune system (Cencioni et al., 2010, Mechoulam and Parker, 2013). Therefore, modulation of the ECS has been widely explored for therapeutic involvement in human health and disease (Di Marzo, 2008, Pertwee, 2012). Both CB1 and CB2 are G-protein coupled receptors. They share ~ 44% sequence identity, and recently several experimental 3D-structures have been reported for each of them (Hua et al., 2020, 2017, Shao et al., 2019). Because the CB2 receptor (unlike CB1) is primarily expressed in the spleen and immune cells, rather than the central nervous system, it became known as the "peripheral cannabinoid receptor" soon after its discovery (Miller and Devi, 2011). When CB2 receptor expression was found in the neurons and the microglial cells of the brain, this terminology was determined to be inaccurate, and CB2 receptor expression has since been shown to be correlated with neuroinflammation (Picone and Kendall, 2015). Many studies showed upregulation of the CB2 receptors in microglial cells in an *in vitro* model of autoimmune encephalomyelitis (Picone and Kendall, 2015). In addition, CB2 receptor expression has more recently been associated with neurodegenerative diseases such as Huntington's and Alzheimer's disease (Di Marzo et al., 2015, Savonenko et al., 2015). CB2-selective Positron Emission Tomography (PET) tracers in an Alzheimer's mouse model have demonstrated increased expression of CB2 receptors, concomitant with the formation of amyloid-beta plaques (Savonenko et al., 2015). This study suggests that CB2 PET tracers may have potential as a diagnostic tool for neuroinflammation.

Some of the therapeutic benefits of CB2 receptor agonists that have been studied include analgesic and anti-inflammatory effects (Malfitano et al., 2014, Sagredo et al., 2012, Shoemaker et al., 2007). CB2 receptor agonists have shown efficacy as potential therapeutic agents in peripheral diseases that involve inflammation, such as atherosclerosis (Netherland et al., 2010), renal fibrosis (Barutta et al., 2011), and liver cirrhosis (Alswat, 2013). However, the most clinically advanced CB2 receptor agonist, Cannabinor or PRS 211,375 (Fig. 1), eventually failed Phase IIb tests, in which it had been studied for its potential to manage pain after third molar extractions (Nevalainen, 2014). A possible side effect of Cannabinor was increased urinary frequency, which has also been shown in animal studies (Gratzke et al., 2010, 2011). Another CB2 receptor agonist that failed in Phase II trials, GW-842,166X, was demonstrated to be ineffective in blocking pain due to osteoarthritis and third molar extraction (Han et al., 2015, Ostenfeld et al., 2011). In 2009, a third compound, S-777,469, passed Phase II trials for treating atopic dermatitis. However, since then there have been no new clinical data released and there are no published plans for further testing, its development may have been halted (Haruna et al., 2015, Pertwee et al., 1995, Sekiguchi et al., 2015). Another agonist with a very high selectivity of 4700-fold for the CB2 receptor over CB1, developed by Glenmark Pharmaceuticals, Tedalinab (GRC-10693), was studied for the treatment of osteoarthritis and neuropathic pain and showed effective analgesic and anti-inflammatory actions in completed Phase I trials in 2009 (Morales et al., 2017). Eli Lilly and Co. in 2011 investigated the CB2 cannabinoid agonist LY2828360. While the study did not reveal any statistically significant difference from the placebo, its single daily oral dose caused no observed specific risks or discomforts in patients with osteoarthritic knee pain in Phase II clinical trials. Though its signaling profile is unknown, LY2828360 may prevent opioid tolerance and physical dependence when used with opioid-based pain medications (Lin et al., 2018). In 2012, the Japanese company, Kyowa Kirin Co., Ltd., reported that CB2 agonist KHK-6188 showed efficacy of two different doses relative to placebo and had passed Phase II in a clinical study for patients with neuralgia following a herpes zoster infection, but, as of 2013, development of this agent was discontinued (Nevalainen, 2014).

In 2017, Corbus Pharmaceuticals Inc. announced that they started evaluating the efficacy and safety of small molecule JBT-101 (also known as Lenabasum) as a selective CB2 receptor agonist in autoimmune diseases such as systemic lupus erythematosus (SLE). To date, it has demonstrated favorable safety and tolerability profiles in clinical studies and is currently in Phase III trials for the treatment of Dermatomyositis (DM) and Systemic Sclerosis (SSc) which sometimes occurs as an overlap with myositis. However, the studies are still running (Robinson et al., 2017).

Derivatives of five-membered heterocycles, such as pyrrole (Berggren et al., 2004a; Barth et al., 2007; Francis et al., 2007, 2006), thiophene (Barth et al., 2006a; Barth et al., 2006b; Barth et al., 2006c; Barth and Congy, 2006c), thiazole (Berggren et al., 2004b, (Barth and Rinaldi-Carmona, 2007a), Lange et al., 2005), imidazole (Cheng, 2007, 2006, Dyck et al., 2004, Plummer et al., 2005), oxazole (Gratzke et al., 2010), and 1,2,4-triazole (Dyck et al., 2004, (Barth and Rinaldi-Carmona, 2007b), Jagerovic et al., 2006, 2004, Pavon et al., 2006), are well known for featuring in small molecules that modulate the CB receptors' activity. A few published studies have reported the derivatives of 1,2,3-triazole as CB receptor modulators. Hou et al. (2009) reported N1 and N2 substituted 1,2,3-trizoles are CB1 receptor antagonists. Recently, Morales and colleagues presented the first bitopic ligands for the CB2

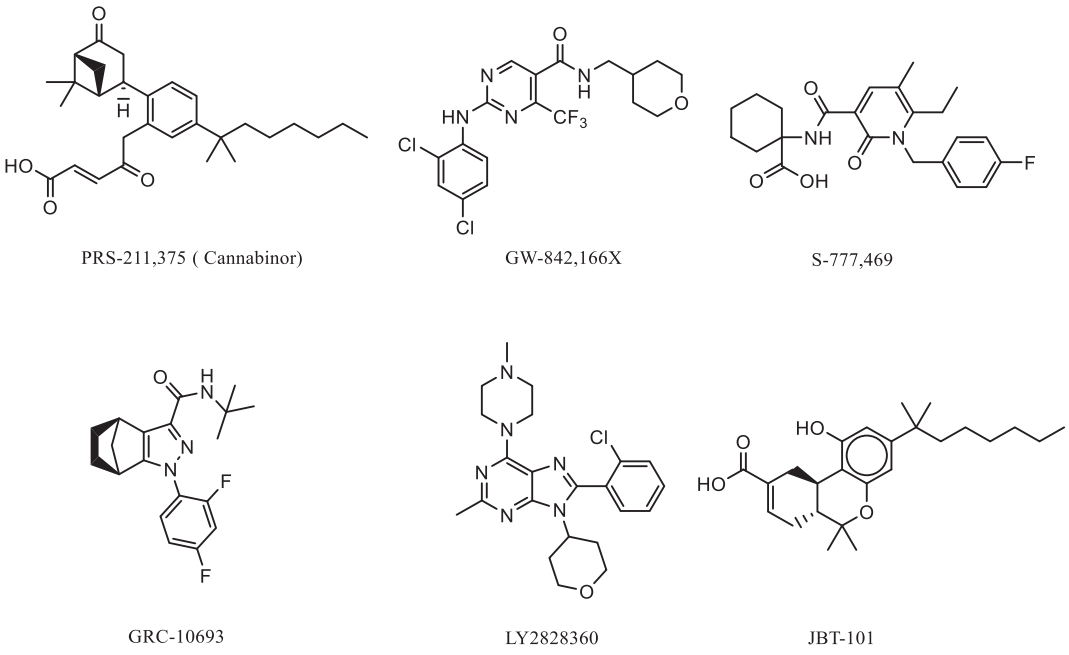


Fig. 1 Some reported CB2-selective agonists.

receptor, which bind to the orthosteric site as well as to a less conserved site within the same receptor unit (Morales et al., 2020). Herein, we report our study on the resynthesis and biological evaluation of a series of substituted triazoles possessing an affinity for CB receptors. Later, we also report that these 1,4,5-trisubstituted triazoles possess high selectivity towards CB2 receptors. We also describe the results of computational modeling of the interactions of the lead compounds with the CB2 receptor, taking advantage of the recently reported X-ray crystal structures of the active state of the CB2 receptor.

2. Materials and methods

2.1. General experimental procedures

^{1}H and ^{13}C NMR spectra were obtained on Bruker model AMX 500 and 400 NMR spectrometers with standard pulse sequences, operating at 500 and 400 MHz in ^{1}H and 125 and 100 MHz in ^{13}C, respectively. $CDCl_3$, DMSO d_6, and CD_3OD were used as solvents, and TMS was used as an internal standard. All chemicals used were from Sigma-Aldrich (Poole, Dorset, U.K.) with the following exceptions. For the binding experiments, [^{3}H]- CP55,940 (174.8 Ci/mmol), [^{3}H]-DAMGO (53.4 Ci/mmol), [^{3}H]-U69,593 (42.7 Ci/mmol), and [^{3}H]-enkephalin (45 Ci/mmol) were obtained from PerkinElmer Life Sciences Inc. (Boston, MA, USA). CP55,940, DAMGO, DPDPE, and naloxone hydrochloride were obtained from Tocris Bioscience (Ellisville, MO, USA).

2.2. Chemistry

The evaluated triazole derivatives **1**–**11** were designed and synthesized at the Yarmouk University and Tafila Technical University, Jordan. The general experimental procedure and experimental data for triazole derivatives **1**–**11** have been previously described (Abu-Orabi, 2002).

2.2.1. General procedure for synthesis of triazoles *1*–*11*

To an ethanolic solution of substituted benzyl azides, 1 equivalent of disubstituted acetylenes was added. The resulting mixture was refluxed overnight. After removal of the solvent, the residue was recrystallized using ethanol-petroleum ether (Abu-Orabi et al., 1999).

2.3. Biological evaluation

2.3.1. Cell culture and membrane preparation

HEK293 cells (ATCC) were stably transfected with plasmids containing cloned human cannabinoid receptor subtypes 1 and 2 (obtained from Origene, Rockville, MD, USA). These cells were maintained in a humidified incubator at 37 °C and 5% CO_2 in a Dulbecco's modified Eagle's medium (DMEM) nutrient mixture F-12 HAM supplemented with 2 mM L-glutamine, 10% fetal bovine serum, 1000 IU/mL penicillin, and 1000 μg/mL of streptomycin, and 0.5 mg/mL G418 antibiotic solution. HEK293 cells stably transfected with opioid receptor subtypes μ, δ, and κ were used to perform the opioid receptor binding assays. These cells were maintained at 37 °C and 5% CO_2 in a DMEM nutrient mixture supplemented with 2 mM L-glutamine, 10% fetal bovine serum, 1000 IU/mL penicillin, 1000 μg/mL of streptomycin, and either 0.5 mg/mL (κ) or 0.2 mg/mL (δ and μ) G418 antibiotic solution. Membranes for the radioligand binding assays were prepared by scraping the cells in cold Tris-HCl, pH 7.4, and then centrifuged at 5200 X g for 10 min at 4 °C. The supernatant was discarded, and the pellet was resuspended in the same buffer, homogenized using a sonic dismembrator model 100 (Fisher Scientific, Pittsburgh, PA, USA) for 30 s, and then

centrifuged at 1000 g for 10 min at 4 °C. The supernatant was saved, and the pellet underwent the suspension and sonication process two additional times under the same conditions. The supernatants were combined and centrifuged at 23,300 g for 40 min at 4 °C. The pellet was resuspended and aliquoted into 2 mL vials and stored at − 80 °C. The total protein concentration was determined using a Pierce BCA protein assay kit (Thermo Scientific, Rockford, IL, USA) according to the manufacturer's instructions (Atta-ur-Rahman et al., 2001). The optimal membrane and radioligand (K_D) concentrations for each receptor batch were established through membrane evaluation and saturation binding experiments.

2.3.2. Radioligand displacement for cannabinoid receptor subtypes

Compounds evaluated in this assay were run in competition binding with both cannabinoid receptor subtypes, CB1 and CB2 (Ross et al., 1999). Cannabinoid receptor binding screening was performed under the following conditions: 10 μM of each compound from independent triplicate dilutions was incubated with 1.6975 nM (CB1) or 1.959 nM (CB2) [^{3}H]-CP55,940, a potent cannabinoid agonist with an affinity for both receptor subtypes, and 5 μg of CB1 or 1 μg of CB2 membrane for 90 min at 37 °C with gentle agitation in a 96-well plate in a 0.2 mL final volume of 50 mM Tris-HCl, 20 mM EDTA, 154 mM NaCl, and 0.2% radioimmunoassay grade BSA at pH 7.4. The reaction was terminated via rapid vacuum filtration through a UniFilter 96 GF/C filter (PerkinElmer Life Sciences Inc., Boston, MA, USA), presoaked with 0.3% polyethyleneimine, followed by 10 washes with 50 mM Tris-HCl, pH 7.4, buffer containing 0.2% BSA. Filters were dried, 25 μL of MicroScint20 was added, and the plates were read using a TopCount NXT microplate scintillation counter (PerkinElmer Life Sciences Inc., Boston, MA, USA). Total binding was defined as binding in the presence of a vehicle (1.0% DMSO). Nonspecific binding was the binding observed in the presence of 10.0 μM CP55,940. Specific binding was defined as the difference between total and nonspecific binding. Percent displacement was calculated using the following formula:

$$[100 - (\text{binding of compound} - \text{nonspecific binding}) \times 100] / \text{specific binding}$$

The K_i values were calculated using a nonlinear curve fit model in GraphPad Prism 5.0 software (GraphPad, La Jolla, CA, USA). Each compound was tested in triplicate unless stated otherwise.

2.3.3. Radioligand displacement for opioid receptor subtypes

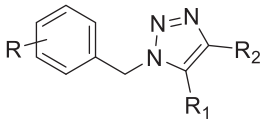
All compounds evaluated in the assay were run in competition binding assays against the opioid receptor subtypes (δ, κ, μ). Opioid binding assays were performed under the following conditions: independent triplicate dilutions of 10 μM compound were incubated with 0.85 nM [^{3}H]-DAMGO (μ), 0.91 nM [phenyl-3,4-^{3}H]-U-69,593 (κ), or 0.99 nM [^{3}H]-DPDPE (δ) for 60 min in a 96-well plate in a 0.2 mL final volume of 50 mM Tris-HCl, pH 7.4, with 15 μg (κ) or 20 μg (μ and δ) of the membrane. The reaction was terminated via rapid vacuum filtration through a UniFilter 96 GF/B filter presoaked with 0.3% BSA, followed by 10 washes with 4 °C 50 mM Tris-HCl, pH 7.4. Filters were dried, 50 μL of MicroScint20 was added, and the plates were read using a TopCount NXT microplate scintillation counter. Total binding, specific binding, and percent displacement were calculated using formula 1, given above.

Nonspecific binding was the binding observed in the presence of 10 μM DAMGO (μ), U-69,593 (κ), or DPDPE (δ). The competitive binding assay was performed by testing 12 triplicate 3-fold serial dilutions of 300 μM compound and 3 μM control (naloxone hydrochloride) with 15 μg of δ membrane and 1.87 nM [^{3}H]-DPDPE. We ran each compound once (on one plate) in triplicate to determine the K_i. The K_i values were calculated using a nonlinear curve fit model in GraphPad Prism 5.0 software (GraphPad, La Jolla, CA, USA).

2.4. Functional assay for G-protein independent β-arrestin recruitment

Optimization of the CB2 tango construct was conducted prior to performance of the assay as described by Kroeze et al. (performed in the National Institute of Mental Health Psychoactive Drug Screening Program at the University of North Carolina) to measure GPCR mediated β-arrestin translocation activity. Briefly, HTLA cells, which express a tTA-dependent luciferase reporter and β-arrestin2–TEV fusion gene, were transfected with the CB2 tango construct overnight. Subsequently, the cells were plated in DMEM supplemented with 1% dialyzed FBS and incubated for at least 6 h. For measurement of agonist activity, solutions containing the test compounds were prepared in the Tango assay buffer (20 nM HEPES, 1x HBSS at pH 7.4) at 5x final concentration and incubated with the cells overnight. Similarly, for measurement of antagonist activity, cells were incubated for 30 min with solutions containing the test compounds made at 6x final concentration before addition of 10 μL of the final EC80 concentration of the reference agonist, CP55940 (Kroeze et al., 2015).

Each compound was tested in triplicate; whereupon, the experimental data was analyzed using a four-parameter nonlinear regression fit in GraphPad Prism 9.1. EC_{50} values were determined from the fitted model. Each compound was tested in triplicate unless stated otherwise.



1 R = 2,3,4,5,6-pentamethyl; $R_1 = CO_2tBu$; $R_2 = CO_2tBu$
2 R = 2,3,4,5,6-pentamethyl; $R_1 = CO_2CH_3$; $R_2 = CO_2CH_3$
3 R = 2,6-dichloro; $R_1 = CONHNH_2$; $R_2 = CONHNH_2$
4 R = 2,6-dichloro; $R_1 =$ -CO_2CH_3; $R_2 = CO_2CH_3$
5 R = 3-fluoro; $R_1 = CO_2CH_3$; $R_2 = CO_2CH_3$
6 R = 3-fluoro; $R_1 = CO_2tBu$; $R_2 = CO_2tBu$
7 R = 3-fluoro; $R_1 =$ H; $R_2 = CO_2CH_3$
8 R = 3-fluoro; $R_1 = CONHNH_2$; $R_2 = CONHNH_2$
9 R = 4-fluoro; $R_1 = CO_2CH_3$; $R_2 = CO_2CH_3$
10 R = 4-fluoro; R_1 = H; $R_2 = CO_2CH_3$
11 R = 4-fluoro; $R_1 = CO_2tBu$; $R_2 = CO_2tBu$

Fig. 2 Triazole derivatives (**1**–**11**).

Table 1 Radioligand Displacement Assay of the Synthesized Compounds (**1**–**11**) for Human Cannabinoid Receptors (Subtypes CB1 and CB2) and Opioid Receptors (Subtypes δ, κ, and μ).

	(% Displacement at 10 μM) Cannabinoid receptors		Binding affinity (K_i ± S.E.M.) nM	(% Displacement at 10 μM) Opioid receptors		
Compound	CB2	CB1	CB2	δ	κ	μ
1	38.5	30.6		16.0	1.7	22.3
2	2.0	3.4		1.9	3.4	20.7
3	[a]	[a]		[a]	[a]	[a]
4	[a]	17.4		41.7	9.0	[a]
5	[a]	[a]		39.5	2.1	[a]
6	95.5 ± 7.5	26.6 ± 8.9	105.3 ± 22.6	36.8 ± 5.2	15.5 ± 6.5	19.1 ± 5.8
7	41.3	5.3		[a]	[a]	[a]
8	23.3	[a]		15.5	25.1	24.4
9	[a]	[a]		0.6	8.7	23.2
10	3.6	[a]		[a]	[a]	20.3
11	99.3 ± 6.8	25.5 ± 8.4	116.4 ± 19.5	1.1	[a]	[a]
Naloxone[b]				106.4	101.6	97.0
CP55,940[c]	102.6	104.3	1.25 ± 0.14			

[a] No percent displacement at 10 μM; [b,c] Positive controls.

2.5. Molecular modeling and docking study

The active state (agonist bound) (PDB ID: 6KPC) X-ray crystal structures of CB2 receptor was downloaded from the Protein Data Bank (www.rcsb.org) and used for computational molecular modeling (Hua et al., 2020). The CB2 protein structure was prepared by adding hydrogens and missing side chains and adjusting bond orders and proper ionization at physiological pH 7.4 using the Prep Wizard module in the Schrödinger suite 2018–1 (Sastry et al., 2013). Compounds **6** and **11** were sketched in Maestro and energy-minimized using the LigPrep module of the Schrödinger suite 2018–1 using the OPLS3e (optimized potential for liquid simulations 3e) force field (Schrödinger, 2018a, 2018b). The docking of the compounds **6** and **11** into the CB2 receptor was performed using the Induced Fit docking protocol module of the Schrödinger software (Friesner et al., 2006, 2004). The grid for the CB2 receptor was prepared using the centroid of the co-crystallized ligand in the CB2 X-ray structure. The van der Waals radius-scaling factor and partial charge cutoff were kept to be 1 and 0.25, respectively. No additional constraints were used when preparing the grid or for docking. Further, the binding free energy (Prime MM-GBSA free energy) of the docked structures was calculated using the Prime module of the Schrödinger software (Jacobson et al., 2004), considering minimization of polar hydrogens only.

3. Results and discussion

The discovery of the cannabinoid receptors has aided the discovery of novel cannabinoid receptor agonists and antagonists with potential therapeutic value (Palmer et al., 2002, Pertwee, 2000, Wiley and Martin, 2002). A vast number of CB1 and CB2 receptor ligands with a wide range of chemical structures are now available (Goya and Jagerovic, 2000, Goutopoulos and Makriyannis, 2002). The research demonstrated that five-membered heterocyclic rings show a broad spectrum of activities, including modulation of the CB receptors (Berggren et al., 2004a, 2004b, Cheng, 2007, 2006, Dyck et al., 2004, Francis et al., 2007, 2006, 2006a, 2006b, 2006c, Francis and Murielle, 2007a, 2007b, Jagerovic et al., 2006, 2004, Lange et al., 2005, Pavon et al., 2006, Plummer et al., 2005). In this project, we were interested in the discovery of bioactive compounds that serve as modulators for CB receptors. We are not aware of any reported derivatives of 1,2,3-triazole having been explored for these biological effects. A series of pure 1,2,3-triazole derivatives (Fig. 2) were submitted for *in vitro* radioligand binding affinity assays using the opioid receptors (subtypes μ, κ, and δ) and cannabinoid receptors (CB1 and CB2) (Tarawneh et al., 2015).

The results are shown in Table 1. At a concentration of 10 μM, both compounds **6** and **11** significantly (>95%) displaced [^{3}H]-CP55,940 for CB2 receptors, while showing no significant (<30%) displacement of [^{3}H]-CP55,940 for CB1 receptors. To follow up on the promising results, the *in vitro* CB2 K_i for compounds **6** and **11** were determined, and this screening revealed both compounds **6** and **11** as potent and selective CB2 ligands with K_i ± S.E.M. values of 105.3 ± 22.6 nM and 116.4 ± 19.5 nM, respectively (Fig. 3). The other compounds in this study showed < 50% displacement of the radioligand at both CB1 and CB2 receptors at 10 μM; therefore, they were not further tested for binding affinity.

Replacing the *tert*-butyl esters on R1 and R2 in **6** with methyl groups to form compound **5** led to the loss of biological activity. Similar to **5**, changing the position of the fluoro group on the benzene ring to the *para* position as found in **8**, while R1 and R2 were retained as in **5**, did not significantly improve the activity. Replacing the fluoro group on the benzene ring in **6** to form pentamethylbenzyl **1**, with both R1 and R2 retained as in **6** and **11**, also resulted in the loss of the activity.

To understand the functional behavior (agonists/antagonists/inverse agonists) of compounds **6** and **11**, these compounds were tested using a GPCR Tango assay (G-protein independent β-arrestin translocation assay) against the CB2 receptor. The CB2 functional activity of **6** and **11** from GPCR Tango assay revealed that these compounds act as CB2 agonists with an EC_{50} of 1.83 ± 0.16 (S.E.M.) and 1.14 ± 0.52 (S.E.M.) μM, respectively (Fig. 4).

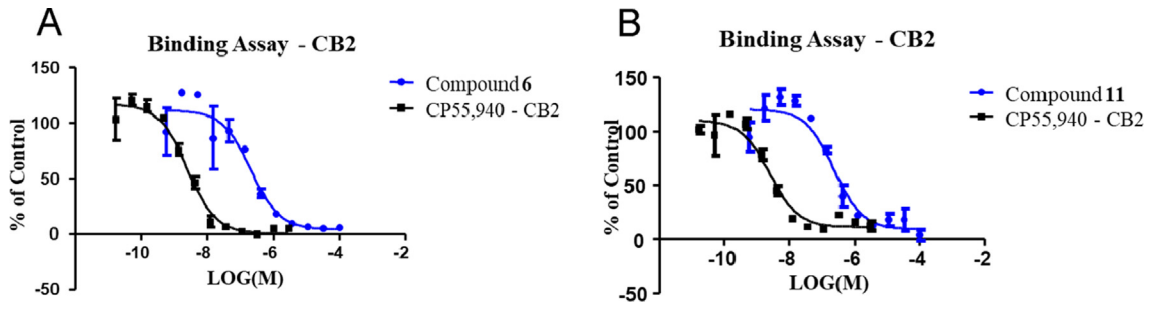


Fig. 3 The binding displacement curves obtained when **(A) 6** and **(B) 11** were rescreened in the cannabinoid receptor 2 radioligand binding assay. CP55,940 was used as a positive control.

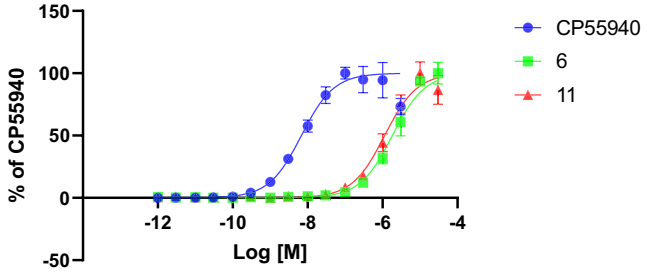


Fig. 4 Concentration-response curves for compounds **6** and **11** from the GPCR Tango agonist assay for CB2 receptor.

The experimental X-ray crystal structure of the CB2 receptor was used in order to evaluate the ability of the synthesized compounds to bind well. To understand the putative binding modes and interactions profiles of compounds **6** and **11**, molecular docking and binding free energy calculations were performed using the active-state X-ray crystal structure (PDB ID: 6KPC) of the CB2 receptor. As per our docking study, the docked poses of **6** and **11** were found to be very similar in the active-state CB2 model. The prime MM-GBSA binding free energy results are presented in Table 2. The binding poses of **6** and **11** (Fig. 5A and 5B, and Fig. 6) showed π–π stacking (of the *m*-fluoro/*p*-fluoro benzene moiety) with Phe87, Phe91 and Phe183. The fluorobenzene also showed strong hydrophobic interactions with Phe94, Val113, Phe117, Phe183, Trp194, Met265, Phe281, Cys288, and Ser285. The *tert*-butyl moiety attached at the C5 position of **6** and **11** formed strong hydrophobic interactions (CH···C, C···C, and CH–π) with key residues, Phe91, Phe94, Phe106, Val113, and Ile110, while the *tert*-butyl moiety attached at the C4 position exhibited strong hydrophobic interactions with Tyr25, Ile27, Phe94, His95, Leu182, Lys278, Phe281, and Ala282 of the CB2 receptor. Furthermore, we compared the docked pose of **6** and **11** with that of the co-crystallized ligand of the CB2 receptor (8DO) into the active site of the CB2 receptor (Fig. 6B) and found that they overlaid in a similar fashion and exhibited identical π–π stacking interactions with Phe87 and Phe183. However, **6** and **11** did not form H-bonding with Ser285, a key residue for CB2 activity which did H-bond with8DO, the CB2 co-crystalized ligand (Fig. 5C), possibly the reason for their lower potency as CB2 agonists.

4. Conclusion

As part of the continuous search for new chemotypes as CB2 selective agents, a series of 1,2,3-triazole derivatives that have not previously been evaluated for their radioligand displacement affinity on cannabinoid receptors were synthesized and screened against cannabinoid receptors. The results of the screening revealed that compounds **6** and **11** produced a concentration-dependent inhibition of specific [^{3}H]-CP55,940 CB2 binding with K_i values of 105 nM and 116 nM, respectively. The CB2 functional activity of **6** and **11**, as measured by a GPCR Tango assay revealed that these compounds act as CB2 agonists with an EC_{50} of 1.83 ± 0.16 and 1.14 ± 0.52 μM, respectively. Replacing the *tert*-butyl esters on R1 and R2 with methyl groups led to the loss of biological activity. Changing the fluoro group on the benzene ring to pentamethyl, with both R1 and R2 retained, as in **6** and **11**, also resulted in the loss of the activity. In addition, the binding mode of the compounds in the active state of the CB2 receptor was investigated through molecular modeling. The data revealed that the poses of **6** and **11** were very similar in active state X-ray crystal structures and fitted well into the active site of the CB2 receptor. In summary, the triazole derivatives **6** and **11** can be considered as lead molecules for further development of CB2 receptor agonists possessing excellent efficacy. They showed selectivity to CB2 receptors over CB1 receptors, as well as over opioid receptors.

Table 2 GlideScores and Prime MM-GBSA free energies of compounds **6** and **11** interacting with the CB2 receptor.

Compound	CB2-R K_i ± S.E.M (nM)	GlideScores (kcal/mol)	Prime MM-GBSA Free Energy (kcal/mol)
		Active-state CB2 (6KPC)	Active-state CB2 (6KPC)
6	105 ± 22.6	−8.40	−60.61
11	116.4 ± 19.5	−8.55	−60.79

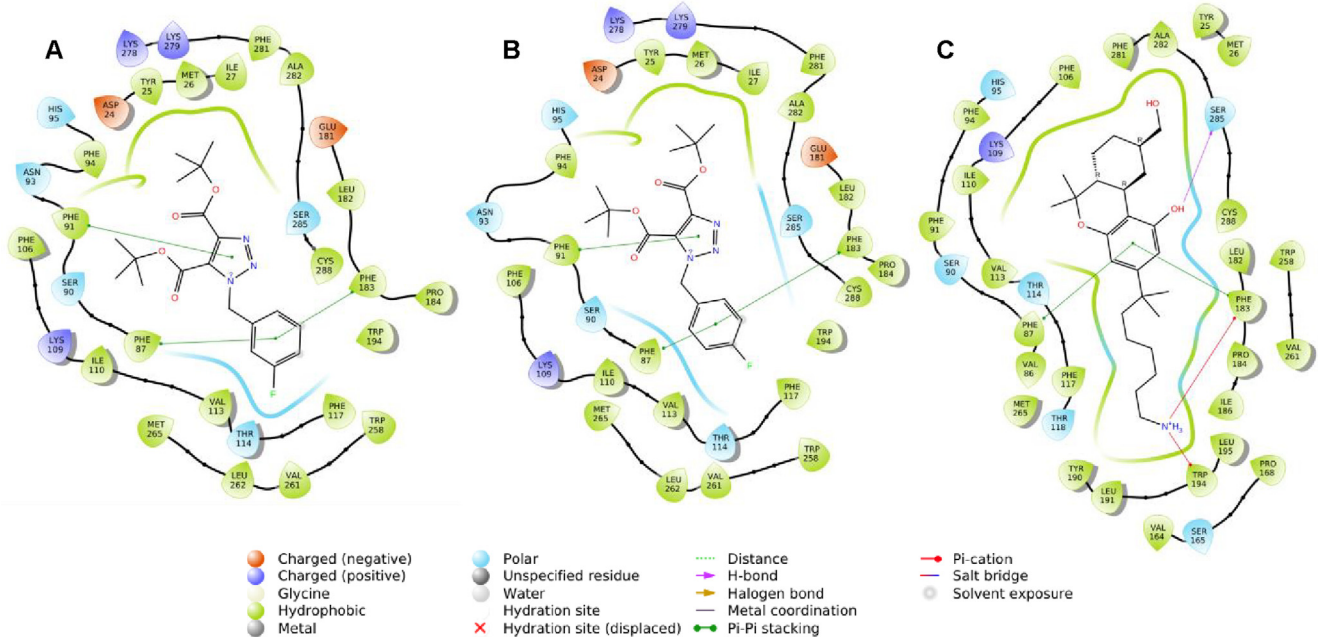


Fig. 5 2D interaction diagrams of (A) **6** and (B) **11** and (C) the cocrystallized ligand of CB2 receptor (8DO) with the active-state CB2 crystal structure.

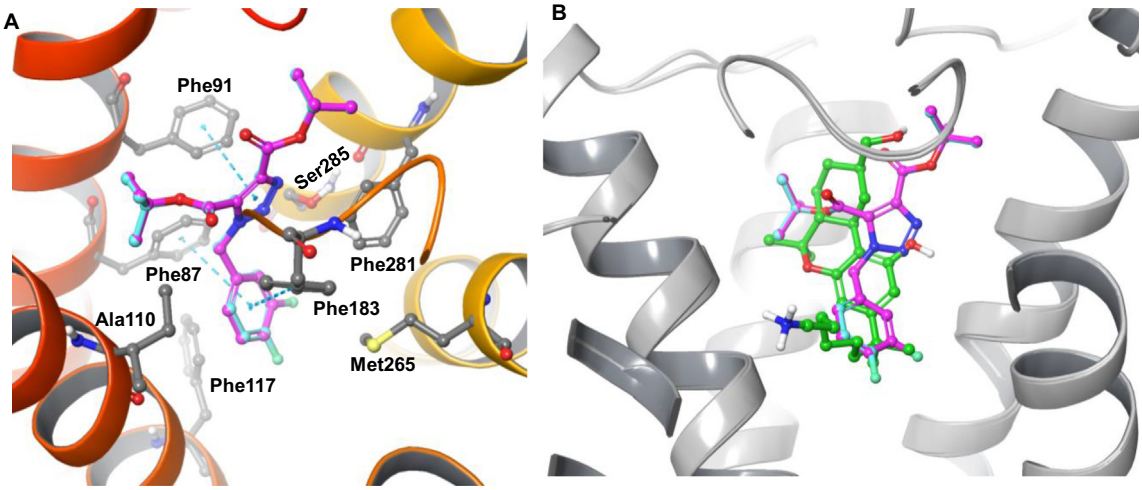


Fig. 6 Overlay representation of (A) compounds **6** (carbon in magenta) and **11** (carbon in cyan) and (B) co-crystalized ligand of CB2 receptor 8DO (carbon in green), and compounds **6** (carbon in magenta) and **11** (carbon in cyan) within the binding site of the CB2 active-state X-ray crystal structure. The key amino acid residues are shown in gray. The nonpolar hydrogens are not shown, for clarity.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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