1. Introduction

1.1 Drug design

In the pharmaceutical industry, discovery and development of a new potential therapeutic agent is the most complex and tedious process which involves a lot of money, time and manpower. This typical drug discovery cycle from the first synthesis to its introduction into the market takes approximately US $800 million to US $1.8 billion with around 10-15 years of hard work.[1] Around 40-60 % of molecules fail in the pharmacokinetic parameters such as absorption, distribution, metabolism and excretion as well as due to toxicity reasons. Hence, the number of approved new molecular entities is decreasing day by day. To address these issues there is a need to develop alternative strategies for the development of a new molecular entity having an effective drug profile.[2]

Drug design may be defined as "*an inventive process of finding new medications based on the knowledge of a biological target.*" Drug design frequently relies on computer aided drug design, also known as molecular modeling, which can be considered as an effective strategy for the discovery and development of a drug molecule with higher cost benefit, time saving as well as reduction in the chances of failure in the final stages.[3] Drug design can be broadly classified in two categories as listed below (Fig. 1.1):

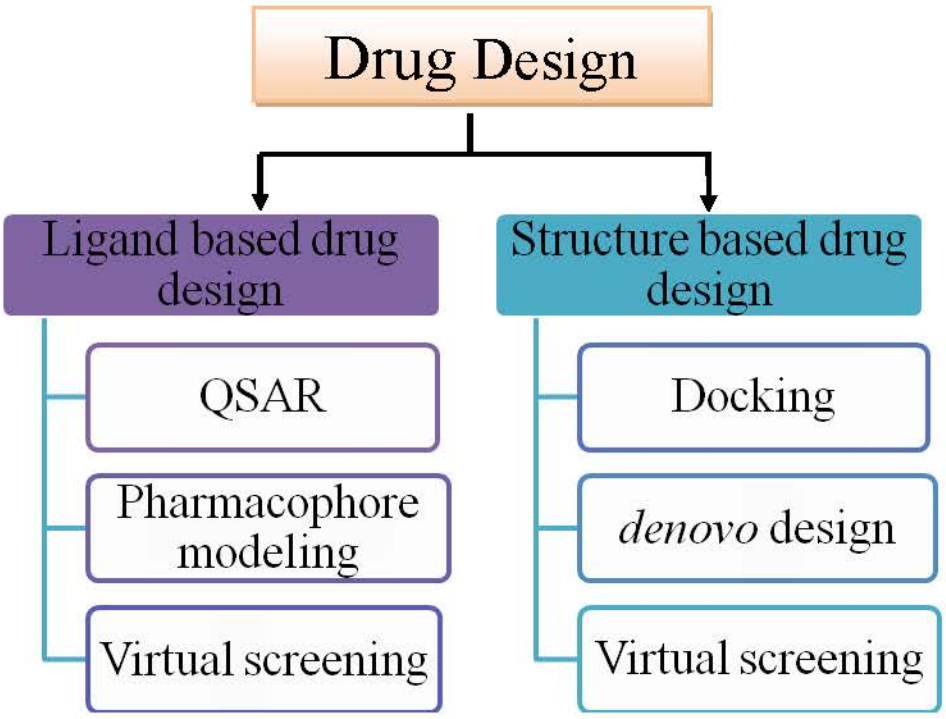


Fig. 1.1 Different types of drug design techniques.

1.1.1 Ligand based drug design

Ligand based drug design is also known as indirect drug design. This strategy is utilized when the structure of the enzyme or receptor is unknown. It depends on the knowledge or information of other molecules that bind to the same biological target. A dataset of structurally similar compounds having a wide range of biological activity is used to develop a reliable ligand based model.[1,4,5]

1.1.1.1 Quantitative structure activity relationship (QSAR)

More than fifty years ago, Corwin Hansch founded Quantitative structure activity relationship (QSAR) modeling. QSAR is defined as a mathematical correlation between the chemical structures and biological activity of the compounds which is used to predict the biological activity of new compounds. QSAR is the most commonly used computational tool in the medicinal chemistry. In QSAR modeling, chemical descriptors are the core of the technique. Different types of chemical descriptors have been identified which reflect various levels of chemical structure representation. These include a range of parameters from molecular formula (1D) to two-dimensional structural formula (2D) to three-dimesional conformational dependent (3D) properties. The most popular 3D-QSAR approaches are CoMFA and CoMSIA models.[1,6] PHASE, MOE, Scigress and MAPS are some other commonly used 3D-QSAR softwares.

1.1.1.2 Pharmacophore modeling

Ehrlich introduced the concept of pharmacophore for the first time in 1909. According to Ehrlich, a pharmacophore is defined as 'a molecular framework that carries (phoros) the essential features responsible for a drug's (pharmacon) biological activity'.[7] Recently, IUPAC has defined pharmacophore as 'an ensemble of steric and electronic features that is necessary to ensure optimal supramolecular interactions with a specific biological target and to trigger (or block) its biological response.'[8] Pharmacophore model can be established either by ligand based pharmacophore modeling or structure based pharmacophore modeling. Pharmacophore model can be developed by using a set of active compounds which are superposed on each other and then extracting the common chemical features which are essential for the biological activity. Pharmacophore modeling has been widely used in virtual screening, *de novo* design, lead optimization and design of multitarget drugs.[9] Some softwares for developing pharmacophore are PHASE, DISCO, Catalyst, LignadScout, GASP etc.

1.1.1.3 Virtual screening

It is always a very difficult task for a medicinal chemist to decide about the compounds to synthesize. A large number of compounds have already been synthesized and a medicinal chemist has to choose compounds out of thousands or millions of existing compounds for the purpose of screening. To overcome this problem, computational chemists have developed some computer based programs that are capable of automatically screening of very large libraries of compounds for a particular type of activity. This process is called as virtual screening. It is a fast and cost effective tool for screening a huge database computationally in search of a novel lead molecule having a particular drug like properties. Different filters such as Lipinski's rule of five, pharmacophore model, QSAR model and docking have been used in the virtual screening process.[4,10]

1.1.2 Structure based drug design

Structure based drug design also called direct drug design, is based on the knowledge of 3D structure of the enzyme or the receptor obtained through X-ray cryastallography or NMR spectroscopy. This approach is based on the ability of the molecule to favourably interact with a specific biological target (enzyme/receptor) and exert desired biological activity. Thus, information about novel compounds can be easily extracted by accurate analysis of the protein binding site.[1,4]

1.1.2.1 Docking

Docking is the most commonly used and well known structure based drug design approach. It is used to predict the possible binding interactions of a compound that binds with in the active site of the biological target. It is quite similar to lock and key model in which the target protein can be considered as lock and the ligand can be considered as a key; the correct orientation of the key (ligand) can open up the lock (target protein). One can easily know the orientation of the ligand in the active site of the target protein which helps in further optimization of the lead molecule. Hence, docking plays a pivotal role in the drug design process.[1,11-13] FlexX, GOLD, GLIDE, Surflex, DOCK are some commonly used softwares for docking studies.

1.1.2.2 *De novo* design

De novo is a Latin word meaning start afresh, from the beginning, from the scratch. This approach is employed when the three dimensional structure of the target

protein is known and the structure of ligand is to be established. So, it is a process in which the 3D structure of the receptor is used to identify new molecules that can bind to the same target protein. This approach is based on a set of predefined molecular fragments. According to the binding site in the target protein, the algorithm starts with the given fragment and decides which fragment to add next (which edge to take) based on precalculated transition probabilities. Each node represents exactly one fragment. The process stops when all potential growth sites are saturated. By combining these molecular fragments, a new molecule is obtained. This approach is widely used in drug designing nowadays.[14,15] Some frequently used softwares for *de novo* design are LUDI, TOPAS, LEA3D, FOG, FLUX, MEGA, AutoGrow etc.

1.1.2.3 Virtual screening

Structure based virtual screening is used to identify putative hits out of thousands or lacs of compounds to the targets of known structure. This approach is based on a comparison of the three dimensional structures of the small molecules with the putative binding pockets.[4] The key steps of structure based virtual screening are:

- Refining of the available 3D-structure of the target protein and a library of compounds for docking.
- Determination of a favourable binding pose for each compound.
- Docking of each compound in the active site of the target protein.
- Ranking of the docked compounds.[4]

Thus, both ligand based drug design and structure based drug design have become integral part of drug design and discovery programs. It is possible to solve various problems with the help of these computational techniques these days. It helps us to design and develop a more potent, selective molecule having lesser adverse effects and better pharmacokinetic profile.

1.2 Obesity

World Health Organization (WHO) has defined 'overweight and obesity' as abnormal or excessive fat accumulation in body that may impair health.[16] The number of overweight and obese population is increasing with an alarming rate day by day due to lack of sufficient physical work, sedentary life style and consumption of high-fat/energy diet.[17,18] According to WHO reports in 2014, more than 1.9 billion adult population was overweight, of which over 600 million adults were obese, while 41

million children below the age of 5 were overweight or obese. Body mass index (BMI) is used to measure overweight condition and obesity by a simple index of weight-for-height. According to WHO, BMI equal or less than 18.5 kg/m^2 indicates underweight condition whereas normal weight range is 18.5 kg/m^2 to 24.9 kg/m^2. BMI from 25 kg/m^2 to 29.9 kg/m^2 indicates overweight condition whereas from 30 kg/m^2 to 34.9 kg/m^2 indicates obesity. BMI in the range of 35 kg/m^2 to 39.9 kg/m^2 indicates severe obesity condition while BMI of 40 kg/m^2 or more indicates morbid obesity. Obesity is the fifth most common leading risk factor for global deaths these days.[16] A list of top 10 most obese countries is given in Table 1.1.[19] Obesity is associated with a number of noncommunicable diseases such as diabetes mellitus, gout, dyslipidemia, osteoarthritis, lower back pain, poor mobility, hypertension, ischemic heart disease and stroke, sleep apnea, hernia, hepatic strearosis, severe pancreatitis and certain types of cancers[20,21] indicating that obesity has become one of the major epidemic health problems now a days.[22] Based on the above facts it is clear that obesity can not be a mere cosmetic issue.

Table 1.1 List of top 10 most obese countries in the year 2014.[19]

Rank	Top 10 most obese countries		Top 10 most obese OECD countries	
	Country	**% of obese adult population**	**Country**	**% of obese adult population**
1	Cook Islands	50.8	United States	33.7
2	Palau	47.6	New Zealand	29.2
3	Nauru	45.6	Australia	28.6
4	Samoa	43.4	United Kingdom	28.1
5	Tonga	43.3	Mexico	28.1
6	Niue	43.2	Canada	28.0
7	Marshall Islands	42.8	Chile	27.8
8	Qatar	42.3	Czech Republic	26.8
9	Kiribati	40.6	Slovakia	25.7
10	Tuvalu	40.3	Ireland	25.6

OECD: Organisation for Economic Co-operation and Development

1.3 Different approaches for the treatment of obesity

Treatment of obesity is directly associated with the severity of overweight, coexisting chronic diseases and fuctional limitations. The major treatment of obesity includes lifestyle intervention, pharmacotherapy and bariatric surgery.[23]

1.3.1. Lifestyle intervention

Lifestyle intervention is mainly based on the modifications in the eating behaviour and physical activity which are the initial parameters for the weight management. The dietary therapy included decrement in the energy intake as well as increment in the energy expenditure so that negative energy balance can be produced. Obesity can be controlled by reducing diet in a specific manner. A successful reducing diet regimen can lose around 5 % to 10 % of the initial weight.[23,24]

Physical activity plays an important role in the treatment of obesity. An extra 2000 steps per day walk would expend about 100 kcal more. A 10 % or more initial weight loss can be achieved by an extra 6000 to 8000 steps daily walk which expends around 300 to 400 kcal per day. So, increased physical activity is more helpful to initial weight loss.[24]

Behaviour therapy approach is also very helpful to achieve weight loss. Behaviour therapy defines a set of principles and techniques in the modifications of the eating, activity and thinking habits. Behaviour therapy includes clinic setting, self-help groups, commercial weight loss programs and internet based programs.[25]

Thus, the two main strategies are monitoring food intake and increasing physical activity for controlling the obesity condition.

1.3.2 Pharmacotherapy

Researchers are engaged in finding of newer targets for the treatment of obesity. Various therapeutic targets have been identified for the management of obesity but none of them have offered a satisfactory anti-obesity agent till date. Hence, the quest for development of a "magic bullet" which could help lose body weight effectively is still on.[26] Various therapeutics such as glucagon-like peptide 1 (GLP-1) analogs, cholecystokinin (CCK-1) agonists, amylin analogs, peptide YY agonists, neuropeptide Y agonists, MCH1 receptor antagonists, ghrelin antagonists, MC4 receptor agonists, β_3 AR agonists, 5-HT_{2B} receptor agonists, 5-HT_{2C} agonists, 5-HT_6 receptor antagonists, dopamine agonists, lipase inhibitors, μ-opioid receptor antagonists, cannabinoid 1 (CB1) receptor antagonists, anticonvulsants, sympathomimetic agents, AgRP (agouti-related protein) inhibitors, mixed dopamine and noradrenaline reuptake inhibitors, $MetAP_2$ (methionine aminopeptidase) inhibitors, mixed noradrenaline/serotonin reptake inhibitors and mixed noradrenaline dopamine and serotonin reuptake inhibitors have been reported in the literature.[27] FDA approved phentermine (sympathomimetic amine)

in 1959 as an anti-obesity agent for short-term use but due to the risk of cardiaovascular effects and abuse potential, it was withdrawn from European market.[27,28] In 1999, FDA approved orlistat, a gastrointestinal and pancreatic lipase inhibitor acting peripherally (the first long-term use anti-obesity agent) and is available in the market. But unfortunately, orlistat has also showed a number of side effects such as gastrointestinal adverse effects like steatorrhea, flatulence, faecal urgency, faecal incontinence, malabsorbtion, abdominal pain, dyspepsia, upset stomach and reduction in absorption of fat soluble vitamins.[29,30] Lorcaserin, a selective 5-HT_{2C} receptor agonist was initially rejected due to its carcinogenicity in 2010 but FDA approved lorcaserin in 2012 on re-filing.[31] As, a number of mechanisms are involved in food intake modulation so, a combination therapy is supposed to be effective for the treatment of obesity. A significant weight loss and a favourable safety profile can be obtained by using multiple targeting agents.[28] FDA approved Qnexa, a combination of phentermine and topiramate after ensuring its safety profile in July 2012.[28] Contrave, a combination of naltrexone and bupropion has been approved as anti-obesity agent in 2014.[32] Recently, liraglutide, a glucagon-like peptide-1 receptor agonist has been approved as anti-obesity agent in 2014.[32] A list of drugs have been approved for the treatment of obesity but due to their serious side effects they were withdrawn from the market. Out of them, the most promising candidate was rimonabant, a CB1 receptor antagonist which was withdrawn due to its CNS side effects in 2008.[33] In 2010, another drug sibutramine, a NA/5-HT reuptake inhibitor was withdrawn due to its high risk of cardiovascular side effects.[29,32] Current status of all the approved and withdrawn anti-obesity drugs is shown in Table 1.2. The current status of approved drugs clearly indicates that there is a narrow range of available drugs for the management of obesity. Hence, it is the demand of the time to discover a potent anti-obesity drug having lesser side effects.[32]

Among all these therapeutic targets, CB1 receptor has proved its clinical significance as one of the potential therapeutic targets for the treatment of obesity. Although rimonabant and other CB1 receptor antagonists have been either withdrawn or terminated at different stages of development programmes, but researchers have not yet fully explored CB1 receptor antagonists. A lot of work is still remaining to be done for the development of CB1 receptor antagonists.[31] Designing of peripherally acting CB1 receptor antagonists could be promising therapeutic agents for the management of obesity.[34] Researchers are also focusing on two new intriguing suggestions, first one is low-dose combination of rimonabant with other anorectic agents such as gut peptide

CCK-8s, 5-HT_{2C} receptor agonists or opioid receptor antagonists. The second suggestion is based on recent genomic studies. According to this study, CNS side effects of rimonabant may be contributed by variants (polymorphisms) of the CB1 receptor gene alone or due to a combined effect of gene for serotonin transporter (SLC6A4).[31] Thus, a lot of scope still exists in the development of CB1 receptor antagonists. The focus of designing of centrally acting selective CB1 receptor antagonists has shifted to development of peripherally acting selective CB1 receptor antagonists for the treatment of obesity.

Table 1.2 Current status of developed anti-obesity drugs with their targets.[31,32]

Sr. No	Targets	Drug	Year of approval	Year of withdrawl	Current status
A. Agonists					
1.	Sympathomimetic agents	Phentermine	1959		**Approved for short-term use**
2.	Cholecystokinin (CCK-1) agonists	Gl181771X			Phase III
3.	Glucagon-like peptide 1 (GLP-1) analogs	Liraglutide	2014		**Available in market**
4.	Neuropeptide Y agonists	Obinepitide			Phase II
		Valneperil			Phase II
		TM30339			Phase I
5.	MC4 receptor agonists	MK-0493			Phase II
6.	5-HT_{2B} receptor agonists	Fenfluramine	1973	1997	
		Dexfenfluramine	1996	1997	
7.	5-HT_{2C} receptor agonists	Lorcaserin	Approved in 2012 on re-filing		**Available in market**
		ATH-X105			Phase II
8.	β_3 AR agonists	LY377604			Phase II
		KRP-204			Phase II
B. Antagonists/Inhibitors					
9.	MCH1 receptor antagonists	NGD-4715			Phase II
10.	5-HT_6 receptor antagonists	BVT.74316			Phase I
		PRX-07034			Phase I
11.	Dopamine (D_3) antagonists	GSK598809			Phase I
12.	CB1 receptor antagonists	Rimonabant	2006	2008	
13.	Neuropeptide Y5 receptor antagonists	S-2367			Abandoned in 2011 (Phase II)
14.	μ-Opioid receptor antagonists	GSK 1521498			Phase I
15.	Sodium glucose transporter-2 (SGLT-2) antagonists	Remogoflozin etabonate (GSK189075)			Abandoned in 2010 (Phase I)

		PF-04971729			Phase I
16.	Lipase inhibitor	Orlistat	1999		**Available in market**
		Cetilistat			Phase III
17.	Mitochondrial transfer protein inhibitor	SLx-4090			Abandoned in 2010 (Phase II)
18.	Agouti-related protein (AgRP) inhibitor	TPN435			Phase I
19.	Methionine aminopeptidase ($MetAP_2$) inhibitors	ZGN-433			Phase I
20.	Diacylglyceride acyltransferase ($DGAT_1$) inhibitors	AZD7687			Phase I
		PF-04620110			Phase I
C. Combination therapy					
21.	Norepinephrine/ dopamine releasing stimulators	Diethylpropion	1959		**Approved for short-term use**
		Benzphetamine	1960		**Approved for short-term use**
		Phendimetrazine	1982		**Approved for short-term use**
22.	NA/5-HT reuptake inhibitors	Sibutramine	1997	2010	
23.	Antiepileptic, dopamine/noradrenaline reuptake inhibitor	Empatic (Zonisamide + Bupropion)			Phase III
24.	5-HT/DA/NA reuptake blocker	Tesofensine			Phase III
		DOV21947			Phase II
25.	Sympathomimetic agent, weak carbonic anhydrase inhibitors (exact mechanism is still unkown)	Qnexa (Phentermine + topiramate)	Approved in 2012 on re-filing		**Available in market**
26.	Dopamine and noradrenaline reuptake inhibitors	Contrave (Bupropion + naltrexone)	Approved in 2014		**Available in market**
27.	Amylinomimetic/leptin analog	Pramlintide/ metreleptin			Phase II programme terminated in 2011

1.3.3 Bariatric surgery

The prevalence of class III (BMI $\geq$ 40 kg/m^2) obesity increased by 70 % from 2000 to 2010. Patients having BMI $\geq$ 40 kg/m^2 or those with a BMI $\geq$ 35 kg/m^2 associated with high risk co-morbid conditions such as type-2 diabetes or cardiopulmonary disease are considered for bariatric surgery. So, bariatric surgery used for reducing excess body weight is considered as one of the most efficacious treatment for the obesity. Based on the anatomical changes, bariatric surgery can be carried out

by three approaches i.e. laparoscopic gastric banding, gastric bypass (laparoscopic or open) and duodenal switch in which the surgery is carried out laparoscopically.[23]

1.4 Endocannabinoids

Endocannabinoid system (ECS) consists of endocannabinoids, enzymes and cannabinoid receptors (CB1R and CB2R). The endocannabinoids are a class of biologically active lipid mediators that bind and activate cannabinoid receptors.[35] The two main endocannabinoids are anandamide and 2-arachidonoylglycerol (2-AG) which act as neurotransmitters or neuromodulators.[33,35,36] These endocannabinoids are formed from arachidonic acid and released from different cells. After performing their functions, these endocannabinoids are metabolised by the enzymes such as fatty acid amide hydrolase (FAAH) and monoacylglycerol lipase (MAG lipase) as shown in Figure 1.2.[33,37] These endocannabinoids regulate food intake modulation and control of energy balance in both central and peripheral orexigenics. They are mainly located in the hypothalamus region whose one of the functions is to control food intake. Release of endocannabinoids in hypothalamic nucleus causes stimulation of feeling for taking food.[33] These endocannabinoids also regulte the peripheral lipid and glucose metabolism after binding to the peripheral CB1 receptors that are present in skeletal muscles, liver, white adipose tissue and pancreas.[38] Thus, increased level of endocannabinoids or over-activation of endocannabinoid system causes obesity[39] which can be controlled by blocking of the over-activity of endocannabinoids by antagonising the CB1 receptor in peripheral tissue. Thus, designing of peripherally acting CB1 receptor antagonists serves as a novel approach for the treatment of obesity.[40,41]

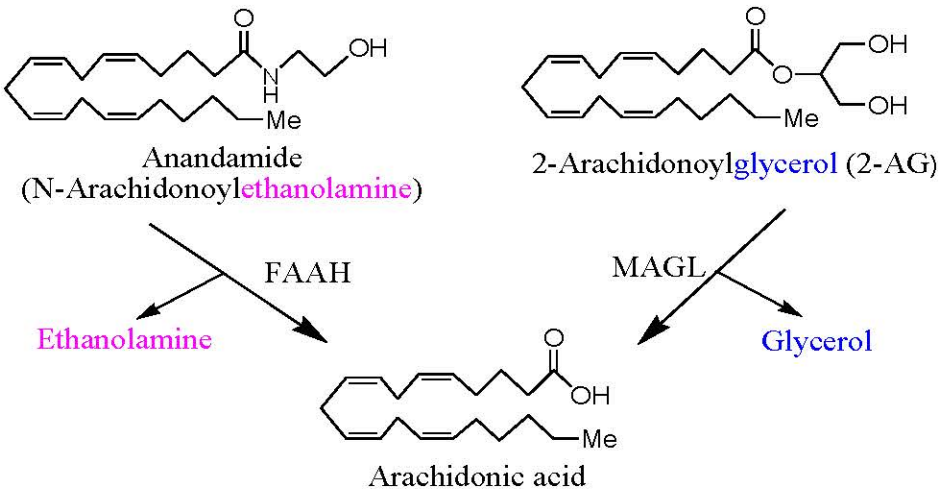


Fig. 1.2 Metabolism of anandamide and 2-arachidonoylglycerol.

1.5 Cannabinoid receptors

Almost 4000 years back, cannabis plant identified as *Cannabis sativa* was used as recreational drug because of its mind-altering effect as well as for therapeutic purposes.[42] More than 60 cannabinoids are isolated from cannabis plant. Out of them, the most active and clinically relevant psychoactive constituent is Δ^9-tetrahydrocannabinol (THC) identified in 1974. Dronabinol, a synthetic THC is commonly used in the treatment of anorexia associated with HIV infection and in post-chemotheraphy nausea and emesis.[43] Discovery of THC opened a lot of research areas in which extensive efforts were made for the identification of its specific receptors, known as cannabinoid type 1 (CB1) and cannabinoid type 2 (CB2) receptors.[44] There is a possibility of existence of a third cannabinoid type 3 (CB3) receptor but it has not been cloned yet.[45,46] CB1 and CB2 receptors were cloned in the years of 1990 and 1993 respectively.[47] The CB1 receptor is predominantly present in brain areas such as hippocampus, cerebellum, cortex and basal ganglia as well as in peripheral tissues including adipose tissue, urinary bladder, testis, liver, eye, ileum, pancreas and skeletal muscles. The CB2 receptor is present in cells of the immune system, spleen, thymus, bone marrow, tonsils, pancreas, microglial cells, peripheral nerve terminals, glioma and skin tumor cells as represented in Figure 1.3.[44,48-50]

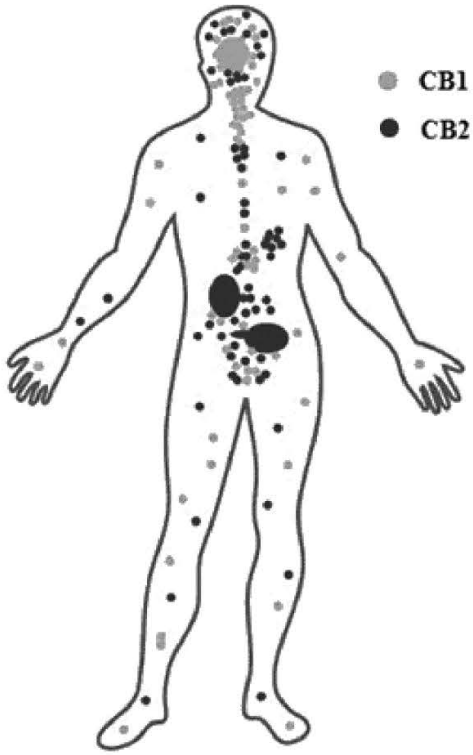
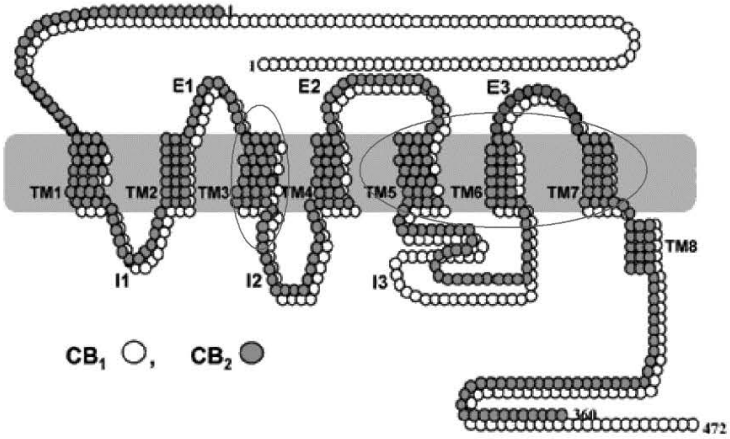


Fig. 1.3 Presence of CB1 and CB2 receptors in human body.

Both CB1 and CB2 receptors are G-protein coupled receptors (GPCRs) containing seven transmembrane α spirals (7-TMs) which are connected with each other by three intracellular loops (I1, I2 and I3) and three extracellular loops (E1, E2 and E3). The intracellular C-terminus region starts from a site of palmitoylation whereas the extracellular N-terminus consists of potential N-glycosylation site (Fig. 1.4). The binding pocket for a ligand is located in the crevice that is composed by the residues present in the TM3-5-6-7. Human CB1 and CB2 receptors are having a 44 % amino acid sequence identity.[22]



Parameters	CB1	CB2
Gene localisation	6q14-q15	1p36
Gene length (kb)	25	39
Exons	4	2
Amino acid sequence length	472	360

Fig. 1.4 Schematic representation of 2D structures of CB1 and CB2 receptors. Red circles represent the ligand binding site.[22]

1.6 Signal transduction mechanisms in CB1 receptors

Both CB1 and CB2 receptors belong to the rhodopsin GPCR family (Class A). Activated CB1 receptors located on the nerve terminals cause inhibition of both excitatory and inhibitory neurotransmissions in most of the brain regions such as cortex, cerebellum, hippocampus, hypothalamus, nucleus accumbens and striatum.[51] In neurons and peripheral tissues, activation of CB1 receptors causes inhibition of adenylate cyclase that decreases the production of cAMP, leading to attenuation of the protein kinase A (PKA) signalling cascade.[51,52] It also stimulates various members of mitogen-activated protein kinase family (MAPks) including p38 MAPK, p42/p44 MAPK, c-jun *N*-terminal kinase (JNK) and extracelluar signal-regulated kinase-1 and -2 (ERK 1/2).[52,53] Through $G_{i/o}$ protein, CB1 receptors are also coupled with ion channels, positively to A-type and inwardly-rectifying K^+ channels which decreases neuronal excitability and negatively to N-type and P/Q-type Ca^{2+} channels and D-type K^+ channels as shown in Figure 1.5.[54-57]

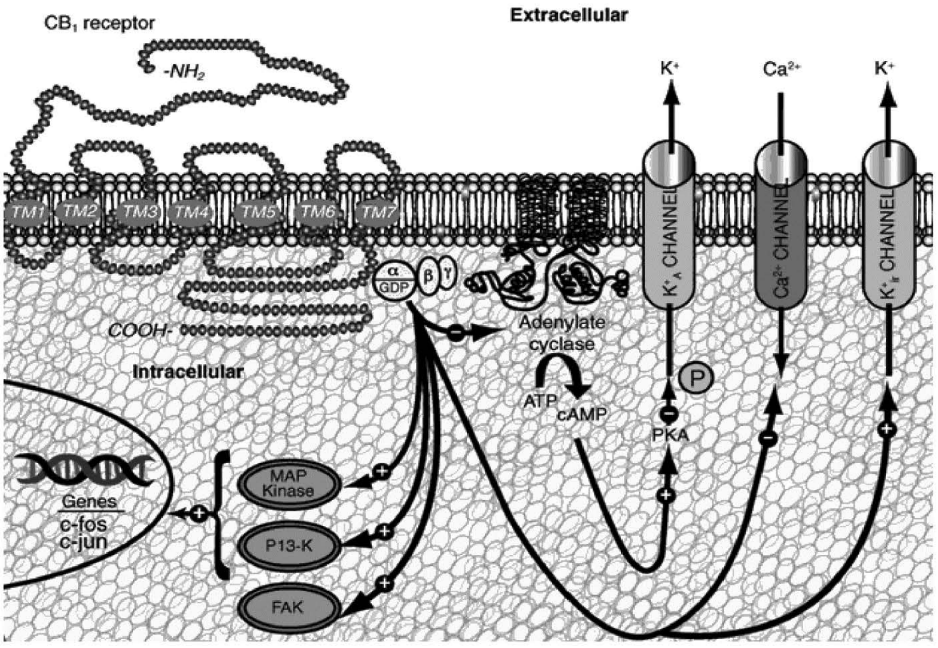


Fig. 1.5 Mechanism of action of CB1 receptor.[55]

1.7 Role of CB1 receptors in obesity

It is well established that ECS and especially CB1 receptor play an important role in energy homeostasis and modulation of food intake and fat metabolism.[58] The endogenous signalling transduction system in ECS acts on both central and peripheral nervous system. In human obesity, the ECS activity is increased.[59-62] The hypothalamus and the limbic systems are the main two functional levels in the central site to control the food intake. The hypothalamic ECS modulates the feeding by increasing the orexigenic signals and decreasing the satiety signals.[63] The hypothalamic ECS becomes activated after fasting for a short time which stimulates the appetite subsequently.[64] The limbic system finds a potential role in the control of over food intake.[63,65] In the Ventral Tegmental Area (VTA) of GABAergic terminals, stimulation of CB1 receptors causes increased dopaminergic neuronal activity which enhances the release of dopamine in the nucleus accumbens that results in increased consumption of food intake.[66] Thus, it has proved that the interaction between dopamine and mesolimbic endocannabinoid systems regulate food intake.

ECS regulates energy balance by modulation of carbohydrate and lipid metabolism as well as peripheral lipogenic mechanism in peripheral sites. CB1 receptors activation in the peripheral tissue boosts the adiponectin modulation, insulin secretion, lipid storage, lipogenesis and glucagon secretion.[63,65] CB1 receptors' stimulation in adipocytes decreases adiponectin, increases synthesis and storage of

triglycerides and facilitates glucose uptake which results in obesity.[67] Blocking of CB1 receptors induced reduction in plasma leptin level which decreased food intake.[68] It has been observed that stimulation of CB1 receptors activates lipoprotein lipase and increases the sequestering of free fatty acids through adipocytes. Thus, blockage of CB1 receptors in adipose tissue causes decrease in the free fatty acid level into the circulation that lowers the fat storage and enhances insulin sensitivity.[64,67,69-71] The endocannabinoids acting on CB1 receptors decrease the satiety signals generated by cholescystokinin in the GIT.[72] The level of ghrelin is also enhanced by the CB1 agonism which stimulates food intake.[73] Activation of CB1 receptors produces slow peristalsis and prolonged transit times in intestine that may cause obesity[64] and stimulates various lipogenic factors including sterol response element-binding protein-1C (SREBP-1C) in liver, which increases synthesis of fatty acid causing fatty liver.[74-76] ECS also regulates glucose metabolism and insulin sensitivity.[77] Antagonism of CB1 receptors increases basal oxygen consumption and glucose uptake in skeletal muscles which improves insulin sensitivity and increases energy expenditure. It is clear from the above facts that peripheral CB1 receptors play a pivotal role in the modulation of metabolism.[63,65] The different roles of ECS in central as well as peripheral nervous systems are shown in Figure 1.6.[64] ECS activity can be reduced by CB1 receptor antagonists resulting in increased energy expenditure and decreased food intake.[77] This indicates that development of CB1 receptor antagonists could be a potential approach in the treatment of obesity.

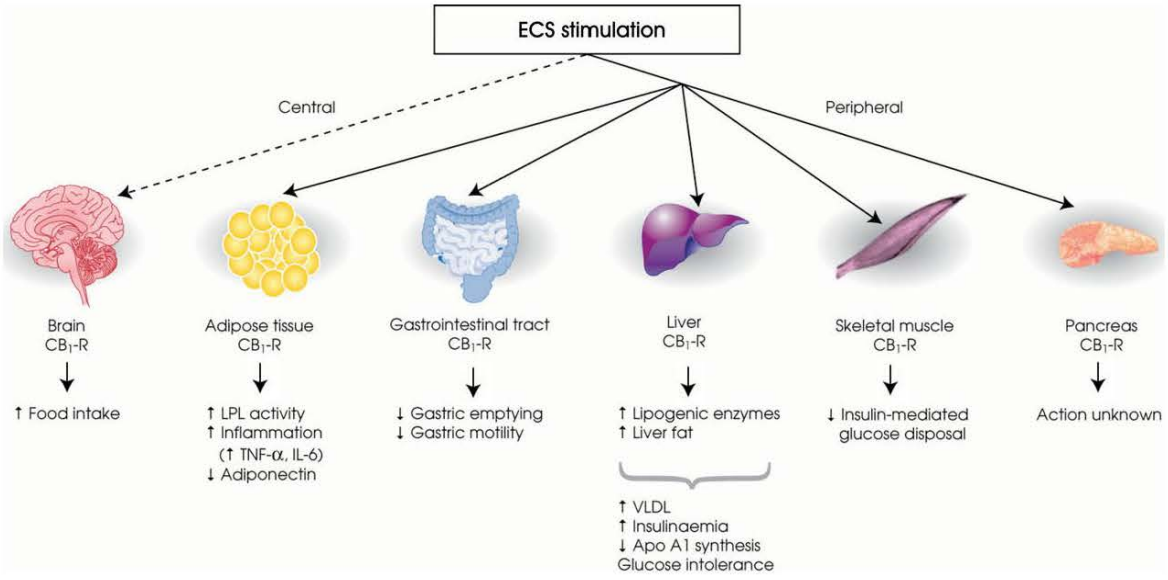


Fig. 1.6 Effect of overactivity of the ECS at both central and peripharal levels and the effect of the CB1 receptor blockade.[64]

1.8 Selectivity issues

Selectivity plays a very critical role in the development of CB1 receptor antagonists. Mainly two types of selectivity must be considered during the designing of CB1 receptor antagonists. The first selectivity is related to the selectivity of CB1 over CB2 receptors. There are a number of side effects observed if the compound antagonizes the CB2 receptors. Blockade of CB2 receptors might increase the risk of cardiometabolic conditions such as myocardial infarction and/or atherosclerosis,[78,79] lowered apoptosis of peritoneal macrophages that accentuates foam cell formation,[80] increased risk of autoimmune disorders such as colitis,[81] increased dermal thickness and leucocyte infiltration resulting a fibrosis like condition.[82,83] In the renal capsule, $CB2^{-/-}$ mice exhibited higher risk of oxidative/nitrosative stress, cisplatin-induced kidney inflammation, and cell death and dysfunction.[84] This clearly indicates that it is necessary to design selective antagonists for CB1 receptor over CB2 receptor.

The second selectivity issue that must be considered for designing is to obtain peripherally acting CB1 receptor antagonists over centrally acting because, CB1 receptor antagonists acting on CNS showed psychiatric side effects such as irritability, anxiety, depression or even suicidal tendency. Along with these, nausea as well as neurological alterations such as vertigo and headaches were also associated with centrally acting CB1 receptor antagonists.[33] Hence, designing of selective peripherally acting CB1 receptor antagonists that do not cross the blood brain barrier (BBB) would be useful for the treatment of obesity.

McAllister et al.[85] reported the binding site of CB1 receptor through molecular modeling approaches in which an aromatic domain is formed by the F3.25, F3.36, W4.64, Y5.39, W5.43 and W6.48 residues of transmembrane helix (TMH) 3-4-5-6 of the cannabinoid receptor. Rimonabant, a selective CB1 receptor antagonist and WIN55212-2, a CB1/CB2 receptor agonist both bind within this microdomain (Fig. 1.7). Rimonabant showed one hydrogen bonding with K3.28 and direct aromatic stacking interactions with F3.36, Y5.39 and W5.43 residues whereas direct aromatic stacking interactions of WIN55212-2 was observed with F3.36, W5.43 and W6.48 residues. Mutation in F3.36 residue produced 3-fold and 9-fold loss in affinity for rimonabant and WIN55212-2 respectively which indicated that F3.36 residue was having direct interactions with rimonabant and WIN55212-2 both. Mutation in W5.43 residue showed deleterious effect upon rimonabant binding and 8-fold loss in affinity for WIN55212-2 which indicated that W5.43 residue was located in the centre of the

aromatic cluster. According to the model, W5.43 residue was having direct stacking interactions with both 4-cholophenyl and 2,4-dichlorophenyl rings attached to the pyrazole ring of rimonabant. Mutation in W6.48 residue exhibited 7-fold and 4-fold loss in activity for rimonabant and WIN55212-2 respectively. The mutation studies clearly suggested that F3.36, W5.43, and W6.48 residues are essential residues of binding pocket for both rimonabant and WIN55212-2. Along with this, mutation in K3.28 residue showed 17-fold loss in binding affinity for rimonabant but the binding affinity of WIN55212-2 was retained indicating that K3.28 residue is essential for rimonabant for hydrogen bond interactions whereas it does not show interactions with WIN55212-2.[86,87]

On the other side, CB2 receptor binding site was composed of Leu108, Ser112, Pro168, Leu169, Trp194 and Trp258 residues located in TMH 3-4-5. The CB2 receptor selectivity is obtained through the interaction of S3.31 and F5.46 residues. The selectivity of CB2 receptor is obtained when a group successfully formed a hydrogen bond with S3.31 residue and a lipophillic group of the compound interacted with F5.46 residue.[88-90] Thus, molecular modeling studies played an important role in the development of novel selective CB1 receptor antagonists.

Designing of CB1 receptor antagonists must consider selectivity for peripheral sites over central action as well as selectivity towards CB1 receptors over CB2 receptors. Hence, development of peripherally acting selective CB1 receptor antagonists could be a safe approach for the treatment of obesity.

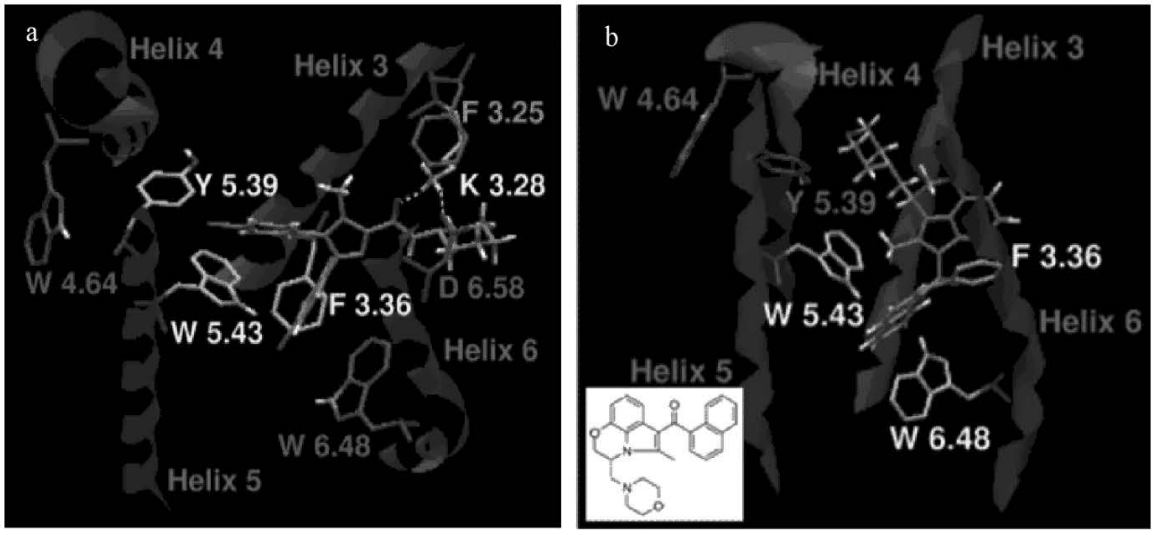


Fig. 1.7 Orientation of rimonabant (a) and WIN55212-2 (b) in the active site of CB1 receptor.[85]

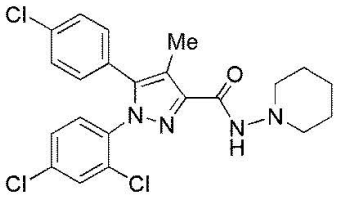
1.9 Some centrally and peripherally acting CB1 receptor antagonists

The chemical structure of THC was discovered in 1964. Further, modifications in the structure of THC were carried out to develop a selective CB1 receptor antagonist in the late 1980's but the obtained results were disappointing.[91] After long time research efforts of Rinaldi-Carmona et al. from Sanofi Recherche, rimonabant (**1**) was discovered as the first potent and selective CB1 receptor antagonist in 1994.[92] European Commission approved rimonabant as an anti-obesity agent in 2006. Unfortunately, rimonabant exhibited serious CNS side effects including suicidal tendency, so European Medicine Agency immediately withdrew the drug from the market in 2008. Due to its potential therapeutic efficacy, rimonabant still remains a promising lead for the designing and development of novel therapeutic agents for the treatment of obesity.[91] Further, Merck Research Laboratory discovered taranabant (**2**) as a CB1-inverse agonist having anorectic effect[93] but it was suspended in phase III clinical trials for the same CNS side effects as rimonabant. Other CB1 receptor antagonists such as surinabant (**3**),[94] otenabant (**4**)[95] and ibipinabant (**5**)[96] were also suspended in different phases of development programme.[91]

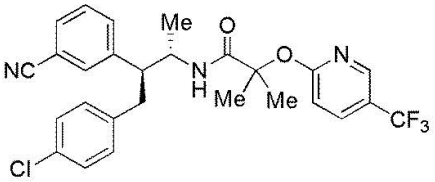
Recently, 7TM Pharma discovered TM38837 (**6**) as a peripherally acting CB1 receptor antagonist having no CNS penetration (brain plasma ratio = 1:33).[97] TM38837 does not cross BBB at 100 mg dose.[98] Another compound AM6545 (**7**), a rimonabant derivative was also discovered as a peripherally acting neutral CB1 receptor antagonist[99,100] having very promising properties. AM6545 is able to increase glucose tolerance and insulin sensitivity, lower leptin level, reverse fatty liver as well as improve the plasma lipid profile. The main aim of designing of neutral CB1 antagonists is to develop compounds that would be devoid of BBB penetration so that psychiatric side effects could be decreased.[101]

Currently, researchers are focusing on designing and synthesis of peripherally acting CB1 receptor antagonists so that CNS adverse effects could be minimized. It has been proved that polar compounds have poor brain penetration. On the other hand, higher lipophilicity of a compound enhances brain penetration.[102] Hence, the new strategy for the designing of peripherally acting selective CB1 receptor antagonists is to increase polar surface area (PSA) and lower the lipophilicity. Designing of neutral compounds is also one of the strategy for peripherally acting CB1 receptor antagonists that are devoid of CNS adverse effects having no or less brain penetration.[101,103,104] Another alternative could be to design charged compounds, because charged moieties

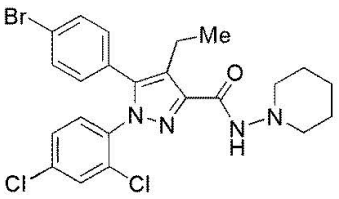
do not cross BBB. These findings have generated enough interest to work further in this direction. Thus, the current research is focused on discovering of peripherally acting CB1 receptor antagonists which could prove as potential therapeutic agents for the management of obesity.



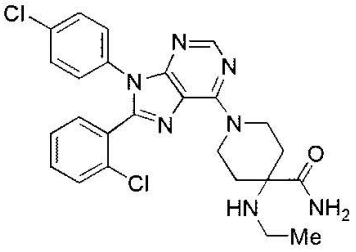
Rimonabant (**1**, SR141716A)



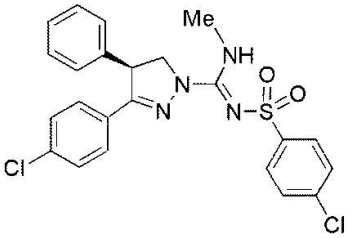
Taranabant (**2**, MK-0364)



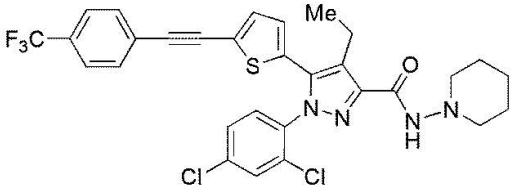
Surinabant (**3**, SR147778)



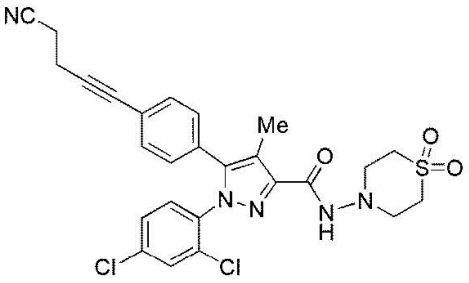
Otenabant (**4**, CP-945,598)



Ibipinabant (**5**, SLV319, BMS-646,256)



TM38837 (**6**)



AM6545 (**7**)