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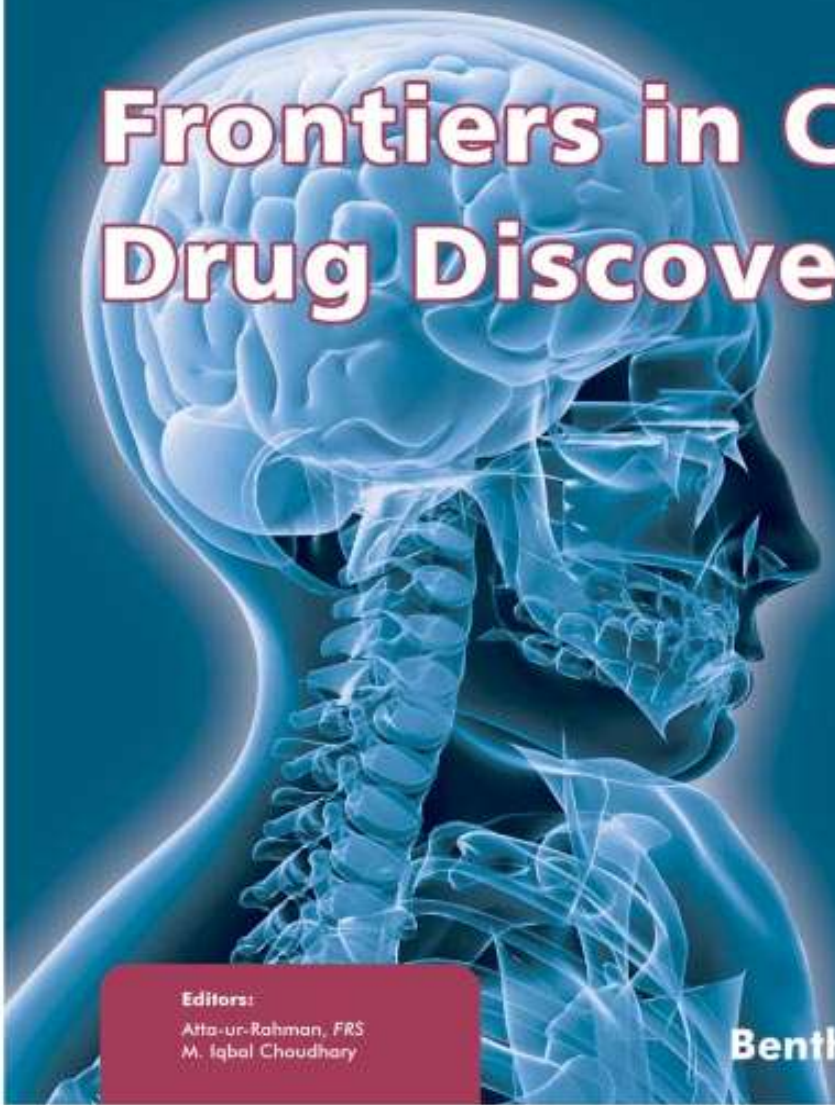
Frontiers in CNS Drug Discovery



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ABSTRAK

G protein-coupled receptors (GPCRs) comprise the largest family in the receptorome (the subset of the genome encoding membrane receptors). These signal transducing molecules convey extracellular signals into the cell interior by activating intracellular networks such as heterotrimeric G protein-dependent signaling pathways. They are widely distributed in the nervous system where they mediate a myriad of key processes including cognition, mood, appetite, pain and synaptic transmission. Currently, at least 30% of marketed drugs are GPCR modulators. With global aging, the CNS drug market is set to grow. GPCR ligands for CNS receptors feature prominently in the pipeline of major pharmaceutical companies. Among GPCRs widely investigated as drug targets include the metabotropic glutamate, adenosine and cannabinoid receptors, as evidenced by recently patented ligands for these receptors. Metabotropic glutamate receptors regulate signaling by glutamate, the major excitatory brain neurotransmitter, while adenosine is a ubiquitous neuromodulater mediating diverse physiological effects. Recent patents for ligands of these receptors include mGluR5 antagonists and adenosine A1 receptor agonists. Cannabinoid receptors used to be one of the most important GPCR drug discovery targets for treating obesity and metabolic syndrome, but the unexpected withdrawal of several CB1 antagonists/inverse agonists has prompted alternative approaches. These recent patents are the outcome of the continuing focus of many pharmaceutical companies to identify novel GPCR agonist, antagonist or allosteric modulators useful to treat psychiatric and neurological diseases for which more effective drugs are urgently needed.

Keywords: G protein, receptor, signaling, drug target, drug discovery, ligand, agonist, antagonist, metabotropic glutamate, adenosine, cannabinoid

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GPCR Drug Pipeline: New Compounds for CNS Diseases

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Abstract: G protein-coupled receptors (GPCRs) comprise the largest family in the receptorome (the subset of the genome encoding membrane receptors). These signal transducing molecules convey extracellular signals into the cell interior by activating intracellular networks such as heterotrimeric G protein-dependent signaling pathways. They are widely distributed in the nervous system where they mediate a myriad of key processes including cognition, mood, appetite, pain and synaptic transmission. Currently, at least 30% of marketed drugs are GPCR modulators. With global aging, the CNS drug market is set to grow. GPCR ligands for CNS receptors feature prominently in the pipeline of major pharmaceutical companies. Among GPCRs widely investigated as drug targets include the metabotropic glutamate, adenosine and cannabinoid receptors, as evidenced by recently patented ligands for these receptors. Metabotropic glutamate receptors regulate signaling by glutamate, the major excitatory brain neurotransmitter, while adenosine is a ubiquitous neuromodulater mediating diverse physiological effects. Recent patents for ligands of these receptors include mGluR5 antagonists and adenosine A_1 receptor agonists. Cannabinoid receptors used to be one of the most important GPCR drug discovery targets for treating obesity and metabolic syndrome, but the unexpected withdrawal of several CB_1 antagonists/inverse agonists has prompted alternative approaches. These recent patents are the outcome of the continuing focus of many pharmaceutical companies to identify novel GPCR agonist, antagonist or allosteric modulators useful to treat psychiatric and neurological diseases for which more effective drugs are urgently needed.

Keywords: G protein, receptor, signaling, drug target, drug discovery, ligand, agonist, antagonist, metabotropic glutamate, adenosine, cannabinoid.

INTRODUCTION

G Protein-Coupled Receptors

With approximately 5%, or about 1000 [1] out of the 20,000-25,000 protein-coding genes of the human genome [2] encoding for G-protein coupled receptors (GPCRs), this protein superfamily represents the largest class of cell surface proteins in humans. GPCRs transduce a diverse range of extracellular signals that include light, ions, odorants, neurotransmitters, hormones, chemokines, nucleotides, lipids, amino acids and proteins [3].

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Atta-ur-Rahman / M. Iqbal Choudhary (Eds.)

Their tissue distribution is wide, with particularly high representation in the brain [4]. Not surprisingly, they mediate a wide range of physiological processes ranging from vision to taste, reproduction, metabolism, hormone release, muscle contraction, inflammation, growth, differentiation and cell proliferation [5]. In the CNS, GPCRs play important roles in cognition, analgesia, appetite, mood, blood pressure, synaptic transmission and neuronal excitability. As at least 30% of marketed drugs have GPCRs as their molecular target, they are among the most intensely investigated drug targets in the pharmaceutical industry [6].

GPCRs share a common molecular architecture of seven transmembrane helices connected by intra- and extracellular loops, an extracellular N-terminus and an intracellular C-terminus [7]. They have been recently classified phylogenetically under the GRAFS system into 5 main families: glutamate, rhodopsin, adhesion, frizzled/taste2 and secretin [8]. Prominent GPCRs in the nervous system includes the adrenergic, muscarinic, serotonin, dopamine, metabotropic glutamate, adenosine, opioid and cannabinoid receptors.

G Protein Signaling

In the classical GPCR signaling paradigm, the liganded receptor undergoes conformational changes that activate membrane-bound signal transducing heterotrimeric guanine nucleotide-binding proteins (G proteins), which consist of an α subunit and a $\beta\gamma$ dimer. The 16 known mammalian Gα subunits are classified into 4 families (α_i, α_s, α_q, and α_{12}), with five β and eleven γ subtypes [9]. The Gα subunit is activated when it binds GTP in exchange for GDP, which then leads to the dissociation of G proteins both from each other and the receptor. Both can then modulate the activities of a variety of effector molecules ranging from ion channels to enzymes such as adenylyl cyclase, guanylyl cyclase, phospholipases, phosphodiesterases, and phosphoinositide-3 kinase (PI3K). In many cases, this leads to the generation of second messengers such as cyclic AMP, cyclic GMP, calcium, diacylglycerol and inositol 1,4,5-triphosphate. These can give rise to signaling pathways which eventually produce a cellular response, such as enzyme secretion or cell proliferation [5]. G protein signaling is terminated when the Gα-bound GTP is hydrolyzed, bringing the Gα subunit to its basal GDP-bound state, a process accelerated by GTPase-activating proteins such as RGS (Regulator of G protein signaling) proteins [10]. This promotes the re-formation of the $\alpha\beta\gamma$ heterotrimer for coupling to the receptor. Signaling is also turned off when second messenger molecules are degraded, or receptor desensitized through phosphorylation by G protein-coupled receptor kinases (GRK) which promotes binding by arrestins that occlude binding to G proteins. After their activation, many GPCRs are targeted by arrestins for internalization via clathrin-mediated endocytosis [11].

GPCR signaling is regulated by a host of accessory proteins which may influence ligand affinity, receptor-G protein coupling, receptor-effector coupling, receptor dimerization and receptor targeting to subcellular compartments [12]. GPCRs can also exist as homooligomers or heterooligomers, where the heterooligomeric receptors may have different functional characteristics compared to the contributing receptors. The discovery of various partner or scaffold proteins has made it increasingly clear that GPCRs can signal independently of G proteins, hence the shift towards the naming of these receptors as heptahelical/serpentine or 7 transmembrane receptors (7TMRs) [13]. Numerous studies have shown that binding of arrestins not only desensitize GPCRs but also serves as a scaffold for activating signaling pathways *via* JNK (Jun N-terminal kinase), PI3K, p38 and Akt [14]. The beta arrestin-bound β_2 adrenergic receptor activates ERK (extracellular signal related kinases) 1/2 independently of G proteins [15].

GPCR Ligands

GPCR ligands are generally divided into agonists and anta-gonists. They can be orthosteric ligands which bind at the ligand-binding or active site, or allosteric modulators that regulate receptors by binding at another site. An agonist can be defined as a drug which upon binding its receptor produces a biological response, in contrast to an antagonist which produces no response. In comparison to a 'full' agonist, a partial agonist produces less than the maximal response at full receptor occupancy. In a system with spare receptors or receptor reserve, a maximal response can be obtained without occupying all the receptors. According to the two state model for receptor activation, receptors switch between a resting state (R) and an activated state (R*) [16]. An antagonist binds equally well to both conformations, whereas an agonist preferentially binds R* over R, shifting the equilibrium towards R*. In 1980, the ternary complex model for GPCRs was proposed, where agonist binds with high affinity to the receptors that are coupled to G protein, whereas antagonist binding is independent of G protein [17]. This model was extended to 'constitutively activated' receptors which isomerize to the R* state in the absence of an agonist [18]. The main effect of a (neutral) antagonist is to reduce agonist occupancy of the receptor by blocking the active site. Inverse agonists are those that possess 'negative intrinsic activity' in that upon binding to constitutively activated receptors, they shift the equilibrium from R* towards R [19]. In agonist directed trafficking or biased agonism, receptors can attain several activated states, each corresponding to a different signal route, such that each agonist may preferentially direct receptor activation towards a specific signaling pathway [20].

GPCR Drug Discovery

GPCRs form one third of novel drug targets launched in the decade of 1990-2000. At least 30% of all current experimental and marketed small-molecule drugs act on GPCRs, including drugs for a wide array of CNS disorders. Hence GPCR modulators represent the largest single drug class. The GPCR family is estimated to be 15% of the 'druggable' subset of the human genome (genes expressing proteins able to bind drug-like small molecules) [21]. Hence GPCRs are major drug discovery targets and the focus of intense research by both academia and the pharmaceutical industry.

The completion of the sequencing of the human genome has resulted in the identification of 'orphan' receptors by sequence homology. These receptors number in the hundreds and are the subject of increasing patent files, but whose ligands and functions are not yet known. After its identification, a receptor's physiology, function and disease-relevance can be postulated based on its anatomical distribution, expression levels during disease and any identified ligands. The identification of potential ligands is usually accomplished by high throughput screening of these receptors against corporate compound libraries via receptor binding or functional assays [22]. Screening of libraries against constitutively active receptors can identify antagonists or inverse agonists. Where receptors are found to participate in a signaling complex or pathway, the additional proteins involved increases the number of potential drug targets. The structure of identified ligands can be used as a template to design synthetic small-molecule modulators – be it agonists, antagonists, inverse agonists or allosteric regulators. Drug design is facilitated by the availability of GPCR structural data, which can come from either structural studies or *in silico* modeling. Promising lead compounds are then validated by *in vitro* and *in vivo* assays. However, ligand identification remains the important first step in the drug discovery process. Table **1** summarizes recent patents for ligands of three CNS GPCRs, to be surveyed in this review.

METABOTROPIC GLUTAMATE RECEPTOR

Background

L-Glutamate is the major excitatory neurotransmitter in the mammalian brain. It plays a vital role in the mediation of excitatory synaptic transmission, as most central neuronal circuits involve glutamatergic transmission at some level [23]. It binds to and activates two groups of postsynaptic receptors. The first group is the ionotropic glutamate (iGlu) receptors comprising NMDA (N-methyl-D-aspartate), AMPA (alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid), and Kainate receptors, which are ligand-gated ion channels transducing glutamate binding into cation influx in postsynaptic neurons. The second group is the metabotropic glutamate (mGlu) receptors which mediate the slower actions of glutamate. They are GPCRs belonging to the Glutamate Receptor family, which also includes two GABA (gamma-aminobutyric acid) receptors, a calcium-sensing receptor and probably taste receptors. These receptors are characterized by a large N-terminal extra-cellular domain [24].

There are eight mGlu receptors subdivided into three groups based on sequence homology, pharmacology and intracellular signaling mechanisms. Group I mGlu receptors (comprising mGluR1 and mGluR5) are coupled to $G\alpha_q$ to activate phospholipase C (PLC) for mobilization of intracellular calcium, while Groups II (mGluR2 and mGluR3) and III (mGluR4, 6, 7 and 8) receptors couple to $G\alpha_i$ for inhibition of adenylyl cyclase [25].

Table 1. Recent Patents for GPCR Ligands

Target GPCR	GPCR subtype	Type of Ligand	Patent Holder	Patent Number	Date of Publication	Ref.
Metabotropic Glutamate	mGluR5	Antagonist	Hoffmann-La Roche, Inc.	US7153874	Dec 26, 2006	[35]
Metabotropic Glutamate	mGluR5	Antagonist	Hoffmann-La Roche, Inc.	US7091222	Aug 15, 2006	[36]
Metabotropic Glutamate	mGluR5	Antagonist	NPS Pharmaceuticals, Inc.; Astrazeneca AB.	US7112595	Sep 26, 2006	[37]
Adenosine	A_1	Agonist and Partial Agonist	CV Therapeutics, Inc.	US7022681	April 4, 2006	[47]
Cannabinoid	CB_1	Antagonist and/or Inverse Agonist	Merck & Co., Inc.	US7057051	June 6, 2006	[67]

Drug and Disease

The importance of glutamate as an excitatory neurotransmitter suggests that mGlu receptors participate in a wide variety of CNS functions, regulating both presynaptic control of glutamate release, as well as postsynaptic control of neuronal responses to glutamate. Hence they have been suggested to be involved in a variety of pathophysiological processes and disease states affecting the CNS, where much of the pathology is thought to be due to excessive glutamate-induced excitation of CNS neurons.

Metabotropic glutamate receptors mediate basal excitatory synaptic transmission and play multiple roles in synaptic plasticity including long-term potentiation (LTP) and long-term depression (LTD). These are thought to underlie learning and memory, thus making them potential drug targets for CNS disorders such as Alzheimer's disease [26].

Metabotropic glutamate receptors are also seen as potential targets in the treatment of both convulsive and non-convulsive seizures because they modulate iGluR at glutamatergic synapses only under certain conditions, and so may selectively reduce hyperactive glutamatergic synapse communication with the cortex of thalamus without significantly affecting normal response rates [27].

Since mGlu receptors are differentially distributed in several basal ganglia nuclei where they regulate neuronal signaling, and the direct and indirect pathways of the basal ganglia act as a fine tuning mechanism in movement control, the pharmacological manipulation of these receptors may restore the balance between the direct and indirect pathways and thus relieve the symptoms of Parkinson's disease and related movement disorders, without the side effects of current dopamine replacement therapies [28]. Group I mGlu receptor antagonists and Group II mGlu receptor agonists have been proposed as suitable candidates [29].

Recent Patent

The mGluR5 has been implicated in synaptic plasticity, learning and memory, and shown to be necessary for some forms of LTP and LTD in different brain regions, making them potential therapeutic targets for many CNS disorders [30]. Studies in animal models have suggested that mGluR5 antagonists may be useful in various psychiatric and neurological disorders, chronic pain, substance abuse/withdrawal and obesity [31].

The majority of mGlu receptor ligands are amino-acid derivatives that bind to the glutamate-binding site situated within the large N-terminal domain of the receptor. Highly selective ligands for mGlu receptors had been a challenge to develop, due in part to their highly conserved glutamate binding site. In recent years, an increasing number of selective agonists, antagonists and allosteric modulators have been developed which activate or inhibit specific mGlu receptor subtypes [26]. MPEP (2-methyl-6-(phenylethynyl)-pyridine) is the first potent and selective mGluR5 antagonist, while the newer MTEP (3-[2-methyl-1,3-thiazol-4-yl)ethynyl]pyridine is reported to be more selective for mGluR5 over mGluR1, and with fewer off-target effects such as inhibition of NMDA receptors [32]. These com-pounds belong to a novel family of non-amino acid-like allosteric mGluR5 modulators. They are non-competitive antagonists which bind at sites within the seven transmembrane spanning domain, independently of glutamate binding at the N-terminal extracellular domain [33]. They are postulated to negatively modulate the receptor by blocking conformational changes effected by agonist binding [34]. Pharmacological characterization of these antagonists has enabled better understanding of their potential uses, the functions of mGLuR5 and ways to develop more selective ligands.

Patent US7153874 [35] is for novel imidazole derivatives potentially useful for treatment or prevention of mGluR5-mediated diseases. These are the mGluR5 antagonists 4-[1-Aryl-imidazol-4-ylethynyl]-2-alkyl-pyridine and 1-heteroaryl-imidazol-4-ylethynyl]-2-alkyl-pyridine derivatives. A second patent (US7091222) from the same inventors was for compounds that differed from those in US7153874 by the nature of side-chain substituents [36]. In competition experiments with radiolabeled MPEP for binding mGluR5, the compounds from both patents were reported to block MPEP binding with K_i values in the

range of 16-122nM, suggesting a relatively high affinity for the allosteric binding site used by MPEP. Patent US7112595 is for heteropolycyclic antagonists of Group I mGlu receptors, particularly the mGluR5 [37]. These compounds typically incorporate an oxadiazole moiety in between the benzene rings of a benzaldazine platform.

Overall, these patented compounds bear structural resemblance to the benzaldazine analogs reported to constitute a novel family of selective allosteric mGluR5 modulators that bind at the MPEP binding site [38]. Metabotropic glutamate receptor ligands which are amino acids or its derivatives tend to have poor blood-brain barrier penetration. These compounds are all non-amino-acid derivatives. They are likely to be selective allosteric mGluR5 antagonists that bind at a transmenbrane domain site to negatively modulate the receptor. These should serve as useful tools to understand modulation of mGluR5, and may find important therapeutic uses.

ADENOSINE RECEPTOR

Background

Originally known as P_1 purinoceptors, four adenosine A_1 receptor subtypes have been cloned, pharmacologically characterized, and designated A_1, A_{2A}, A_{2B} and A_3, following their chronological discovery [39]. They are classified within the Rhodopsin family, under the α subgroup and the MECA receptor cluster which also comprise the melanocortin, endothelial differentiation and cannabinoid receptors [24].

The A_1 and A_3 subtypes couple to the $G\alpha_i$ family subunits to inhibit adenylyl cyclase while A_{2A} and A_{2B} subtypes stimulate adenylyl cyclase via $G\alpha_s$. Other signaling pathways include the activation of mitogen-activated protein (MAP) kinase by the A_{2A} subtype via p21(ras) and rap1[40].

Adenosine is an endogenous purine nucleoside which is an important neuromodulator in the nervous system. It is present in every cell type where it is produced during metabolic stress and released into the extracellular space, to produce its effect by binding to adenosine receptors. Its range of physiological effects include neurotransmitter release, vascular smooth muscle tone, heart rate, atrial contractility, lipolysis and regulation of platelet, kidney and white blood cell function. The A_1 subtype is the most comprehensively studied.

Drug and Disease

A_1 receptors are most densely expressed in the brain (cortex, hippocampus and cerebellum). Their activation inhibits many neurons postsynaptically by inducing or modulating ionic currents, and presynaptically by reducing transmitter release. A_1 receptor agonists mimic these inhibitory effects and are potentially useful neuroprotective agents. Analogs of adenosine have been shown to have sedative and anti-convulsant effects and to modulate dopaminergic control of movement [41]. Antagonists lead to excitatory effects, and may be used to enhance cognition in geriatric therapy, and for various forms of dementia, such as Alzheimer's disease [42]. Blockade of striatal A_{2A} receptors have produced neuroprotective effects [43], suggesting its relevance in neurodegenerative disorders such as Parkinson's disease. The A_{2B} subtype is the least well characterized because of the lack of potent, selective agonists [44]. In humans, the A_3 subtype has the most restricted distribution, with low density in the brain.

The adenosine receptors are important targets in drug research. Intervention of adenosine metabolism is a potential tool for treating CNS disorders such as epilepsy, sleep-, movement- (Parkinsonism or Huntington's disease) or psychiatric disorders (Alzheimer's disease, depression, schizophrenia or addiction) [45]. The development of new ligands has so far been directed by medicinal chemistry and many have been generated by introducing modifications to the structure of the lead compounds adenosine and methylxanthine [46]. The prototypical full A_1 receptor agonists are the N^6-substituted adenosine analogs CCPA (2-chloro-N^6-cyclopentyl-adenosine), CPA (N^6 -cyclopentyladenosine) and R-PIA ((R)-N^6 – (2-phenylisopropyl)adenosine). A partial agonist is postulated to give less unwanted effects by not evoking all possible responses through activating only a subset of receptors in a system with receptor reserve.

Recent Patent

Both agonists and antagonists for the A_1 receptor have great therapeutic potential in a wide range of clinical conditions. The adenosine receptors have been investigated as drug targets for many years, but two factors make it difficult to develop useful agents. First, the receptors are ubiquitously expressed, potentially producing multiple physiological effects in peripheral tissues. Second, existing A_1 receptor agonists tend to activate at least one other subtype [41].

Patent US7022681 [47] is for compounds based on the structure in Fig. (**1**). They represent optimization of the lead compound N^6-substituted adenosine, and are described as A_1 receptor agonists and partial agonists. These are patented for possible use in such CNS disorders as pain, epilepsy and emesis. Their usefulness will be dependent on a high degree of selectivity for the A_1 receptor, while the partial agonists may give lesser unwanted effects by evoking mainly the desired therapeutic effect, and deliver sustained results through reduced receptor downregulation and desensitization [48].

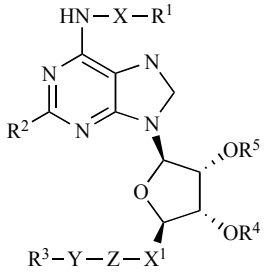

Fig. (1). Structural formula of A_1 receptor agonists in Patent US7022681.

CANNABINOID RECEPTOR

Background

Two cannabinoid receptor subtypes have been cloned, CB_1 and CB_2, both coupled to $G\alpha_i$ subunits for inhibition of adenylyl cyclase and activation of MAP kinase. CB_1 receptors also inhibit presynaptic N- and P/Q-type calcium channels and activate inwardly rectifying potassium channels. Other signal transduction pathways involve focal adhesion kinase, PI3K, sphingomyelinase and nitric oxide synthase (reviewed by Mackie [49]). CB_1 receptors are expressed mainly in neurons, while CB_2 receptors are also found in the brain

but principally they are expressed in non-neuronal tissues, primarily on immune cells. Brain CB_1 receptors have been shown to undergo constitutive activation [50]. There is emerging evidence for additional types and subtypes of cannabinoid receptors [51].

The main psychoactive component of the *Cannabis sativa* plant, Δ^9- tetrahydro-cannabinol (THC), is a cannabinoid receptor agonist. The search for an endogenous ligand led to the discovery of an endogenous cannabinoid system comprising the receptors, the endogenous ligands (endocannabinoids) and their associated molecular targets, transport system and degrading enzymes [52]. The first two endocannabinoid families with cannabimimetic activity discovered are the acyl ethanolamides (such as anandamide (arachidonoyl ethanolamide or AEA)) and the acyl glycerols exemplified by 2-arachidonylglycerol (2-AG). They are agonists at both CB_1 and CB_2 receptor subtypes, with AEA being a partial agonist and 2-AG a full agonist. Both endocannabinoids are expressed in the brain, with 2-AG levels about 100 times higher than AEA [53]. The third endocannabinoid in the series is the ether-type 2-arachidonylglyceryl ether (noladin ether) [54].

Endocannabinoids are produced when needed, by cleavage of membrane lipid precursors [55]. Their action is terminated in part by uptake into cells, a process involving a putative endocannabinoid membrane transporter (EMT), for which inhibitors have been developed. Anandamide and related ethanolamides are degraded by fatty acid amine hydrolase (FAAH) while 2-AG is degraded by monoacylglycerol lipase (MAG lipase or MGL). FAAH is better characterized than MGL, and inhibitors have been developed.

Drug and Disease

Neuronal stimulation induces synthesis of endocannabinoids which act as retrograde signaling agents by activating presynaptic CB_1 receptors to inhibit neurotransmitter release in a selective and restricted manner [56]. Cannabinoid receptor agonists as well as endocannabinoids activate brain signaling pathways linked to neuronal repair and neuroprotective responses [57]. As such, cannabinergic agents have been shown to increase neuronal viability from ischemic events, stroke, traumatic brain injury, Alzheimer's disease, Parkinson's disease and motor neuron disorders. Accordingly, enhancement of endocannabinoid responses via inhibition of FAAH or EMT have been shown to reduce neuronal damage from excitotoxicity [58]. In contrast to CB_1, less is known about the physiological roles of CB_2 receptors, which most likely include modulation of cytokine release from immune cells [59]. As CB_2 receptor agonists have been reported to promote analgesia, they may find clinical application in chronic pain [60].

Development of selective inhibitors of endocannabinoid transport and degradation may allow treatments that avoid the psychoactive properties of cannabinoid agonists. It may also avoid receptor desensitization associated with agonist stimulation by working through endocannabinoids normally released in response to an insult [58].

When development of antagonists/inverse agonists of the cannabinoid receptors represented one of the most active areas in drug development, most major pharmaceutical companies were believed to be undertaking parallel CB_1 antagonist development programs [52]. Given that cannabis enhanced appetite, it was postulated that CB_1 receptor antagonists could be used in obesity as an appetite suppressant. The first CB_1 receptor antagonist/inverse agonist reported was rimonabant (SR141716A or Accomplia®), a diarylpyrazole derivative with a more than 1000-fold selectivity (at sub-micromolar concentration) over CB_2 subtypes and non-cannabinoid receptors [52]. Large clinical trials showed that it promoted sustained weight loss and improvement in lipid profiles, central obesity, insulin

resistance and incidence of metabolic syndrome [61]. Since CB_1 antagonists also attenuate the rewarding properties of opioids, it has been postulated to be useful in 'craving' disorders such as smoking cessation and drug abuse [62].

Recent Patents

With rimonabant as a prototype, many analogs and other CB_1 antagonists have been developed, mostly for the therapeutic indications of obesity and smoking cessation. Following rimonabant were its analogs such as AM251 [63], AM281 and SR147778 (surinabant) [64], all diarylpyrazoles with high receptor affinity. Other derivatives include the 3,4-diarylpyrazoline SLV-319 (ibipinabant), the fused bicyclic derivative otenabant and the acyclic amide taranabant [65].

Patent number US7057051 [66] is for compounds which are antagonists and/or inverse agonists of the CB_1 receptor. Unlike the aforementioned compounds, these are substituted imidazoles based on the structural formula in Fig. (**2**). These compounds are rimonabant analogs with an imidazole ring in place of the pyrazole ring of rimonabant derivatives. Aside from that, most of the compounds retain the structural determinants of rimonabant's potency and CB_1 receptor selectivity, as described by Lan *et al*: a para-substituted phenyl ring at the 5-position, a carboxamide at the 3-position and a 2,4-dichlorophenyl substituent at the 1-position [67] of the imidazole ring corresponding to rimonabant's pyrazole ring. They were also reported to show pharmacokinetic and pharmacodynamic properties suitable for human drug therapy, and are patented for use in diseases mediated by the CB_1 receptor. However, recent events have cast doubt on the utility of rimonabant-like compounds in obesity. Rimonabant was withdrawn (following its suspension by the European Medicines Agency in October 2008) in the wake of significant psychiatric side effects, notably anxiety and depression. This led to the withdrawal from clinical development of taranabant, otenabant, ibipinabant and surinabant by their respective developers [68].

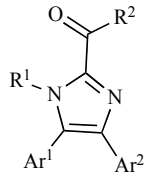

Fig (2). Structural formula of substituted imidazoles of Patent US7057051.

As all these were antagonists with inverse agonist activity, it is postulated that compounds that are either neutral antagonists, or inverse agonists without antagonist activity may give a more acceptable side effect profile. Consistent with this, animal studies have shown nausea and vomiting caused by neutral antagonists but not antagonists with inverse agonist activity [69]. A second strategy to limit psychiatric side effects would be to use antagonists that do not penetrate the central nervous system but instead block possible overactivity of the endocannabinoid system in peripheral cells and organs. One such agent has been shown to reduce food intake in rats [70]. Lastly, given that blockade of CB_1 receptors in humans can result in depression, it may be possible to treat depression by activating these receptors. As administration of CB_1 receptor ligands would affect all available CB_1 receptors, a more selective effect may be obtained by enhancing the action of physiologically-released endocannabinoids. EMT or FAAH inhibitors would be potential candidates for this role.

CURRENT AND FUTURE DEVELOPMENTS

GPCRs constitute the single largest class of small-molecule drug targets [21]. With the availability of the human genome sequence, it has been reported that out of the estimated 367 human GPCRs for endogenous (non-olfactory) ligands (endoGPCRs) [4], about 200 have had their ligand identified, leaving about 160 with unknown natural ligand, the so-called orphan receptors. These receptors are the focus of many pharmaceutical companies that use high throughput binding assays to determine the endogenous ligands ('deorphanising' the receptor). New approaches to ligand development will likely combine the traditional medicinal chemistry methods with advances in the fields of genomics, proteomics and bioinformatics. Due to the high cost of a high throughput screening campaign and the high attrition rate of lead compounds during clinical testing, computational approaches are increasingly used to screen only candidate molecules possessing drug-like properties.

A second major effort is to screen for small molecule ligands without prior knowledge of endogenous ligands, by high throughput functional assays. This is commonly done with cell-based assays, which is continuously improving with novel technological advances. A new non-invasive assay which does not require labeling, utilizes microelectrodes embedded to the bottom of microwell plates to measure changes in cell impedance, which sensitively reflect changes in cell morphology upon agonist treatment [71]. The use of standardized cell culture using frozen cell division-arrested cells offers reduced data variability Advances in screening technologies hold promise of improving and expediting the process of uncovering novel modulators of GPCR function. Since currently only about 10% of endoGPCRs have been targeted by drugs [4], the race to identify novel GPCR-directed ligands carries immense potential for new drug discovery [72].

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