REVIEW OF RECENT DEVELOPMENTS OF CB2 SELECTIVE AGONISTS AND LEAD OPTIMIZATION AND MOLECULAR DOCKING STUDIES OF GPR55 SELECTIVE AGONISTS

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Abstract

This study consists of two Parts; the literature section of the thesis is focused on the review of recent development of selective agonists for the cannabinoid CB2 receptor. The experimental part is focused on the optimization and molecular docking studies of GPR55 lead compound. CB2 selective agonists have been revealed to have improved therapeutic effects without CNS side effects at doses within the therapeutic window. They have been implicated to be good targets in novel drug discovery for osteoarthritic pain, neuropathic pain, post-surgical pain, and cancer just to mention a few. In this first part of the study, review of the latest advances made in the development of CB2 cannabinoid selective agonists and chemical scaffolds is made for scientific materials that appeared from 2010 to early 2013. It was observed that a lot of work has been done in recent years on CB2 with notable improvement in ligands with high selectivity and affinity with the receptor.

The second part of the study was about the optimization of a GPR55 lead compound for improved affinity, activity, selectivity and potency. Researches have revealed the therapeutic potential of GPR55 as a novel target for the treatment of inflammation, pain, and cancer. It is also important in bone physiology and remodeling. Recent evidence suggests a possible therapeutic use of GPR55 ligands for a novel drug discovery. In the synthetic part, varying primary amines were reacted with either 4-nitrobenzenesulfonyl chloride or 4-nitrophenyl chloroformate to displace the chlorine group. The nitro moiety was converted to an amine group and the resulting compound is then coupled to 1,1-biphenyl-4-carbonyl isothiocyanate. Yields were relatively high in the first steps of the synthesis ranging from 38% to 100%. The coupling step was relatively slow with yields ranging from 5% to 100%. A molecular docking study was done on the produced ligands on GPR55 homology model using Molecular Operating Environment suite (MOE) and Schrödinger suite of software. The studied ligands had high degree of interactions with pocket residues, the GPR55 ligands are hope to have improved affinity and selectivity for the GPR55 receptor. An in vitro study on the produced ligands in CB1 and CB2 models showed no recordable activity in these media. Any recordable activity in the in vitro testing of the ligands in GPR55 model will go further to prove the selective nature of our produced compounds.
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Dedication
I dedicate this piece of material to the God Almighty for his protection, to my mum Alice Arthur, my wife Ivy In Kum Eyiah and to all my siblings, loved ones and enemies.
**Abbreviations**

2-AG: 2-Arachidonoylglycerol  
2-AGE: 2-Arachidonoylglyceryl ether  
ABHD12: α/β-Hydrolase domain-containing protein 12  
ABHD6: α/β-Hydrolase domain-containing protein 6 (2-arachidonoylglycerol hydrolase)  
Abn-CBD: Abnormal-cannabidiol  
ACEA: Arachidonoyl-2’-chloroethylamide  
ACPA: Arachidonoylcyclopropylamide  
AEA: Arachidonoylethanolamide  
AM251: trans-4-[3-Methyl-6-(1-methylethenyl)-2-cyclohexen-1-yl]-5-pentyl-1,3-benzenediol  
AM281: 1-(2,4-Dichlorophenyl)-5-(4-iodophenyl)-4-methyl-N-4-morpholinyl-1H-pyrazole-3-carboxamide  
cAMP: Cyclic adenosine monophosphate  
CB1: Cannabinoid receptor 1  
CB2: Cannabinoid receptor 2  
CBD: Cannabidiol  
CBR: Cannabinoid receptor  
CCs: Classical cannabinoids  
CHO cells: Chinese hamster ovary cells  
CYP2D2: Cytochrome P450 subtype 2D2  
DAGL: Diacylglycerol lipase  
DCM: Dichloromethane  
DIPEA: N,N-Diisopropylethylamine,  
DMH-CBD: Dimethylheptyl-cannabidiol  
FAAH: Fatty acid amide hydrolase  
GPR55: G protein-coupled receptor 55  
GTPγS: Guanosine 5’-O-[gamma-thio]triphosphate  
HU-210: (6aR)-Trans-3-(1,1-dimethylheptyl)-6a,7,10,10a-tetrahydro-1-hydroxy-6,6-dimethyl-6H-dibenzo[b,d]pyran-9-methanol  
LPI: Lyosphosphatidylinositol  
Lyso-PLC: Lyso-platelet Activating Factor-Phospholipase C  
MAFP: Methyl arachidonyl fluorophosphonate  
MAGL: Monoacylglycerol lipase  
AM 251: N-(piperidin-1-yl)-5-(4-iodophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazole-3-carboxamide  
NADA: N-arachidonoyl dopamine  
NAPE-PLD: N-acyl phosphatidylethanolamine phospholipase D  
NAPE-PLD: N-acyl phosphatidylethanolamine-selective phospholipase D.  
NCCs: Non classical cannabinoids  
NPT: Constant temperature $T$, constant pressure $P$ and constant Number of Particles  
O-1602: trans-4-[3-Methyl-6-(1-methylethenyl)-2-cyclohexen-1-yl]-5-methyl-1,3-benzenediol
OAEA:  O-Arachidonoylethanolamine
OLDA:  N-Oleoyl dopamine
PAA:  Palmitoylthanolamide-preferring acid amidase
PEA:  Palmitoylthanolamine
PIA:  Palmitoylisopropylamide
PI-PLC:  Phosphoinositide phospholipase C
PLA2:  Phospholipases A2
PLC:  Phospholipases C
PMSF:  Phenylmethylsulphonyl
PPAR:  Peroxisome Proliferator Activated Receptors
PSB-SB-1204:  7-(1-Butylcyclopentyl)-5-hydroxy-3-(2-hydroxybenzyl)-2H-chromen-2-one
SAR:  Structure-activity relationship
THC:  Tetrahydrocannabinol
WIN55212-2:  (R)-(+)-[2,3-Dihydro-5-methyl-3-(4-morpholinylmethyl)pyrrolo-[1,2,3-de]-1,4-benzoazin-6-yl]-1-naphthalenylmethanone
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I Literature Section

Recent Development of Selective Agonists for the Cannabinoid CB2 Receptor

1. Introduction

Research in early 1990s discovered the existence of endogeneous cannabinoid system (ECS) in humans (Murineddu et al., 2012). Phytocannabinoids from cannabis, endocannabinoids from the body and chemically produced synthetic analogues form the major classes of ligands to the cannabinoid receptors (Pacher et al., 2006, Petwee 2010). The ECS discovery boosted scientific research into other areas and undiscovered therapeutic potential of the cannabinoid system (Pacher 2006). ECS has been implicated to play critical role in several pathophysiological states (Rempehl et al., 2012). Ligands that target cannabinoid receptors have been efficient in preclinical and clinical models for the treatment of different forms of pain (Hsieh et al., 2011), as well as neuroprotection for the management of Alzheimer’s and Parkinson’s diseases (Rempehl et al., 2012). ECS has also been therapeutically efficient in preclinical studies for the management of Huntington’s disease, stroke and atherosclerosis, glaucoma, osteoporosis and spinal cord injury (van der Stelt et al., 2011; Pertwee, 2010). Recent studies have also revealed that small molecule cannabinoid agonists exert anti-cancer effects on tumor cells (Van Dross et al., 2012). Up-regulation of CB mediated response has been claimed to promote survival of oligodendrocytes which support and insulates axon cells in the management of multiple sclerosis. CB mediated signals have been claimed to enhance uterus development, promotes sleep and appetite (Murillo-Rodríguez et al. 1998; Kirkham and Tucci, 2006).

The cannabinoid receptor type 1, abbreviated as CB1, is found primarily in the brain and a minor population in the peripheral tissues (Franklin and Casrasco, 2013). In the CNS; they act as potent synaptic modulators (De May and Ali, 2013). Structurally, CBRs have their binding sites located in their transmembrane domain. CB2 receptors are found primarily on the cells of the immune system and peripheral tissues and minor population in the CNS, (You et al., 2011; Pertwee 2010; Odan et al., 2012). CB1 and CB2 receptors share 68% protein homology at the transmembrane level (Pacher et al., 2006). They have been implicated in a number of pain, physiological and neuropsychiatric disorders (Franklin et al., 2012).

Endocannabinoid system (ECS) comprises of 7 transmembrane rhodopsin-like G-protein coupled CB1 and CB2 cannabinoid receptors (Onaivi et al., 2012; Hollinshead et al., 2012; Teng et al., 2011), ligands and enzymes. Ligands that interact with these receptors are classified as cannabinoids; synthetic and endocannabinoids. The synthetic analogues can be classified into classical cannabinoids, nonclassical cannabinoids and aminoalkylindoles (Pertwee 2010). N-arachidonoylethanolamine and 2-arachidonyl-glycerol (2-AG) form the endogeneous ligands to the endcannabinoid system 1-2 (Fig. 1), (Murineddu et al., 2012). The endocannabinoids are synthesized by NAPE-PLD, PLA2, PLC, DAGL, PI-PLC and
Lyso-PLC enzymes (Onaivi et al., 2012), they are metabolized and cleared from the body by monoacylglycerol lipase (MAGL), ABHD6, ABHD12 and FAAH enzymes (Onaivi et al., 2012; Murineddu et al., 2012). Other less characterized cannabinoid receptors are GPR55, PPARs and endothelial anandamide receptor (Onaivi et al., 2012; You et al., 2012).

Figure 1. Endocannabinoids

All cannabinoid receptors are G protein-coupled receptors (GPCRs) and share a similar structural topology, composed of seven transmembrane (TM) helices packed into a 7-TM helical bundle, with three intracellular and three extracellular loops. But at the molecular level, there exists a lower level of inter specie sequence homology between human and mouse for CB2. Human (hCB2; 360 residues) shares 82% identity of the entire CB2 receptor protein sequence with mouse mCB2 protein (mCB2; 347 residues) (Onaivi et al., 2012). Humans also shares 81% identity of the overall protein residue with rat rCB2 protein. There is CB2 ligand affinity variations between species in preclinical studies and it might be partially due to the inter specie amino acid sequence variations as discussed above.

CB1 selective modulators have been effective as analgesic agents in preclinical and clinical studies; Dronabinol is an analogue of Δ⁹-THC and has been used as drug for many purposes. Dronabinol is a pure isomer of THC, (–)-trans-Δ⁹-tetrahydrocannabinol, and it is sold as Marinol (a registered trademark of Solvay Pharmaceuticals). It is used clinically as drugs for the management of anorexia associated with AIDS and cancer patients and as analgesic agents (Pourkhalili et al., 2012; Rempel et al., 2012). Nabiximols (trade name Sativex) is a patented cannabinoid spray developed by GW Pharmaceuticals for multiple sclerosis (MS) patients. Principal components of Sativex are: tetrahydrocannabinol (THC) and cannabidiol (CBD). However, CB1 selective ligands/drugs are associated with undesirable CNS side effects like dizziness, dry mouth, tiredness, increase rate of suicide and depression (Hollinshead et al., 2012; Rempel et al., 2012). These may lead to withdrawal of the drug from the market since the side effects might be a bit intense that patients tend to abandon the drug and discontinue its usage. Anti-pain drugs like morphine and acetaminophen (poison) have their own serious side effects like seizures, difficulty in breathing and liver poisoning.
The search for cannabinoid based analgesic drugs that are free from these psychoactive side defects continues.

Recent preclinical and clinical studies have revealed CB2 selective agonists to have anti-inflammatory and analgesic properties (Watson et al., 2011; You et al., 2011; Manley et al., 2011; Rempel et al., 2012). Research suggests that, ligands optimized for CB2 receptor will overcome the undesirable CB1 linked CNS side effects (Murineddu et al., 2012; Franklin and Carrasco, 2012). The aforementioned claim is partly due to the peripheral distribution of these CB2 receptors (Odan et al., 2012; Onaivi et al., 2012). It could also partly be due to specific residues at the binding pocket of CB2, making it possible to increase selectivity through hydrogen bonding and by altering logP. It is true that the CB1 and CB2 receptors share high level of active site homology (about 68%) but there has been notable advancement in the development of high selective and potent CB1 and CB2 ligands in the last decade.

GPR55; the orphan receptor was originally identified as a putative third cannabinoid receptor (CB3) that couples to Gα13 subunit (Moriconi et al., 2010). The putative cannabinoid receptor (GPR55) was first identified and cloned in 1999 by Sawzdargo et al; (Sawzdargo et al., 1999; Elbegdorj et al., 2013). It was later claimed to be a cannabinoid receptor (Brown et al., 2007). GPR55 is one of the less characterized cannabinoid receptors (Onaivi et al., 2012; Moriconi et al., 2010). The putative cannabinoid receptor GPR55 affects osteoclast function in \textit{in vitro} and bone mass in \textit{in vivo} studies, (Ross, 2011; Sharir and Abood, 2010).

**Objectives of the thesis**

The analgesic and other therapeutic potential of cannabinoid based ligands is immense. CB2 selective agonists have been revealed to have improved therapeutic effects without CNS side effects. As the quest for new and safe drugs still continues, CB2 receptor has thus become a novel analgesic drug target (You et al., 2012) for this new line of development. The objective of this literature review is to review the recent development in CB2 selective agonists. Also to summarize the recently disclosed, novel chemical scaffolds as CB2-selective agonists in scientific journals that appeared from 2010 and early 2013.

**2. Therapeutic potential of CB2 agonists**

The biological activity and the degree of distribution of CB2 receptors makes it promising therapeutic target for treating neuropathic pain (Hsieh et al., 2011; Cichero, et al 2011), inflammation (You et al., 2011, Curto-Reyes et al., 2010), and immune disorders, glaucoma, GI disorders, and cardiovascular diseases (Mercier et al., 2010). Others include osteoarthritis-pain control (Hollinshead et al., 2012), rheumatoid arthritis, hepatic disease, neurodegenerative diseases, migraine (You et al., 2011), MS and liver fibrosis, bronchodilation, chronic pain just to mention a few. The past decades and recent discoveries have made a lot of innovations into CB2 research from structure elucidation to high selective
and potent CB2 ligands. Pharmos pharmaceuticals initiated a phase 2 clinical trials on a CB2 selective drug candidate ‘cannabinor’ for the treatment of neuropathic visceral, inflammatory and post-surgical pain in Jan. 2007, (Pharmos 2005; Pharmos 2007). But based on the available data at the time of review, the scientific world is yet to witness a CB2 selective drug for the treatment of pain, Alzheimer’s and Parkinson’s diseases among others. CB2 selective drugs will help alleviate CNS side effects like dizziness, dry mouth, tiredness, increased rate of suicide and depression which are usually associated with CB1 selective ligands, Worm et al., 2009.

3. Early CB2 receptor ligands: Classical (CCs), non-classical (NCCs), and indole based cannabinoids.

Cannabinoids with Δ9-THC-like tricyclic structures form the CCs (classical cannabinoid) 3-10 (Fig. 2) class (Hollinshead et al., 2012) represented by Δ9-THC, nabilone, ajulemic acid, dexanabinol and HU-210 (Thakur, 2009, Pertwee 2010; van der Stelt, 2011). Research suggests that phenolic-OH group and C-3 side chain are important pharmacophores for CB-receptor selectivity, efficacy and the molecules’ affinity for ligand-receptor interaction. Cannabinoid ligands of Classical (C) and non-classical (NC) cannabinoids 11-14 (Fig. 3), usually exhibits stereoselectivity in pharmacological assays; (-)-trans and (+)-cis enantiomer forms. The (-)-trans enantiomer component usually forms the more active stereoisomer (Pertwee, 2010).

Figure 2. Classical cannabinoids (ref: Thakur et al., 2009; Pacher et al., 2006)
Previous studies as reviewed by Thakur et al. proposed that ‘OH’ substitution at C9 or C11 as in (HU-210) of the CCs increases CB2 receptor selectivity and affinity (Fig. 2). Another pharmacophore for improved CB2 receptor selectivity and affinity is the ring junction stereochemistry of CCs (Thakur et al., 2009). Phenolic ‘OH’ and side chain group in CCs are critical pharmacophores for CB1 receptor selectivity. Masking of the phenolic OH as an alkyl ether or removing the OH entirely is shown to decrease CB1 receptor affinity and increase CB2 selectivity.

THC and other classical cannabinoids with CB1 selectivity are known for their analgesic property with associated CNS side effects. Maximization of their positive properties and minimization of the side effects may lead to development of novel therapeutic agents. THC derivatives were used by Huffman at Clemson University to develop a class of CB2 selective agonists from \( \Delta^8 \)-tetrahydrocannabinol series, (Huffman et al., 2010). The biological activity of the compounds were tested with \textit{in vitro} \( ^{34}\text{S}\)GTP\(\gamma\text{S} \) binding assay using CB2-CHO cell membrane culture.

JWH-015 (16) and JWH-267 (17) (Fig. 4.) are indole based compounds and have reduced CB1 receptor affinity and increased CB2 receptor selectivity to over 200 folds (Thakur et al., 2009). Indole based compounds represented by 16 -19 (Fig. 4) are therapeutically useful in controlling pain, autoimmune diseases, inflammation and other diseases mediated by CB2 receptors. The represented indoles are also known to be selective for CB2 receptor, (Blaazer et al., 2011).
4. Recent findings on CB2 agonists

The promising therapeutic potential of CB2 agonists and that they are devoid of the unwanted psychotropic side effects, has raised interest from several research groups. CB2 mediated transductions have been implicated in nociceptive pain, inflammation and nerve injury management (You et al., 2011, Curto-Reyes, 2010). It has been reported that, some level of CB1 affinity is required by CB2 selective compounds to be analgesically active (Watson et al., 2011; Trotter et al., 2011; Manley, 2011). Watson noted that highly selective CB2 agonists were analgesically less active because the signs for pain were observable in the animals that were given the highly selective CB2 ligand as compared to the test animals that were given less selective CB2 ligand. Recent publications suggest that CB2 agonist can be used as pain tranquilizers and they act through modulation of peripheral neurons and microglia cells (van der Stelt et al., 2011; Beltramo et al., 2006). This literature review provides a summary of scientific publications covering CB2 selective agonists, which have been published from 2010 to date.

4.1. Arylpiperazine-containing purine derivatives

Arylpiperazine-containing purine derivatives were researched by Hollinshead and his coworkers from Lilly Research Laboratories. They discovered and optimized CB2 selective compounds with improved pharmacokinetic properties that can be of use in the management of osteoarthritis (Hollinshead et al., 2012). Preclinical studies with compound 21 (Fig. 5) on [S\(^{35}\)]-GTP\(^{35}\)S CB1 functional assay showed improved CB2 and selectivity over CB1 (Hollinshead et al., 2012). The identified lead structure 22 (Fig. 5) was metabolically unstable with a short half-life and inhibited CYP2D2 enzyme. But on the other hand, it had high
affinity for hCB2 and rCB2 in the *in vitro* [3H]-CP-55,940 based CHO cell binding assay. In addition, 22 was analgesically active when tested in a rodent monoiodoacetic acid (MIA) model of osteoarthritic (OA) knee joint pain (Hollinshead et al., 2012). The improved series did not show any measurable hypothermia in the biological testing which is an indicative of their CB2 selectivity. R-stereoisomer form of the represented compound 20 (Fig. 5) was found to be more potent than the S-isomer 21. There were also improvements in the half-life of the optimized compounds.

Figure 5. Purine based CB2 selective agonists

4.2. 1-(4-(Pyridin-2-yl)benzyl)imidazolidine-2,4-dione derivatives

Literature reveals imidazole based compounds to have numerous pharmacological properties like analgesic, anti-neoplastic and anti-inflammatory activity among others which are key therapeutic effects for CB2 agonists (Bhatnagar et al., 2011; Ashnagar and Bruce, 2011). 1-(4-(pyridinyl)benzyl-2)-imidazolidine-2,4-dione derivatives with improved activity and selectivity for CB2 receptor were discovered by group of researchers at Merck Research Laboratories. The identified hit compound 23 (Fig. 6) prior to optimization was found to be CB2 selective but with poor pharmacokinetic properties; low oral bioavailability (rFpo = 4%) and off-target response in hERG affinity testing. The biological activity was tested with the inhibition of forskolin-induced cAMP functional assay. The novel CB2 selective lead compound with potent analgesic property and improved pharmacokinetic properties was discovered to be 24 (Fig. 6) (van der Stelt et al., 2011). In vivo study with the lead compound 24 showed potent anti-pain property in rat spinal nerve ligation model, (van der Stelt et al., 2011). They emphasized that these agonists have high therapeutic potential for the control of pain and spasticity. In a related development, RaQualia Pharma developed benzoimidazolylsulfone derivatives as agonists selective for CB2. Their findings were proved with receptor binding assay (Kazuo and Yasuhiro 2010).
4.3. 3-Carbamoyl-2-pyridone derivatives

The research group at Shionogi Pharmaceutical Research Center developed CB2 selective agonist from 3-carbamoyl-2-pyridone derivatives through structure activity relationship (SAR) approach. Activity was influenced by altering the substituted groups at positions 1, 5 and 6. The optimized CB2 selective agonist 25 (Fig. 7), (S-777469) had reduced CNS side effect and a promising antipruritic agent, (Odan et al., 2012). The group reported in another publication about the discovery of a related compound 26 (Fig. 7), (S-444823) in a related study. The compound did not cross the blood brain barrier and was without any CNS side effect like dizziness, dry mouth, tiredness/fatigue, muscle pain and palpitations. The lead proved to be active and potent antipruritic agent. The verification of the binding affinities was determined in the cAMP binding assay.

![Figure 7. Carbamoyl-2-pyridone derivatives](image)

4.4. 7-Alkyl-3-benzylcoumarins

Coumarin based drugs have legendary report as an anti-asthma, anti-tumor, anti-inflammatory, anti-osteoporosis and analgesic agents (Farinola et al., 2005). This led Rempel and his coworkers at Bonn PharmaCenter to make a study on coumarin based compounds to determine their selectivity for the CB2 receptor. They developed CB2 selective agonists represented by 27 (Fig. 8); (PSB-SB-1204). The activity of the lead series was determined in
radioligand and cAMP binding assays for CB1 and CB2 receptor affinity. The optimized lead from 7-alkyl-3-benzylcoumarins was discovered to have improved potency and selectivity for CB2 receptor. Coumarin based scaffolds have desirable therapeutic properties that can be optimized to improve selectivity and affinity for cannabinoid receptors for diverse therapeutic effect (Rempel et al., 2012).

Figure 8. Benzylcoumarin based derivative

4.5. Desulfated haplosamate based derivative

Chianese and coworkers at University of Napoli discovered haplosamate based derivative as a CB2 selective agonist. Haplosamate steroid has long history of isolation from marine sponge, Dasychalina sp; desulfated derivative of the compound 28 (Fig. 9) was found to be CB2 selective agonist, hCB2 (EC$_{50}$ = 2.82 μM). It was reported that the selectivity for CB2 is associated to the desulfation of the natural haplosamate compound. Undesulfated haplosamate was reported by Chianese et al. and also by Andersen et al. to be CB1 selective, (Chianese et al., 2011). The cannabimimetic activity of this class of steroids (haplosamate A); 29 (Fig. 9) was found to be linked to the steroid ring: removal of the ring lead to complete loss of cannabimimetic activity.

Figure 9. Haplasamate based CB2 selective agonists
4.6. Diazepane based compounds

Diazepane based compounds are known to have therapeutic activity in managing some conditions like anxiety, insomnia, seizures, *status epilepticus* and muscle spasms. This scaffold was used by Riether and their coworkers and also by Renée Zindell in a related study at Boehringer Ingelheim Pharmaceuticals to the discovery of selective agonists for cannabinoid receptor 2. The compounds were analgesically active, good immune modulators for skin inflammation as well as anti-pruritic in mouse model. The lead series 30 (Fig. 10) was active but metabolically unstable; optimization was done to amplify the druglike properties and minimize the unwanted properties. The optimized 1,4-diazepane compounds 31 and 32 (Fig. 10) have high CB2 selectivity, potency and were orally bioavailable, (Riether et al., 2011). Aliphatic and aromatic amide groups were potent and selective but less orally available (CB2 EC<sub>50</sub> =111 nM), efficacy (104 %) and T<sub>1/2</sub> of 11 min. The representative compound 32 also showed activity CB1 EC<sub>50</sub> (> 20000 nM).

Similar findings were discovered by Renée Zindell et al. 2011 in a related studies on aryl 1, 4-diazepane compounds for CB2 selectivity with improved pharmacokinetic properties. The lead compound 33 (Fig. 10) from HTS cAMP had EC<sub>50</sub> value of 154 nM and a 130–fold CB2/CB1 selectivity but were metabolically unstable. Compound 34 and 35 (Fig. 10) have low log P values, improved stability and selectivity for CB2 receptor. cAMP and functional cellular assay was used for receptor affinity determination.

![SAR: Parent Hit compound](image1)

![30](image2)  
\[EC_{50} (CB2) = 136 nM, Efficacy = 67\%\]  
\[T_{1/2} < 7 min\]

![31](image3)  
\[EC_{50} (CB2) = 67 nM \]  
\[Efficacy = 104 \%\]  
\[T_{1/2} > 120 min\]

![32](image4)  
\[EC_{50} (CB2) = 111 nM \]  
\[Efficacy = 104 \%\]  
\[EC_{50} (CB1) > 20000 \]  
\[cLogP = 1.4\]  
\[T_{1/2} > 120 min\]

![33](image5)  
\[EC_{50} = 154 nM\]

![34](image6)  
\[hCB2 EC_{50} = 1 nM\]  
\[hCB1 EC_{50} = 1770 nM\]  
\[cLogP = 2.23\]

![35](image7)  
\[hCB2 EC_{50} = 10 nM\]  
\[hCB1 EC_{50} > 10000 nM\]  
\[cLogP = 1.9\]

**Figure 10.** Diazepane based CB2 selective agonists
4.7. Decahydroquinoline amides
A research carried out by J. Manley and coworkers at Merck Research Laboratories led to the publication of the decahydroquinoline amide based compounds as CB2 selective agonists. A comparison of highly selective compound 36 (Fig. 11) to moderately selective CB2 agonist 35 (Fig. 11) revealed that, some level of CB1 agonists’ activity is required by CB2 selective agonists to exhibit analgesic property. Highly selective CB2 agonists did not have analgesic property (Trotter et al. 2011; Gifford et al 1999). It was revealed that, CB2 agonist’s potency also depends on stereochemistry of the decahydroquinoline Ring (Manley et al., 2011). The selective amides proved to be potent analgesic agents in rat model of acute inflammatory pain.

![Figure 11. Decahydroquinoline derivatives as CB2 selective agonists](image)

4.8. Imidazopyridine series
It is imperative to know that CB1 selective agonists are associated with CNS side effects hence researchers are now redirecting their research into CB2 selective ligands. On the contrary, Wesley Trotter and coworkers at Merck Research Laboratories came out with an optimized imidazopyridine series as CB2 selective agonists. They optimized the imidazopyridine series for affinity, efficacy and high selectivity of CB2 over CB1 using SAR approach. They discovered that the analgesic property was confined in moderately selective CB2 agonist 37 (Fig. 12) than highly selective CB2 agonist 38 (Fig. 12), (Trotter et al., 2011, Fig. 12). This finding call for further studies to investigate the level of CB2 selectivity required to be analgesically active and also to avoid CNS side effects.
4.9. Benzimidazole derivatives

Benzimidazole scaffold which has been associated with biological activities like anti-inflammatory, antidepressant, antihypertensive and analgesic properties (Kedar et al., 2010) was used by some group of researchers at Pfizer Global Research and Development in their quest to search for CB2 selective agonists. They brought into the scientific domain a series of benzimidazole based CB2 selective agonists. The augmentation of the pharmacokinetic and binding properties of the hit compounds led to the discovery of nanomolar affinity ($EC_{50} = 2.7\text{nM}$) CB2 selective compounds. The selectivity was reported to be over 3000 folds CB2/CB1 selectivity. Compounds 39, 40 and 41 (Fig. 12) were found to be CNS penetrants with improved pharmacokinetic properties but no data was reported for their analgesic properties (Watson et al., 2011).

4.10. 2,4,6-Trisubstituted 1,3,5-triazine derivatives

Triazine, a known scaffold in the medical domain was used by Yrjölä and co-researchers in the Cannabinoid Research Group at the University of Eastern Finland to design CB2 selective agonists. The 2,4,6-trisubstituted 1,3,5-triazines were potent, selective and efficient for CB2 receptor. The compound 42 (Fig. 13) ($-\log EC_{50} = 7.5$, $E_{\text{max}} = 255\%$) was selected for further development and compound 43 (Fig. 13) (1-adamantyl-analogue) was the most potent CB2 agonist ($-\log EC_{50} = 8.5$ $E_{\text{max}} = 241\%$). Compound 44 (Fig. 13) was one of the active compounds in the series. The agonists proved active and selective for CB2 receptor. Testing of the ligands’ activity was done with non-transfected CHO cells and CB2 selectivity studies were determined in rat cerebellar membranes, (Yrjölä et al., 2013).
4.11. 2-Azetidine carboxamides and pyridine based derivatives

Derivatives of plant based, non-protein amino acid ‘Azetidine-2-carboxylic acid’ were used by Hickey and coworkers for the discovery patenting of 2-azetidinecarboxamide compounds as CB2 selective agonists. The compounds were potent in the treatment of inflammation and pain. It has been exemplified as below 45 (Fig. 14) (Hickey et al., 2010). The invented compounds were treated in human CHO cells expressing human CB2R on a cAMP forskolin bioassay activity model for binding activity. Bartolozzi et al also discovered pyridine based compounds as CB2 agonists. Theses anti-inflammatory and anti-pain ligands had high affinity in a nanomolar range under the cAMP synthesis model. It is exemplified by compound 46 (Fig. 14) (Bartolozzi et al., 2010).

4.12. 4-Oxo-1,4-dihydropyridines as selective CB2 agonist

Research by El Bakali et al. led to the identification of 4-oxo-1,4-dihydropyridines as a novel CB2 selective agonists represented by 47 (Fig. 15). They realized that the functionality of the series was based on the C-6 substitution and that the affinity is altered with changes in the C-6 substituent group, (El Bakali et al., 2010). In a related study, Pasquini and coworkers made a discovery on a highly selective CB2 agonist from 4-quinolone based compounds. The adamantyl substituted derivative; compound 48 (Fig. 15) proved to be selective and effective anti-inflammatory agent in the preclinical test using formalin model (Pasquini et al., 2010).
4.13. Substituted 1-alkylcinnolin-4(1H)-one derivatives
Sanofi Aventis patented 1-alkylcinnolin-4(1H)-one derivatives as CB2 selective agonists which has been implicated to be useful therapeutically. Compound 49 (Fig. 16) has been suggested to be useful in controlling autoimmune diseases, allergy, neurodegenerative and neoplasm. CB2 agonist activity was determined using cyclic adenosine monophosphate (cAMP) assay (Barbagallo et al., 2010).

A discovery related to Sanofi Aventis was carried out by Vernhet, Claude and colleagues leading to a discovery of CB2 selective compound. The 1-benzylcinnolin-4(1H)-one derivatives compound was found to be active. Compound 50 (Fig. 16) proved to be effective for the control of autoimmune, allergic, infection, neurodegenerative, cardiovascular and gastrointestinal diseases, neoplasm, obesity and diabetes (Vernhet et al 2010).

4.14. Substituted oxindole as CB2 agonists
Oxindoles have been credited to be antiproliferative, anticancer, anti-inflammatory agents (Rudrangi, 2011, Blaazer, et al., 2010). Patent application by Wyeth (now Pfizer) claims oxindole compounds such as 51 (Fig. 17) to be CB2 selective agonists. Compound 50 was effective anti-cancer and anti-pain agents which could be accredited to the oxindole scaffold used. Determination of the bioactivity was carried out using the forskolin cAMP binding assay (Zhang et al., 2010). The compound proved to be effective in controlling acute and chronic pain.
5. Conclusions
Large volumes of research papers have been published on CB2 ligands in recent years with high selectivity and affinity. Different scaffolds have been reported to have improved potency and analgesic properties. Notable among them are benzylimidazolidine, oxindoles, alkylcinnolins, 1,3,5-triazines, coumarin and diazepane scaffolds. *In vitro* and *in vivo* studies are also being carried out for CB2 selective compounds with the quest of developing a clinically active CB2 selective drug.

Figure 17. Substituted oxindole as CB2 agonists
II: Experimental Part
II: Experimental Part

Lead Optimization and Molecular Docking Studies of GPR55 Selective Agonists

1. Introduction

Historically, GPR55 which is a novel lysophosphatidylinositol (LPI) receptor with cannabinoid sensitivity is proposed to be a putative 3rd cannabinoid receptor. It belongs to the rhodopsin-like subfamily of the G-protein coupled receptors. It has a major population in the cells of human striatum and CNS. Human GPR55 (hGPR55) was originally identified and cloned in 1999 (Pingwei and Abood 2013; Sawzdargo et al., 1999). But research on the chemistry and pharmacology of GPR55 became a bit silent for some time until recently (Elbegdorj et al., 2013). Thereafter, articles and patents have been made concerning the structure, pharmacology and the therapeutic potential of this orphan receptor. Some publications have been made concerning the membership of GPR55 to the cannabinoid family; Ryberg et al. published that GPR55 interacts with $\Delta^9$-THC $57$ (Fig. 18), CP55940, HU210 and O-1602 which are known CB1 and CB2 ligands. In a parallel homology modeling study by Elbegdorj et al., they confirmed $\Delta^9$-THC, CP55940, HU210 and O-1602 to be ligands to this putative CB receptor (GPR55) with affinities ranging from low micromolar to as low as 1 nM molar EC$_{50}$ values.

GPR55 as well has activity with cannabidiol and abnormal cannabidiol which are non-ligands to either CB1 or CB2 (Ryberg et al., 2007; DeMorrow et al, 2010). These parallel studies and others not listed confirm the membership of GPR55 into the cannabinoid family. But current study on this receptor still create some doubt in the scientific community as to whether GPR55 qualified to be the 3rd cannabinoid receptor; this is because it shares very low sequence homology with CB1 and CB2 cannabinoid receptors. Based on the current repertoire, the confirmed state of GPR55 is that of a novel LPI receptor and a putative cannabinoid receptor (Nevalainen and Irving 2010; Elbegdorj et al., 2013).

It is true that not much GPR55 selective ligands (both agonists and antagonists) are known both endogenous or synthetic, $52$-$57$ (Fig. 18) and only handful of endogenous selective ligands have been published $61$-$62$ (Fig. 20). GPR55 is said to be the true receptor for the endogenous lipid lysophosphatidylinositol (LPI) and 2-arachidonoyl derivative of LPI (2-AGPI) in GPR55 knockout mice model (Saori et al., 2010).
Figure 18. Previously discovered classical and synthetic ligands with GPR55 activity. Compounds 51, 55, 56 and 57 were used as reference ligands in the docking studies.

Structurally, hGPR55 has its tertiary protein structure composed of 7 transmembrane α-helices as a common structure for the G-protein family. The primary structure of the receptor protein is made of 319 sequence amino acids (Sharir and Abood, 2010; Sawzdargo et al., 1999). The transmembrane part of the receptor constitutes major part of the receptor. The pocket for ligand binding opens at extra membrane loop region and runs deep into the transmembrane region (Kotsikorou., et al., 2011; Elbegdorj et al,. 2013). This explains why the binding pocket is lined with hydrophobic residues and the nature of the known ligands is lipophilic. The receptor shares only low sequence identity of 13.5% with CB1 and 14.4% with CB2. But rather, it is quite homologous to other G-protein receptors like 27% with GPR35 and 30% with GPR23 (Elbegdorj et al., 3013; Kotsikorou et al., 2011; Oka et al 2010). The mechanism of downstream signaling, the nature, and the scope of these effects of GPR55 is currently unknown. But it is believed that the environment and the cell lines involved play important role (Nevalainen and Irving, 2010). Previous studies have revealed that, for agonists affinity, residues K2.60(80) for charge interaction, H170, Q6.58(249), K2.60(K2.60A), S2.64(84), Gln 7.36 (271) and Lys 2.60 (80) are important (Table 1). The residues Y3.32(101), S6.47(238), S7.42(277), N7.43(278), D7.49(284) and V6.51(242), (Table 1) are also important for H-bonding, pi-Hydrogen and for hydrophobic interactions, (Kotsikorou et al., 2011; Elbegdorj et al., 2013). These interactions are critical for ligands’ binding, affinity and efficiency.
Therapeutically, GPR55 is a target to reckon with when it comes to target for novel drug discovery. Study by Kotsikorou et al. revealed antagonists of GPR55 to be therapeutically effective for inflammatory and neuropathic pain control (Kotsikorou et al., 2010, Ford et al., 2010). They realized that GPR55 knockout mice were resistant to pain as compared to the wild type. This shows that GPR55 can be a good target in neuropathic pain control. It has been reported that, GPR55 antagonists would be important in controlling osteoporosis since GPR55 plays important role in bone mass and bone degradation, (Elbegdorj et al., 2013; Whyte et al., 2009). This is also shared by Sharir and Abood, 2010. It has also been reported in a parallel study that there is a linkage between the proliferation of the highly metastatic breast cancer cell line (MDA-MB-231) and GPR55 up-regulation; this suggests a possible therapeutic target for breast cancer control (Ruth A. Ross, 2011; Piñeiro and Falasca, 2012). GPR55 is therefore an important therapeutic target that should not be underestimated in future drug design for pain and cancer management.

Current studies on GPR55 ligand reveals that, some of the ligands are known CB receptor ligands, Fig. 18 and 19 while only very few are GPR55 selective. Most of the previously discovered GPR55 ligands were highly lipophilic and they also do interact with CB1 receptor. The discovery of less lipophilic and selective GPR55 chemotypes in drug discovery would be a wave into novel drug development (Kotsikorou et al., 2011; Lipinski et al., 2001). CB1 and CB2 ligands like Δ⁹-THC, CP55940 55 (Fig. 19), 2-AG, anandamide, JWH-015 and others 52-58 (Fig. 18) and 62-63 (Fig. 20) also do have GPR55 activity. The discovery of the endogenous true and selective ligands of GPR55; LPI and 2AG-LPI 60-61 (Fig. 20) have also been made, (Kotsikorou et al., 2011). Other classes of ligands with GPR55 activity include cannabidiol analogues 58-59 (Fig. 19), classical and synthetic analogues and some endocannabinoids (Nevalainen and Irving, 2010, Fig. 18).

![Figure 19. Previously discovered cannabidiol analogues as GPR55 ligands](image-url)
GPR55 is expressed in the CNS with sizeable populations in the peripheral tissues; adrenal tissue, intestines, bone marrow and the immune cells (Kargl et al., 2012; Thakur et al., 2009). GPR55 selective agonists have also been published. Benzoylpiperazine (GSK494581A and GSK575594A) have been proven to be GPR55 agonists with some glycine transporter subtype 1 (GlyT1) inhibitor activity (Brown et al., 2011). The results of HTS screenings in PubChem BioAssay database offer new scaffolds for developing selective GPR55 agonists and antagonists (PubChem AIDs 1961 and 2013). Probe reports list three different scaffolds for both selective GPR55 agonists (ML184-186, PubChem AID: 1965) and antagonists (ML191-193, PubChem AID: 2026) (Jenkins et al., 2012; Elbegdorj et al., 2013). Current researches have revealed the therapeutic potential of GPR55 as a target for the treatment of inflammation, pain, and cancer. It is also important in bone physiology and remodeling. Recent evidence suggests a possible therapeutic use for GPR55 antagonists for the treatment of osteoporosis.

### 1.1 Aims of the study

The informed decision for this study is derived from the previous discoveries by this cannabinoid research group and also from the previously discovered GPR55 agonists. This study is an aspect of an ongoing project and it is an optimization step for the previously discovered lead compound 76 by our cannabinoid research group. The compounds for this study are products of SAR and are derivatives of sulfonamide and carboxamide scaffolds. This study is therefore aimed at synthesizing and evaluating new compounds which are hoped to be selective agonists for GPR55 receptor. Other objectives were to perform molecular modeling (docking) studies on the ligands, characterization of analogues by NMR, mass and elemental analysis and also to undertake biological evaluation of the compounds. It is expected that the compounds will have activity on the GPR55 receptor; also the optimized
compounds will have improve pharmacokinetic properties and higher selectivity. It is also expected to be inactive in CB1 and CB2 receptor models in the biological activity studies. The thesis is focused on the development of a library of compounds for GPR55 receptor based on the previously discovered lead compound by the cannabinoid research group.

2. Chemistry of synthetic experiments

2.1. Chemistry

Synthesis of sulfonamide derivatives

Synthesis of carbamothioyl compounds (70a-70f) were carried out in 4 distinct steps. 4-nitrobenzenesulfonyl chloride 64 was reacted with amine 65 (a-f) with catalytic amount of pyridine. It was refluxed under the stated conditions to dislodge the chlorine group to yield 66. HCl was formed in the process into the reaction solution. Aryl nitro intermediate 66 is reacted to Sn to reduce the nitro group into aromatic amine (aniline) 67. Biphenyl carbonyl chloride 68 is converted to thiocyanate intermediate 69 by reacting it with potassium thiocyanate. Coupling of the two intermediates 67 and 69 is carried out at the stated conditions to form the final product 70 (a-e), (Bissinger et al., 2011).

Scheme 1. General scheme for the synthesis of sulfonamide derivatives 70a-70e under the stated conditions and solvents.
Synthesis of carboxamide derivatives

Synthesis of 73a-73b was carried out by reacting 4-nitrobenzoyl chloride 71 with the base 72 and catalytic amount of pyridine. It was refluxed under the stated conditions to dislodge the chlorine group. HCl was formed in the process into the reaction solution. The aryl nitro intermediate 73 was reduced with Tin/HCl to amine (aniline) 74. Biphenyl carbonyl chloride 68 was converted to thiocyanate intermediate 69. Coupling of the two intermediates 74 and 69 is carried out at the stated conditions to form the final product 76, (Bissinger et al., 2011)

Scheme 2. General scheme for the synthesis of carboxamide derivatives. Synthesis of carboxamide derivatives 76a-76b were carried out under the stated conditions and chemical solvents

Synthesis of N-((4-(N-benzylsulfamoyl)phenyl)carbamoyl)-[1,1'-biphenyl]-4-carboxamide (71f)

A solution of 1,1'-biphenyl]-4-carbonyl chloride 68 in MeOH/ NH3 was stirred overnight. Volatiles were removed to result in white solid intermediate 69f. A suspension of 69f in DCM was reacted with oxalyl dichloride and refluxed. Volatiles were removed. Toluene was added to the resulting precipitate to remove the remains of the oxalyl chloride to yield 70f, (Klabunde et al., 2005). 70f was coupled to 67f to yield the final product 71f, (Bissinger et al., 2011).
2.2. Formation of Sulfonamide and amide bond

The experiment was based on the synthesis of GPR55 analogues with biphenyl moiety. Biphenyl based compounds have good interactions with proteins and they help increase ligand–receptor interactions. The reactions followed a similar pattern beginning with the formation of a sulfonamide functional group from primary amine (R-NH₂) and a sulfonyl chloride. Acyl chloride plays similar role as sulfonyl chloride in amide bond formation, (Montalbetti et al., 2005). The amine served as a nucleophile (Nucleus-loving) whiles the sulfonyl chloride served as an electrophile, March J. 1992. The lone pair of electrons is donated by the nucleophilic nitrogen and it initiates the attack on the electrophilic sulfur leading to the release of the hydrochloric acid (HCl), Scheme 4. Non-nucleophilic tertiary amine ‘‘Hünig’s base’’ like methylmorpholine, or N,N-diisopropylethylamine (DIPEA) traps the released HCl to prevent the reconversion of the sulfonyl chloride or acyl chloride to its reactant form. Pyridine served as catalyst to fasten product formation.
Conversion of nitroarenes into an aromatic primary amine was done in absolute ethanol and concentrated HCl using tin as catalyst. Tin gets oxidized in the process; from ‘0’ to ‘+2’ oxidation state initially, \( \text{Sn} + 2\text{HCl} \rightarrow \text{Sn}^{2+} + 2\text{Cl}^{-} + \text{H}_2 \). After product formation, 30% NaOH was added to the mixture to form the amphoteric compound \( \text{Sn(OH)}_4 \) which is converted to \( \text{Sn(OH)}_6^{2-} \) ion upon further NaOH additions.
2.4. Coupling of thiocy anate and aniline

Reaction between potassium thiocyanate and biphenylcarbonyl chloride 68 affords 1,1-biphenyl-4-carbonyl isothiocyanate through nucleophile-electrophile interaction leading to the release of KCl as by-product. Coupling reaction is then carried out between 1,1-biphenyl-4-carbonyl isothiocyanate intermediate 69 and aniline 67 to form a thiourea functional group in the final product, 70a-70e.
3. Molecular Modeling

3.1 Molecular Docking Studies

The docking study was done using a homology model of GPR55 receptor protein built with Modeller 9v1 on a nociception receptor template with 24.82% of sequence identity. Ligands were modeled using LigPrep module of Schrodinger suite software. Four known GPR55 ligands were selected as reference ligands. Molecular docking was carried out with Molecular Operating Environment (MOE) programme and Glide programme in Schrodinger suite of software. The best docking poses were selected on the basis of scoring as well as taking into consideration the presence of interaction with residues from the receptor binding pocket.

### Table 1. Pocket residues of GPR55 Receptor

<table>
<thead>
<tr>
<th>Residue</th>
<th>Amino acid</th>
<th>Residue</th>
<th>Amino acid</th>
</tr>
</thead>
<tbody>
<tr>
<td>H170</td>
<td>Histidine</td>
<td>Y3.32(101)</td>
<td>Tyrosine</td>
</tr>
<tr>
<td>Q6.58(249)</td>
<td>Glutamine</td>
<td>S6.47(238)</td>
<td>Serine</td>
</tr>
<tr>
<td>K2.60, (K2.60A)</td>
<td>Lysine</td>
<td>S7.42(277)</td>
<td>Serine</td>
</tr>
<tr>
<td>S2.64 (84)</td>
<td>Serine</td>
<td>D7.49(284)</td>
<td>Aspartate</td>
</tr>
<tr>
<td>Q7.36 (271)</td>
<td>Glutamine</td>
<td>N7.43(278)</td>
<td>Asparagine</td>
</tr>
<tr>
<td>Lys 2.60 (80)</td>
<td>Lysine</td>
<td>V6.51(242)</td>
<td>Valine</td>
</tr>
<tr>
<td>F3.33 (102)</td>
<td>Phenylalanine</td>
<td>K2.60(80)</td>
<td>Lysine</td>
</tr>
<tr>
<td>F6.48(239)</td>
<td>Phenylalanine</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Ref.: (Kotsikorou et al., 2011; Elbegdorj et al., 2013)
Picture 1. Some docking poses of ligands A-D. Compound ‘A’ is ligand 74a (Scheme 2), ‘B’ is 70c (Scheme 1), ‘C’ is ligand 70a (Scheme 1), and ‘D’ is ligand 75a (Scheme 2). The straw colour in the compound is carbon skeleton, red is oxygen, yellow is sulphur and blue is for nitrogen atom. For picture ‘B’, the green colour represents carbon skeleton.
Picture 2. Pictures E-H shows the orientation of the docked newly synthesized ligands E-F and reference ligands G-H in the pocket of GPR55 receptor when they were docked. Docked compound ‘E’ is ligand 70d (Scheme 1) and F is ligand 70e (Scheme 1). Docked compound G is 52 in Fig. 18 and reference H is compound 56 in the Fig. 18. The straw colour in the compound is carbon skeleton, red is oxygen, yellow is sulphur and blue is for nitrogen atom.
3.2. Molecular Docking Results and Discussion

In figure 21 and Picture 1, there is H-pi interaction between ligand 70a and phenyalanine-102, charge hydrogen and hydrophobic interaction between ligand 70a and residue D-98, hydrogen bonding with serine 267 and hydrogen-pi interaction with histidine 170. The high degree of ligand-receptor interaction is critical for receptor binding and binding affinity. Selectivity of the ligand to the receptor will be improved since there are high numbers of hydrogen bonding. Hydrogen bonding plays important role in target selectivity.

Figure 21. The interactions between compound 70a with some of the pocket residues of GPR55 receptor. The dotted lines with arrows are for hydrogen bond. The arrow shows some of the pocket’s conserved residues that form the binding site which are interacting with the ligand.
Compound 70e (Fig. 24) picture 2, forms charge hydrogen bond with tyrosine 101. Not much was seen with the methyl group at the para position in terms of binding interactions. The ligand '70e' (Figure 24) also forms hydrogen bond with valine 109 and tryptophan 177, Figure 24. The ligand was entirely buried in the pocket which is also important for ligand activity.

Figure 24. The Figure shows the interaction of the ligand 70e with some residues in the binding pocket by hydrogen-pi interactions, pi-pi interactions and H-bonding. The arrow show a key conserved residue 'tyrosine 101' interacting with the ligand in the binding pocket of GPR55.

Compound 70c (Fig. 22, Picture 1) forms charge hydrogen bond with glutamate 98. Not much was seen with the metoxy oxygen in terms of binding interactions. The ligand '70c' also forms hydrogen bond with serine 267 and glutamine 249. The hydrogen bond interactions are important for receptor selectivity; Figure 22. In Figure 22, ligand 76a (Fig. 23, Picture 1) underwent hydrogen bond with tyrosine 101, histidine 243 and also pi-pi interactions with histidine 243. The ligand was entirely buried in the pocket which is also important for ligand activity.
Figure 22. The interactions between compound 70c with some of the conserved residues of the pocket of GPR55 receptor. The dotted lines with arrows are for hydrogen bond. The arrows show some of the conserved pocket residues of GPR55 binding site which are interacting with the ligand 70c.

Figure 23. The figure shows the interaction of the ligand ‘76a’ with the conserved residue ‘tyrosine 101’ and histidine 243 in the binding pocket. The arrow shows one of the key conserved residue in the binding pocket of GPR55 interacting with the ligand through hydrogen bonding.

The other ligands had similar interactions with the pocket residues. 70b, structure not shown had pi-pi interactions with histidine 243, charge hydrogen interaction with proline 193 and phenylalanine 102. Ligand 70b was also fully buried in the binding pocket. The indole derivative ligand ‘70d’, has a more rigid structure and underwent hydrogen bonding, hydrogen-pi interactions and pi-pi interactions, picture 2 (E).
It was observed that most of the ligands during docking penetrated deep into the pocket and interacted through hydrophobic, hydrogen bonding or hydrogen-pi interactions. The reference compounds (Fig. 25) showed some level of interactions with the pocket residues. The reference compounds (Figure 25) exhibited hydrophobic and hydrogen interactions with the pocket residues. The deep transmembrane pocket region accommodated the large ligands for binding interactions. This correlates well with literature as observed by Kotsikorou et al. with their LPI study. It is not surprising that there were some interactions and binding as the ligands have structural analogy to the known GPR55 agonist ‘LPI’ and to the lead compound. Indeed, structural analogies do not always lead to ligand activity. It is true that all the studied compounds displayed good receptor-ligand interactions, but one cannot conclusively rely on docking poses alone. This is because; force field scoring function is not reliable.

The reference ligands 56-57 (Fig. 25) are known GPR55 ligands and the showed interaction with the pocket residues. There was stron Hydrogen bond interaction between the halogen of compound E (Fig. 25) and asparagine 280. The nitrogen of fompoind F underwent strong hydrogen bond with tyrosine 106. The two reference compounds were fully embedded in the pocket of the receptor.

**Figure 25.** The Figure shows the interaction of reference ligands ‘E’ and ‘F’ (52 and 56) with some residues in the binding pocket through pi-pi interactions and H-bonding. The ligands were also well fitted to the pocket of the receptor.

### 3.3. Molecular Dynamic Study.

One of the ligand-receptor complexes was selected for molecular dynamic simulations (MD) with Desmond v.3.0.3.1 to check its stability. Calculations were performed using resources of
CSC, Finland. The ligand-receptor complex was placed in POPC membrane and hydrated with water. Ions were added as to a concentration of 0.15 M of NaCl. The system was minimized and subjected to 10 ns MD in NPT ensemble. The molecular dynamic simulation confirmed the stability of ligand-receptor complex and the reliability of a docking pose.

4. Synthetic Experiments
4.1. Materials and general method
All chemicals were commercial grade and were used without further purification. The solvents were dried with 4 Å molecular sieves. Basic glassware and analytical instruments were used including, Mettler Toledo AB204-S analytical balance, Vacuubrand CVC-2 rotary evaporator. Product formation and product purity were ascertained with Ultrashield Bruker Avance 500.1 MHz NMR spectrometer. ThermoQuest CE instrument EA 1110 CHNS-O elemental analyzer was used for the elemental analysis. Mass analyses were performed on Finnigan MAT LCQ quadrupole ion trap mass spectrometer equipped with Electro Spray Ionizer (ESI). Thin layer chromatography (TLC) was carried on pre-coated silica gel plates with aluminum back. Products were separated with solvent-solvent using DCM or by filtration. Column chromatography was carried out either by traditional column technique or by using CombiFlash Companion instrument (serial number 206J20235). DCM/ methanol system at varying proportions of methanol (0% to 20%) was used for column chromatographic separations. Recrystallization method was used for further purification by adding controlled amounts of hexane to saturated solution of the product in DCM.

NMR samples were prepared using DMSO-d$_6$ as solvent with TMS as internal standard. $^1$H NMR spectra was measured for the intermediates and both $^1$H and $^{13}$C NMR spectra for final products. Chemical shifts (δ) were expressed in parts per million (ppm) with reference from the tetramethylsilane (TMS) peak or the solvent residual peak DMSO (2.50 ppm), chloroform (7.26 ppm), Multiplicities are reported as s = singlet, bs = broad singlet, d = doublet, t = triplet, m = multiplet, q = quadruplet. Coupling constants (J) are given in Hertz (Hz).

4.2. 1, 1’-Biphenyl-4-carbonyl isothiocyanate
To a solution of potassium thiocyanate (0.15 g, 1.58 mmol) in 10 mL of acetone was added slowly a solution of 68 (0.33 g, 1.5 mmol) in 10 mL acetone. The mixture was refluxed for 2 hours and was monitored by TLC (CHCl$_3$: methanol 95:5) for production of 1,1’-biphenyl-4-carbonyl isothiocyanate 69. The reaction mixture was allowed to cool to room temperature and was taken to the next step without extraction and purification.
4.3. 4-Nitro-N-phenethylbenzenesulfonamide (66a)

A solution of 64 in DCM (2.26 mmol, 0.29 mL) is added to solution of pyridine (2.26 mmol, 0.30 mL) and 2-phenylethanamine (72a) (2.26 mmol, 0.28 mL) in 10 mL DCM. The reaction mixture was stirred at room temperature for 10 minutes and refluxed for 4 hours at 80°C. The progress of the reaction was monitored by TLC with (95:5, HCl/methanol) solvent system. The mixture was allowed to cool to room temperature and washed with 1M HCl solution. The organic phase was extracted with dichloromethane (DCM), dried with Na₂SO₄ and concentrated with rotary evaporator. The title compound (66a) was purified with flash chromatography (95:5 DCM/methanol) to yield 66a as a light yellow crystalline solid (0.37 g, yield 53.5%).

\( ^1H \) NMR (DMSO-d₆): \( \delta 2.69 \) (t, 2H, \( J = 7.4 \) Hz, ArC\( \text{H}_2 \)), 3.05 (q, 2H, \( J = 6.4 \) Hz, NHC\( \text{H}_2 \)), 7.15 (d, 2H, \( J = 7.7 \) Hz, Ar\( \text{H} \)), 7.18 (t, 1H, \( J = 7.2 \) Hz, Ar\( \text{H} \)), 7.26 (t, 2H, \( J = 7.5 \) Hz, Ar\( \text{H} \)), 8.00 (d, 2H, \( J = 8.7 \) Hz), 8.13 (t, 1H, \( J = 5.6 \) Hz, CH₂NH), 8.40 (d, 2H, \( J = 8.7 \) Hz, Ar\( \text{H} \)).

4.4. 4-Amino-N-phenethylbenzenesulfonamide (67a)

To a solution of (66a) (0.50 g, 1.63 mmol) and Sn (0.41 g, 3.71 mmol) in 10 mL anhydrous ethanol was added concentrated HCl (8 mL). The resulting solution was refluxed until all the tin granules had disappeared. The reaction was monitored with TLC using CHCl₃/methanol (95:5) as the eluent system. The mixture was cooled to room temperature and 30% NaOH solution was slowly added until the mixture became slightly alkaline and clear. The product was extracted with DCM and the organic phase was dried with Na₂SO₄. The solvent was evaporated with rotary evaporator to obtain 67a as a light yellow crystalline solid (0.39 g, yield = 87%).

\( ^1H \) NMR (DMSO-d₆): \( \delta 2.63 \) (t, 2H, \( J = 7.6 \) Hz, Ar\( \text{H} \)), 2.84 (q, 2H, \( J = 6.5 \) Hz, NHCH₂), 5.91 (s, 2H), 6.58 (2H, d, \( J = 8.6 \) Hz, Ar\( \text{H} \)), 7.12 (d, 2H, \( J = 7.6 \) Hz, Ar\( \text{H} \)), 7.15-7.19 (m, 2H), 7.24 (t, 2H, \( J = 7.5 \) Hz, Ar\( \text{H} \)), 7.39 (d, 2H, \( J = 8.6 \) Hz, Ar\( \text{H} \)).

4.5. N-((4-(N-Phenethylsulfamoyl)phenyl)carbamothioyl)-[1,1'-biphenyl]-4-carboxamide (70a)

Synthesis of 69 was carried out according to a procedure 4.2. To the mixture of 69 was added slowly a solution of (67a) in acetone (10 mL). The resulting solution was refluxed overnight and product formation was monitored with TLC (CHCl₃/methanol = 95:5). The mixture was allowed to cool to room temperature and was transferred to 30 mL of acidified water (pH = 5). The mixture was placed on ice bath to obtain a white solid precipitate. It was filtered and washed with small volumes of cold water. Purification was done using flash chromatography with DCM/methanol system (97.5:2.5) as eluent. Further purification was done using recrystallization by adding small volumes of DCM for dissolution. Small volumes of hexane were added dropwise until recrystallization was obtained. Residual solvent and further impurities were washed off using drops of diisopropylether to obtain 70a as a white solid (0.073 g, yield = 10%).

\( ^1H \) NMR (DMSO-d₆): \( \delta 2.68 \) (t, 2H, \( J = 7.5 \) Hz, Ar\( \text{H} \)), 2.98 (q, 2H, \( J = 6.92 \) Hz, NHCH₂), 7.15-7.19 (m, 3H), 7.25 (t, 2H, \( J = 7.6 \) Hz, Ar\( \text{H} \)), 7.43 (m, 1H), 7.51 (t, 2H, \( J = 7.6 \) Hz, Ar\( \text{H} \)), 7.71-7.74 (m, 1H), 7.75-7.78 (m, 2H), 7.80 (d, 2H, \( J = 8.6 \) Hz, Ar\( \text{H} \)), 7.85 (d, 2H, \( J = 8.6 \) Hz, Ar\( \text{H} \)), 7.95 (d, 2H, \( J = 8.5 \) Hz, Ar\( \text{H} \)), 8.08 (d, 2H, \( J = 8.5 \) Hz, Ar\( \text{H} \)).

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11.74 (s, 1H), 12.77 (s, 1H). $^{13}$C (DMSO-d$_6$) δ 35.77, 44.58, 124.79, 126.72, 127.08, 127.49, 127.68, 128.01, 128.97, 129.15, 130.01, 131.23, 137.92, 139.15, 139.19, 141.92, 145.13, 168.29, 179.78; ESI-MS 516 (M+H). Anal. Calcd for C$_{28}$H$_{25}$N$_3$O$_3$S$_2$: C 65.22, N 8.15, H 4.89, O 9.31, S 12.44; found: C 65.14, N 8.22, H 5.01, S 10.91, O 10.72.

4.6. **N-Alllyl-4-nitrobenzenesulfonamide (66b)**

A solution of 64 (0.29 mL, 2.26 mmol) in 8 mL DCM was added to a solution containing pyridine (0.30 mL, 2.26 mmol) in 8 mL DCM and allylamine (65b) (1.29 g, 0.169 mL, 2.26 mmol) in 8 mL DCM. The reaction mixture was stirred at room temperature and refluxed for 4 hours at 80°C. Product formation was monitored with TLC (95:5, DCM/ methanol). The mixture was allowed to cool to room temperature and washed with 1M HCl solution. Water (10 ml) was added and the organic phase extracted with DCM, dried with Na$_2$SO$_4$, and solvent removed with rotary evaporator to get a light yellow product. Product was purified by flash chromatography using the CombiFlash instrument in an isocratic mode with solvent 'A' (DCM) (100% to 90%) and 'B' (Methanol) 0% to 10%. The title compound 66b was obtained as a light yellow solid (0.54 g, yield 99%). $^1$H NMR (DMSO-d$_6$): δ 2.07 (m, 2H), 5.01 (d, 1H, $J=13.6$ Hz, CH=CH$_2$), 5.12 (d, 1H, $J=13.6$ Hz, CH=CH$_2$), 5.61-5.69 (m, 1H, CH$_2$=CH$_2$), 8.02-8.04 (m, 2H), 8.18 (bd, 1H, $J=5.8$ Hz, CH$_2$N), 8.39 (m, 2H).

4.7. **N-Alllyl-4-aminobenzenesulfonamide (67b)**

Concentrated HCl (8 ml) was added to a solution (66b) (0.35 g, 1.45 mmol) and Sn (0.44 g, 3.71 mmol) in 10 mL anhydrous ethanol. It was refluxed for four hours until all the tin granules had disappeared. The reaction was monitored with TLC control (CHCl$_3$/ methanol = 95:5). The mixture was cooled to room temperature and 30% NaOH was slowly added to the solution until the solution became slightly alkaline and clear. The product was extracted with DCM, and the organic phase dried with Na$_2$SO$_4$. The solvent was evaporated with rotary evaporator to obtain a pale yellow solid product. The product was purified with flash chromatography using CombiFlash instrument in an isocratic mode with solvent 'A' (DCM) from 100% to 90%) and 'B' (methanol) 0% to 10%. The purified product 67b was pale yellow liquid that solidified upon standing, (0.47 g, yield 99%). $^1$H NMR (DMSO-d$_6$): δ 3.48 (bd, 2H), 4.98-5.00 (m, 1H), 5.09-5.13 (m, 1H), 5.61-5.74 (m, 1H), 5.89 (s, 2H), 6.59 (d, 2H, $J=8.7$ Hz, ArH), 7.23 (t, 1H, $J=6.0$ Hz, CH$_2$NH), 7.39 (d, 2H, $J=8.7$ Hz, ArH).

4.8. **N-((4-(N-Alllysulfamoyl)phenyl)carbamothioyl)-[1,1'-biphenyl]-4-carboxamide (70b)**

1,1’-biphenyl-4-carbonyl isothiocyanate 69 was synthesized according to a procedure 3.4.1. To a mixture of (69) was added a solution of (67b) (0.47 g, 2.214 mmol) in 8 mL acetone slowly to the reaction mixture. The resulting solution was refluxed overnight and monitored by TLC (CHCl$_3$/ methanol = 95:5). The mixture was allowed to cool to rt, and transferred to 30.0 mL of water that had been adjusted to pH of 5. The white solid precipitate 70b was filtered and washed with small volumes of cold water. Further purification was carried out with column chromatography DCM/ methanol = (100:0 to 95:5) followed by recrystallization.
using DCM / hexane / acetone system. The NMR of this final product had all the needed peaks and the product had a mass of 0.012 g (9% yield) but the recrystallization was not successful and did not pass the elemental analysis. $^1$H NMR (DMSO-d$_6$): δ 3.34 (m, 2H), 5.01-5.03 (m, 1H), 5.14-5.17 (m, 1H), 7.69 (d, 2H, $J$= 7.23 Hz, ArH), 7.72-7.74 (m, 4H), 7.81 (d, 2H, $J$= 8.40 Hz, ArH), 7.86 (d, 2H, $J$= 8.44 Hz, ArH), 8.00 (s, 1H), 8.03 (t, 2H, $J$= 7.2 Hz, ArH), 8.14 (d, 2H, $J$= 8.44 Hz, ArH), 11.60 (s, 1H), 13.06 (s, 1H). ESI-MS 453.23 [M+H]+. Anal. Calcd for C$_{23}$H$_{21}$N$_3$O$_3$S$_2$: C 61.18, H 4.69, N 9.31, O 10.63, S 14.20; found C 64.89, H 5.29, N 7.08, S 10.05, O 9.69.

4.9. $N$-(4-Methoxybenzyl)-4-nitrobenzenesulfonamide (66c)

A solution of 64 (0.50 g, 2.26 mmol, 0.29 mL) in DCM was added to a mixture of pyridine (2.26 mmol, 0.30 mL) in 10 mL DCM and 65c (0.33 g, 2.269 mmol, 0.32 mL) in 10 mL DCM. The reaction mixture was stirred at room temperature and refluxed for 4hrs at 80°C monitoring by TLC (95:5, CHCl$_3$ / methanol). The mixture was allowed to cool to room temperature and washed with 1M HCl solution. A volume of 10ml water was added and the organic phase was extracted with DCM, dried with Na$_2$SO$_4$, and concentrated with rotary evaporator. The product was purified by flash chromatography using 95: 5 (DCM / methanol) solvent system, to furnish the title compound 66c as a pale yellow crystalline solid (0.60 g, 83 % yield). $^1$H NMR (DMSO-d$_6$): δ 3.67 (s, 3H), 3.98 (d, 2H, $J$= 6.0 Hz, C$_{2}$H$_2$NH), 6.77 (d, 2H, $J$= 8.6 Hz, ArH), 7.08 (d, 2H, $J$= 8.6 Hz, ArH), 7.96 (d, 2H, $J$= 8.4 Hz, ArH), 8.32 (d, 2H, $J$= 8.4 Hz, ArH), 8.46 (t, 1H, 6.1 Hz, CH$_2$NH).

4.10. 4-Amino-$N$-(4-methoxybenzyl)benzenesulfonamide (67c)

Concentrated HCl (8 mL) was added to a solution of $N$-(4-methoxybenzyl)-4-nitrobenzenesulfonamide 66c (0.60 g, 1.86 mmol) and Sn (0.33 g, 2.792mmol) in 10 mL anhydrous ethanol. The mixture was refluxed for four hours until all the tin granules disappeared. The progress of the reaction was monitored with TLC (CHCl$_3$ / methanol = 95:5). The reaction mixture was cooled to room temperature and 30% NaOH was slowly add to the solution until the solution became slightly alkaline and clear. Addition of few volumes of the base turned the mixture cloudy and chalky, became colorless upon further additions. The product was extracted with DCM. The organic phase was dried with Na$_2$SO$_4$ and the solvent evaporated with rotary evaporator. Orange solid product was obtained (0.51 g, 94 % yield). $^1$H NMR (DMSO-d$_6$): δ 3.83 (s, 3H), 3.94 (d, 2H, $J$= 6.0 Hz, CH$_2$NH), 5.18 (s, 2H), 6.77 (d, 2H, $J$= 8.5 Hz, ArH), 7.12 (d, 2H, $J$= 8.5 Hz, ArH), 7.93 (d, 2H, $J$= 8.2 Hz, ArH), 8.32 (d, 2H, $J$= 8.2 Hz, ArH), 8.46 (t, 1H, 6.1 Hz, CH$_2$NH).

4.11. $N$-((4-(4-((4-Methoxybenzyl)sulfamoyl)phenyl)carbamothioyl)-[1,1'-biphenyl]-4-carboxamide (70c)

Synthesis of the intermediate 69 was carried out as described in sub-section 4.2 and continued to the next step without extraction. To a mixture of 67C (0.50 g, 1.71 mmol) in acetone was slowly added a solution of 69 in 8 mL acetone. The resulting solution was
refluxed overnight and monitored by TLC (CHCl₃ / methanol = 95:5). The mixture was allowed to cool to rt, and transferred to 30.0 mL of water that had been acidified to a pH of 5. The product 70c was washed, filtered and finally purified by flash chromatography. ¹H NMR and ¹³C (DMSO-d₆) were checked but the amount was too small to go through successfully the routines of purification. It is apparently because coupling was not effectively done. The obtained crystals were mostly unused reactant after flash and subsequent recrystallization. Coupling was unsuccessful.

4.12. 4-Nitro-N-phenethylbenzamide (73a)
2-Phenylethanamine 72a (3.23 mmol, 0.39 g) was dissolved in 5 mL DCM, cooled to 0 °C, and N-methylmorpholine (3.23 mmol, 0.355 ml) was added. 4-nitrobenzoyl chloride 71 (2.69 mmol, 0.5 g) in 5 mL DCM was added in a dropwise manner and stirred at room temperature overnight. It was washed with 2M HCl for three times, once with saturated NaHCO₃ and once with brine. The organic layer was dried over MgSO₄. Solvent was removed in vacuo to obtain the pale yellow solid crystal product 73a (1.12 g, yield; 87%). ¹H NMR (DMSO-d₆): δ 2.85 (t, 2H, J= 7.4 Hz, ArC₃H₂), 3.30 (s, 1H), 3.50 (q, 2H, J= 6.8 Hz, NCH₂), 7.19 (t, 1H, J= 7.2 Hz, ArH), 7.23 (d, 2H, J= 6.9 Hz, ArH), 7.29 (t, 2H, J= 7.4 Hz, ArH), 8.01-8.04 (m, 2H), 8.28-8.31 (m, 2H), 8.88 (t, 1H, J= 5.3 Hz, CH₂NH).

4.13. 4-Amino-N-phenethylbenzamide (74a)
Concentrated HCl (8 ml) was added to a solution of 4-Nitro-N-phenethylbenzamide 73a (0.70 g, 2, 73 mmol) and Sn (0.40 g, 3.71 mmol) in 10 mL anhydrous ethanol. The solution was refluxed for two hours until all the tin granules disappeared. The reaction was monitored with TLC (CHCl₃/methanol = 95:5). The mixture was cooled to room temperature and 30% NaOH slowly was added to the solution until the solution became slightly alkaline and clear. Extraction of the product was done with DCM and the organic phase dried with Na₂SO₄ and the solvent evaporated with rotary evaporator to obtain 74a as a yellow solid (0.37 g) in 25% yield of the desired product. ¹H NMR (DMSO-d₆): δ 2.79 (2H, t, J = 7.5 Hz ArCH₂), 3.41 (2H, dt, J = 7.5, 5.7 Hz), 5.74 (2H, s), 6.51 (2H, d, J = 8.7 Hz ArH), 7.15-7.31 (5H, m), 7.53 (2H, d, J = 8.7 Hz ArH), 8.83 (1H, t, J = 5.6 Hz CH₂NH).

4.14. N-((4-(Phenethylcarbamoyl)phenyl)carbamothioyl)-[1,1'-biphenyl]-4-carboxamide (75a)
To a solution of potassium thiocyanate (1.95 mmol, 0.19 g) in 8 mL of acetone was added slowly a solution of 68 (0.42 g, 1.94 mmol) in 8 mL acetone. The mixture was refluxed for 2 hours and allowed to cool to room temperature. Formation of product 69 was monitored by TLC. The mixture was cooled to rt and was taken to the next step without any further purification. To a solution of 73a in 10.0 mL DCM was added a mixture of 1,1'-biphenyl-4-carbonyl isothiocyanate 69 already in acetone slowly to the reaction mixture. The resulting coupling mixture was refluxed overnight and monitored by TLC (CHCl₃/methanol = 95:5). The mixture was allowed to cool to rt, and transferred unto 30.0 mL of acidified water of a
pH of 5. The precipitates were washed with small volumes of cold water, filter and purify by flash chromatography (CHCl₃/ methanol = 97:5: 2.5). 75a was obtained as a pale yellow crystalline solid (0.04 g) in 5% yield. ¹H NMR (DMSO-d₆): δ 2.85 (t, 2H, J = 7.5 Hz, ArCH₂), 3.49 (q, 2H, J = 6.8 Hz, NHCH₂), 7.19 (t, 1H, J = 7.2 Hz, ArH), 7.24 (d, 2H, J = 7.1 Hz, ArH), 7.29 (t, 2H, J = 7.4 Hz, ArH), 7.43-7.45 (m, 2H), 7.50-7.53 (m, 3H), 7.76-7.79 (m, 1H), 7.81-7.85 (m, 1H), 7.95 (d, 2H, J = 8.4 Hz, ArH), 8.09 (d, 2H, J = 8.4 Hz, ArH), 8.58 (t, 1H, J = 5.5 Hz, CH₂N), 11.66 (s, 1H), 12.74 (s, 1H):

13C (DMSO-d₆): δ 25.91, 40.34, 111.37, 111.86, 118.47, 118.78, 121.38, 123.44, 124.78, 127.08, 127.44, 127.61, 129.61, 130.00, 136.64, 138.14, 139.19, 141.84, 145.12, 168.30, 179.78: ESI-MS 480.5 [M+H], Anal Calcd for C₂₉H₂₅N₃O₂SC 72.63, H 5.25, N 8.76, O 6.67, S 6.69; found C 71.65, H 4.97, N 8.73, S 6.19 O 8.46.

4.15. N-(2-(1H-Indol-3-yl)ethyl)-4-nitrobenzenesulfonamide (66d)

To a solution of 64 (0.54 g, 2.42 mmol) in 8 mL DCM was added to a mixture (solution) of pyridine (2.90 mmol, 0.234 mL) and 2-(1H-indol-3-yl) ethanamine 65d (0.43 g, 2.71 mmol) in 8 mL DCM. The reaction mixture was stirred at room temperature and refluxed for 4hrs at 80°C monitoring by TLC, (95:5, CHCl₃/ methanol). It was allowed to cool to room temperature (rt) and the solvent was evaporated. The product was washed with 1M HCl solution. The organic phase was extracted with DCM, dried with Na₂SO₄, and concentrated with rotary evaporator. The product was purified by flash chromatography using 97.5: 2.5 (DCM/ methanol) solvent system. 66d was obtained as a yellow crystalline solid (0.62 g, yield 74%) after purification. ¹H NMR (DMSO-d₆) δ 3.44 (t, 2H, J = 5.5 Hz, CH₂H), 5.02-5.17 (m, 2H), 7.42-7.45 (m, 1H), 7.51 (t, 1H, J = 7.8 Hz, ArH), 7.77 (d, 2H, J = 8.0 Hz, ArH), 7.85 (d, 2H, J = 8.2 Hz, ArH), 7.97 (d, 2H, J = 8.3 Hz, ArH), 8.09 (d, 2H, J = 8.2 Hz, ArH), 11.72 (s, 1H).

4.16. N-(2-(1H-Indol-3-yl)ethyl)-4-aminobenzenesulfonamide (67d).

To a solution of 66d (0.34 g, 0.984 mmol) and Sn (0.37 g, 3.12 mmol) in 10 mL anhydrous ethanol was added 8 ml of concentrated HCl. The mixture was refluxed for two hours when all the tin granules had disappeared. The reaction was monitored with TLC (CHCl₃/ methanol = 95:5) for product formation. The mixture was cool to rt and was added slowly 30% NaOH until the solution became slightly alkaline and clear. Extraction of the product was done with DCM. Drying of the organic phase was done with Na₂SO₄ and the solvent was evaporated with rotary evaporator to obtain 67d as a yellow solid product (0.31 g, yield 98%). ¹H NMR (DMSO-d₆): δ 2.78 (t, 2H, J = 7.3 Hz, ArCH₂), 3.10 (q, 2H, J = 6.6 Hz, NH₂), 5.88 (s, 2H), 6.59 (d, 2H, J = 8.7 Hz, ArH), 6.98 (t, 1H, J = 7.5 Hz, ArH), 7.03 (t, 1H, J = 7.0 Hz, ArH), 7.07 (bs, 1H), 7.23 (t, 1H, J = 7.9 Hz, ArH), 7.35 (d, 1H, J = 7.7 Hz, ArH), 8.11 (t, 1H, J = 5.5 Hz, ArH), 8.24 (d, 2H, J = 8.7 Hz, ArH), 10.77 (s, 1H).
4.17. N-((4-(N-(2-(1H-Indol-3-yl)ethyl)sulfamoyl)phenyl)-carbamothioyl)-[1,1′-biphenyl]-4-carboxamide (70d)

A solution of potassium thiocyanate (3.82 mg) in 5 ml of acetone was prepared and added slowly to a solution of 68 (8.51 mg) in 5 mL acetone. The mixture was refluxed for 2 hrs and allowed to cool to room temperature. Monitor of the reaction progress for the formation of 69 was done with TLC (CHCl₃/ methanol = 95:5) and was continued to the next step after cooling to rt. 67 in 8.0 mL acetone was slowly added to a mixture of 69 in acetone. The reaction mixture was coupled overnight through refluxing and monitored by TLC (DCM/methanol = 95:5). The mixture was allowed to cool to rt and transferred to 30.0 mL of water that had been adjusted to a pH of 5. Purification was done by flash chromatography (Eluent; 97.25: 2.5) of DCM/methanol and recrystallization to obtain the desired product (0.43 g, 82% yield).

1H NMR (DMSO-d₆): δ 2.80 (t, 2H, J= 7.5 Hz, CH₂H), 3.04 (q, 2H, J= 6.0 Hz, NHH), 6.94 (t, 1H, J= 7.3 Hz, ArH), 7.03 (t, 1H, J= 7.4 Hz, ArH), 7.11 (s, 1H), 7.30 (d, 1H, J= 8.0 Hz, NHH), 7.39 (d, 1H, J= 7.7 Hz, ArH), 7.43 (t, 1H, J= 7.3 Hz, ArH), 7.51 (t, 2H, J= 7.4 Hz, ArH), 7.76 (bm, 3H, J= 6.9 Hz, ArH), 7.81 (d, 2H, 8.3 Hz, ArH), 7.85 (d, 2H, 8.1 Hz, ArH), 7.93 (d, 2H, 8.3 Hz, ArH), 8.09 (d, 2H, 8.1 Hz, ArH), 10.73 (s, 1H), 11.71 (s, 1H), 12.75 (s, 1H).

13C (DMSO-d₆): δ 25.91, 43.96, 111.36, 111.86, 118.47, 118.78, 121.38, 123.44, 124.76, 127.08, 127.43, 127.49, 127.61, 128.97, 129.61, 130.01, 131.28, 136.64, 138.10, 141.86, 145.10, 168.32, 179.75; LC-MS 555.04 [M+H]+, Anal Calcd for C₃₀H₂₆N₄O₃S₂ C 64.96, H 4.72, N 10.10, O 8.65, S 11.56, found C 65.07, H 4.42, N 9.74, S 11.39, O 9.38.

4.18. N-Benzyl-4-nitrobenzamide (73b)

Benzylamine (6,47 mmol 0.693 g) was dissolved in 5 mL DCM, cooled to 0 °C, and N-methylmorpholine (0.59 mL, 5.39 mmol, 0.55 g) was added. 4-nitrobenzoyl chloride 71 (5.39 mmol, 1.0 g) in 5 mL DCM was added in a dropwise manner and stirred at room temperature overnight. The product was washed with 2M HCl for three times, once with saturated NaHCO₃ and once with brine. The organic layer was dried over MgSO₄ and the solvent was removed with rotary evaporator to obtain 73b (1.35 g, 98% yield). The intermediate 73b was taken to the next step without further purification. 1H NMR (DMSO-d₆): δ 4.50 (d, 2H, J= 5.9 Hz, NHH), 7.24-7.26 (m, 1H, ArH), 7.32 (bd, 4H, J= 4.4 Hz, ArH), 8.11 (d, 2H, J= 8.8 Hz, ArH), 8.31 (d, 2H, J= 8.8 Hz, ArH), 9.35 (t, 1H, J= 5.6 Hz, CH₂).

4.19. 4-Amino-N-benzylbenzamide (74b)

Concentrated HCl (8 ml) was added to a solution of 73b (1.95 mmol, 0.50 g) and Sn (2.341 mmol, 0.28 g) in 8 mL anhydrous ethanol. Refluxing of the mixture was done for four hours until all the tin granules disappeared. Reaction progress was checked with TLC (CHCl₃/methanol = 95:5). The mixture was cooled to room temperature and 30% NaOH was slowly added to the solution until the solution became slightly alkaline and clear. The product was extracted with DCM to obtain the desired product 74b as a pale yellow solid (0.441 g, 93.15% yield). 1H NMR (DMSO-d₆) δ 4.43 (d, 2H, J= 6.0 Hz), 5.60 (s, 2H), 6.55 (d, 2H, J=
8.5 Hz, ArH), 7.22-7.24 (m, 1H), 7.29-7.33 (m, 4H), 7.63 (d, 2H, J= 8.6 Hz, ArH), 8.55 (t, 1H, J= 5.90 Hz, CH2H).

4.20. \(N\)-((4-(Benzylcarbamoyl)phenyl)carbamothioyl)-[1,1'-biphenyl]-4-carboxamide (75b)

Preparation of a solution of potassium thiocyanate (0.20 g, 2.09 mmol) in 5 mL of acetone was made. A solution of 1,1'-biphenyl-4-carbonyl chloride (0.47 g, 2.19 mmol) in 5 mL acetone was slowly added and the mixture was refluxed for 2 hours and allowed to cool to room temperature. Reaction progress for 69 formation was checked with TLC (CDM / methanol = 95:5). The coupling of 69 and \(N\)-(2-(1H-indol-3-yl)ethyl)-4-aminobenzene-sulfonamide intermediate 74b was done by slowly adding a solution of 74b in 8.0 mL acetone to the reaction mixture of 69 in acetone. The mixture was refluxed overnight. The coupling was monitored by TLC (CDM / methanol = 95:5). The mixture was allowed to cool to rt and transferred to 30.0 mL of acidified water (pH of 5). The white solid precipitates were washed with small volumes of cold water, filtered and purified by flash chromatography (CHCl3 / methanol = 97.5: 2.5). 76b obtained as pale yellow solid crystal with mass of 0.93 g and a yield of 100 %. \(^1\)H NMR (DMSO-d6): δ 4.51 (d, 2H, J= 5.8 Hz), 7.24-7.28 (m, 1H), 7.35 (bd, 4H, J= 4.1 Hz, ArH), 7.47 (t, 1H, J= 7.3 Hz, ArH), 7.54 (t, 2H, J= 7.6 Hz, ArH), 7.78-7.82 (m, 2H), 7.87 (bd, 4H, J= 7.3 Hz, ArH), 7.96 (d, 2H, J= 8.2 ArH), 8.11 (d, 2H, J=8.2 5 Hz, ArH), 9.08 (t, 1H, J= 5.8 Hz, CH2H), 11.69 (s, 1H), 12.78 (s, 1H); \(^13\)C (DMSO-d6): δ 43.09, 124.08, 127.08, 127.21, 127.50, 127.66, 128.29, 128.77, 128.97, 129.62, 129.99, 131.29, 132.26, 139.20, 140.14, 141.03, 145.08, 165.98, 168.36, 179.54; LC-MS 466.19 [M+H]+.

4.21. \(N\)-(4-Methylbenzyl)-4-nitrobenzenesulfonamide (66e)

To a solution of 4-nitrobenzylsulfonylchloride 64 (1.00 g, 4.51 mmol) in 8 mL DCM was added to a mixture of pyridine (0.44 mL, 5.41 mmol) and p-tolylmethanamine 65e (0.66 g, 5.41 mmol) in 8 mL DCM. The reaction mixture was stirred at room temperature and refluxed for 4hrs at 80 °C monitoring by TLC, (95:5, CHCl3 / methanol). It was allowed to cool to rt and the solvent was evaporated. The product was washed with 1M HCl solution. The organic phase was extracted with DCM, dried with Na2SO4, and concentrated with rotary evaporator. The product was purified by flash chromatography using 97.5: 2.5 (DCM / methanol) solvent system. 66e was obtained as a yellow solid crystals solid with a mass of 1.25 g (90%). \(^1\)H NMR (DMSO-d6): δ 2.25 (s, 3H), 4.03 (d, 2H, J= 6.5 Hz.), 7.02 (d, 2H, J= 8.0 Hz, ArH), 7.07(d, 2H, 8.0 Hz, ArH), 7.98 (d, 2H, J= 8.2 Hz, ArH), 8.29 (d, 2H, J= 8.2 Hz, ArH), 8.39 (s,1H).

4.22. 4-Amino-\(N\)-(4-Methylbenzyl)benzenesulfonamide (67e)

A volume of 8 ml concentrated HCl was added to a mixture of 66e (0.50 g, 1.63 mmol) and tin (0.39 g, 3.26 mmol) in 8 mL anhydrous ethanol. The reaction mixture was refluxed for four hours until all the tin granules disappeared. The advancement of the reaction was
followed with TLC (CHCl₃/ methanol = 95:5). The reaction was brought to room temperature. 30% NaOH was slowly added to the solution until the solution became slightly alkaline and clear. The compound of interest was extracted with DCM and the solvent was evaporated. Purification by column chromatography was done to obtain the desired product 67e (0.44 g, 96 %). ¹H NMR (DMSO-d₆): δ 2.27 (s, 3H), 3.82 (d, 2H, J = 6.6 Hz, NH), 5.92 (s, 2H), 6.61 (d, 2H, J = 9.0 Hz, ArH), 7.11 (q, 4H, J = 7.6 Hz, ArH), 7.45 (d, 2H, J = 9.0 Hz, ArH / H 7.55 (t, 1H, J = 6.4 Hz, NH).

4.23. N-(4-(N-(4-Methylbenzyl)sulfamoyl)phenyl)carbamothioyl)-[1,1'-biphenyl]-4-carboxamide (70e)
To a solution of potassium thiocyanate (0.11 g, 1.11 mmol) in 5 mL of acetone was added slowly a solution of 68 (0.240 g, 1.11 mmol) in 5 mL acetone. The mixture was refluxed for 2 hours and was allowed to cool to room temperature, and was taken to the next synthetic step. To a mixture of N-(2-(1H-indol-3-yl)ethyl)-4-aminobenzene-sulfonamide intermediate 67e, in 8 mL acetone was slowly added a solution of 69 in acetone. The resulting solution was refluxed overnight and monitored by TLC (CHCl₃/methanol = 95:5). The mixture was allowed to cool to rt, and transferred to 30.0 mL of water that had been acidified to a pH of 5. The product was washed, filtered and finally purified by flash chromatography and recrystallization (0.03 g, 5 %). ¹H NMR and ¹³C (DMSO-d₆) were checked but there were still too many close bands in the TLC and purification became very difficult. The overall reaction path was repeated but the final product (70e). ¹H NMR (DMSO-d₆): δ 2.25 (s, 3H), 3.95 (d, 2H, J = 6.4 Hz), 7.07 (d, 2H, J = 8.1 Hz, ArH), 7.11 (d, 2H, J = 8.1 Hz, ArH), 7.43 (t, 1H, J = 7.5 Hz, ArH), 7.51 (t, 2H, J = 7.6 Hz, ArH), 7.76 (d, 2H, J = 7.9 Hz, ArH), 8.18-8.85 (m, 3H), 7.95 (d, 2H, J = 8.5 ArH), 8.07-8.11 (m, 2H), 11.71 (s, 1H), 12.74 (s, 1H); LC-MS 516.13 [M+H]+.

4.24. N-Benzyl-4-nitrobenzenesulfonamide (66f)
A solution containing 64 (1.00 g, 4.51 mmol) in 8mL DCM was added to a mixture of pyridine (0.44 ml, 5.41 mmol) and phenylmethanamine 65f (0.591 ml, 5.41 mmol) in 8 mL DCM. The mixture was stirred at rt and refluxed for 4hrs at 80°C and monitored by TLC, (95:5, CHCl₃ / methanol). The mixture was brought under room temperature and washed with 1M HCl solution. Addition of 10 mL water was made and the organic phase was extracted with DCM, dried with Na₂SO₄, and concentrated with rotary evaporator. The compound was purified by flash chromatography using 99.5: 0.5 % (DCM / Methanol) solvent system to obtain the desired product 66f (1.12 g, 85 % yield). ¹H NMR (DMSO-d₆): δ 4.08 (bs, 2H), 7.21-7.23 (m, 3H), 7.25-7.28 (m, 2H), 8.02 (d, 2H, J = 9.0 Hz, ArH), 8.36 (d, 2H, J = 9.0 Hz, ArH), 8.55 (s, 1H).

4.25. 4-Amino-N-benzylbenzenesulfonamide (67f)
Concentrated HCl (8 ml) was added to a solution of 66f (0.60 g, 2.05 mmol) and tin (0.24 g, 2.05 mmol) in 8 mL anhydrous ethanol. The mixture was refluxed for four hours until all the tin granules disappeared. Progress of product formation was checked with TLC (CHCl₃ /
methanol = 95:5). The mixture was cooled to room temperature and 30% NaOH was slowly added to the solution until the solution became slightly alkaline and clear. Product extraction was done with DCM and purification by column and recrystallization (0.37 g, 69 % yield). \(^1\)H NMR (DMSO-d\(_6\)) \(\delta\) 3.85 (s, 2H), 5.90 (s, 2H), 6.58-6.60 (m, 2H), 7.21-7.23 (m, 3H), 7.26-7.29 (m, 2H), 7.42-7.43 (m, 2H), 7.60 (s, 1H).

4.26. 1,1'-biphenyl]-4-carbaldehyde compound with dinitrogen (69f)
1,1'-biphenyl]-4-carbonyl chloride 68 (1.00 g, 4.62 mmol) was dissolved in a methanol / NH\(_3\) solution (2M, 6 mL) and was allowed to stir overnight. Volatiles were removed and the resulting solid (white) was triturated with ethyl acetate. The solid was filtered out washed with cold ethyl acetate to get the pure white solid product 69f (0.84 g, 92% yield). \(^1\)H NMR (DMSO-d\(_6\)) \(\delta\) 7.46-7.50 (m, 2H), 7.70 (s, 2H), 7.72-7.74 (m, 3H), 7.96 (d, 2H, \(J= 8.2\) Hz, ArH), 8.01 (d, 2H, \(J= 8.2\) Hz, ArH).

4.27. \(N\)-((4-(N-Benzylsulfamoyl)phenyl)carbamoyl)-[1,1'-biphenyl]-4-carboxamide (70f)
To a suspension of 69f (0.31 g, 1.47 mmol) in 5 mL DCM was added oxalyl dichloride (0.17 g, 1.33 mmol) and heated to reflux at 80 °C for 11 hrs. Volatiles were removed. Toluene was added to the resulting mixture and the volatiles were removed in vacuo. This was repeated twice to remove the remains of the oxalyl chloride. The resulting intermediate 70f was dissolved in 5 mL DCM and was added to a solution of 67f (0.35 g, 1.33 mmol) in 1.5 mL DCM. The mixture was refluxed for 2 hrs. After cooling to rm, the precipitate was filtered and washed with acetonitrile and dried in vacuo to give the final product 71f (0.02 g, 0.62 mmol, 46 % yield). \(^1\)H NMR (DMSO-d\(_6\)) \(\delta\) 3.97 (d, 2H, \(J= 6.0\) Hz), 7.20-7.28 (m, 3H), 7.27-7.29 (m, 2H), 7.48-7.52 (m, 3H), 7.70-7.73 (m, 1H), 7.74-7.76 (m, 2H), 7.78-7.79 (m, 3H) 7.85 (d, 2H, \(J= 8.32\) Hz, ArH), 8.06 (t, 1H, \(J= 6.4\) Hz, ArH), 8.14 (d, 2H, \(J= 8.32\) Hz, ArH), 11.12 (s, 1H), 11.19 (s, 1H); LC-MS 486.0 [M+H]\(^+\). The obtained mass was below triplicate measurable limit for elemental analysis.
5.1. Discussion

In recent years, large numbers of research articles have been published on CB2 ligands, which is the topic of the literature review of this thesis and Synthesis of GPR55 agonists. In this study, our attention at this experimental part is tailored towards the optimization of a lead agonist to GPR55 receptor discovered by Yrjölä et al. (SID 17388369). Some agonists and antagonists have already been discovered for GPR55 (Kotsikorou et al., 2011 and Elbegdorj et al., 2013); Figure 14, and CBD is one of the previously discovered antagonists for GPR55 (Elbegdorj et al., 2013). But there is still the need for more research into the structure and pharmacology of this putative cannabinoid receptor. GPR55 ligands may have therapeutic potential in the treatment of pain, bone diseases, and cancer. Among the many potentials of GPR55 receptor is to serve as target for pain control (Evangelia et al., 2010) and for control of osteoporosis (Whyte et al., 2009; Sharir and Abood, 2010).

Discussion of synthetic steps:
The sulfamoylphenyl-carbamothioyl-biphenylamide compounds were synthesized based on the modification of Bissinger et al.’s protocol. Reactivity of phenyl sulfonylchloride with primary amine was found to be high with high yield of product ranging from 53.5% to 99%. This finding was found to correlate well with Bissinger et al., 2011. Exemplified by formation of N-allyl-4-nitrobenzenesulfonamide (66b); 99% yield and N-(4-methoxybenzyl)-4-nitrobenzenesulfonamide; 66c 83% yield. The yellow solid obtained for the reaction of the primary amine and the sulfonyl chloride agrees well with literature work on similar products by Bissinger et al., 2011 and other similar studies.

Pyridine functioned subtly as a catalyst to speed up the rate of product formation. The catalytic activity of pyridine has been elaborated in scheme 4. The lone pair on the nitrogen group in pyridine initiates the catalytic attack by serving as a nucleophilic center. Different approaches have been reported for the conversion of aromatic nitro group to an aromatic primary amine. We also report in this study about one of these methods as elaborated in section 4.4. This method was found to be effective giving high yields.

Synthesis of 70a and some other final products in this research begun with the reaction of 4-nitro-N-phenethylbenzenesulfonamide with a primary amine. This step of synthesis was found to be generally fast and high-yielding. This could partly due to the high reactivity between primary amine and sulfonyl chlorides. There is a nucleophilic attack by the amine on the sulfur group resulting in the release of HCl into the reaction solution. Excesses of reactant amine tend to trap the released HCl in solution to avoid acid hydrolysis of the formed intermediate. As high as 99 % yield was recorded for this step 67b in the synthesis 70b. The halogen activated groups are easy to couple with a fast release of the halogen into the reaction system.

The nitro-amine conversion followed the traditional conversion with Sn, HCl and absolute ethanol, but with a little modification. The strong base was not part of the refluxing system as has been reported in literature but was added after the refluxing stage to neutralize the excess
concentrated HCl in the system. The addition of NaOH was critical in this conversion since it helps to cleave the white chalky intermediate complex into the final amine. The formed amine was basic and soluble in NaOH leading to the clear alkaline solution that was observed.

The coupling of the amine to a thiocyanate functional group was generally successful but not with great success. This is because a lot of side products were formed and they had close bands to the desired product in TLC. The closeness of some of the bands of the final products with the side products resulted in difficulties in purification. Purification by HPLC was not feasible in most cases because the final products were insoluble in HPLC solvents (H2O, Methanol and Acetonitrile). Some final products fell under this category. The high reactive nature of this amine could partly contribute to the formation of many side products during coupling. The coupling reaction for the formation of 70e resulted in many bands which resulted in difficulty in handling it. The obtained final product was bellow analytical quantity (too small for triplicate elemental analysis). An unconfirmed assertion is that, it might be due to uncontrolled substitution of the aromatic ring due to the high coupling temperatures '80 °C'. The occupancy of electron donating methyl group at the para-position increases the electron density of the aromatic ring. This makes the meta-position prone to further substitution. This may lead to the formation of a side product(s).

Because of the highly reactive double bond, allylamine can undergo free radical reactions and polymerization. It has also been reported to undergo electrophilic additions, oxidation and allylic substitution. Synthesis of 70b with an allylamine was not successful since the unprotected amine might have been involved in other side reactions. A repeat of the entire process could still not make any meaningful difference.

**Discussion of biological data:**

LPI 61 is a known endogenous GPR55 receptor agonist with important biological activities in the body and a therapeutic target for different cancer states. The lead compound 76 with affinity of 500 nM is structurally analogous to LPI to some extent with hydrogen bond donors and acceptors and might orient in similar pose inside the binding pocket. The synthesized compounds were designed from the lead compound by SAR as an optimization step. The ligands were docked into the binding pocket and they proved to interact with the receptor. One might hurriedly conclude that the compounds will be active based on the docking interactions. But this is not conclusive enough until it has been proved by biological findings. This is because, force field scoring functions are not reliable due to difficulties in quantifying Vander Waals interactions and hydrogen bonding.

Biological evaluation of study ligands were carried out in vitro firstly in the CB1 and CB2 binding assays and they did not show any recordable activity in these systems. For GPR55 selectivity, the ligands should not have any activity in CB1 and CB2 assays. A detection of any activity in the GPR55 model for these ligands will go further to confirm the molecular docking results. It will also further show the selective nature of these ligands to the GPR55.
receptor. The results for in vitro studies in GPR55 binding assay are not ready but it is hoped that they will correlate with the docking results. This stems from the results of the molecular docking study as the ligands interacted with some of the conserved residues in the pocket. The studied ligands interacted with the receptor protein and the results are hoped to be proved experimentally with high degree of selectivity and affinity.

The pocket of the receptor is lined with hydrophobic residues and the designed ligands tends to have relatively high lipophilicity (high logP) which might contribute partly to their insolubility in HPLC solvents. The partition coefficient (logP) is essential by giving prior knowledge about the compounds solubility, permeability and the in vivo mechanism of transport. According to Lipinski's “rule of five” a compound with logP > 5 is likely to have poor oral bioavailability (Lipinski et al, 2001). The calculated log P (clogP) values of the synthesized products with a free online software ‘molinspiration v.2011.06’ was found to range from 3.89 for 70b to 5.24 for 65d. They fall within the Lipinski’s rule of five. The deep transmembrane binding pocket is lined with a lot of hydrophobic residues which are essential for ligand binding (Gether and Kobilka, 1998).

6.0. Conclusion

There have been a lot of research articles on CB2 and GPR55 receptors concerning their structures, biological activities and the therapeutic importance. But until now, there haven't been many if there is any CB2 or GPR55 selective drug in the market. GPR55 has been cloned for over a decade and increasing research activities are ongoing in this area since it is a novel target for cancer, osteoporosis and analgesic drug discovery. The aims of the study were accomplished by creating a small library of compounds hoped to be selective for
GPR55 receptor. The molecular docking studies and the first part of *in vitro* biological study results in CB1 and CB2 for the produced GPR55 ligands are very promising. It is hoped that the biological testing for the ligands in GPR55 model results will show high level of affinity and selectivity for the GPR55 receptor.
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**Some web sites visited**


