“A REVIEW ON MAGL INHIBITOR”

A PROJECT SUBMITTED TO

NIRMA UNIVERSITY

In partial fulfillment of the requirements for the degree of

Bachelor of Pharmacy

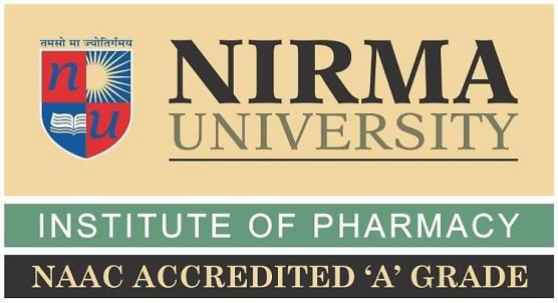
BY

RATHOD GAURAV P. (16BPH017)

Semester VIII

UNDER THE GUIDANCE OF

PROF. MANJUNATH GHATE



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MAY 2020

CERTIFICATE

This is to certify that ***"A REVIEW ON MAGL INHIBITOR"*** *is the bonafide work carried out by* **RATHOD GAURAV P.** *(16BPH017), B.Pharm semester VIII under our guidance and supervision in the Institute of Pharmacy, Nirma University, Ahmedabad during the academic year 2019-2020. This work is up to my satisfaction.*

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CERTIFICATE OF SIMILARITY OF WORK

This is to undertake that the B.Pharm. Project work entitled ***"A REVIEW ON MAGL INHIBITOR"*** *Submitted by* ***RATHOD GAURAV P.(16BPH017)****, B.Pharm. Semester VIII is a bonafide review work carried out by me at the Institute of Pharmacy, Nirma University under the guidance of "Prof. Manjunath Ghate". I am aware about the rules and regulations of Plagiarism policy of Nirma University, Ahmedabad. According to that, the review work carried out by me is not reported anywhere as per best of my Knowledge.*

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DECLARATION

*I, **RATHOD GAURAV P. (16BPH017)**, student of VIIIth Semester of B.Pharm at Institute of Pharmacy, Nirma University, hereby declare that my project entitled **"A REVIEW ON MAGL INHIBITOR"** is a result of culmination of my sincere efforts. I declare that the submitted project is done solely by me and to the best of my knowledge, no such work is done by any other person for the award of degree or diploma or for any other means. I also declare that all the information was collected from various primary sources (journals, patents, etc.) has been duly acknowledged in this project report.*

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Last but not least, I am sincerely grateful to INSTITUTE OF PHARMACY, NIRMA UNIVERSITY to providing such high-quality facilities and academic atmosphere. Thank you one and all.

A REVIEW ON MAGL INHIBITOR

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ABSTRACT

Monoacyl glycerol lipase (MAGL) enzyme belongs to Serine Hydrolase superfamily. MAGL play a role in catabolism of 2-AG (2-Arachidonyl glycerol). 2-AG is a neurotransmitter in endocannabinoid system. Which have some physiological actions. MAGL gives the Arachidonic acid and Glycerol by degrading 2-AG. This Arachidonic acid producing pro-inflammatory agents like prostaglandins, throxanes. So, the major role of MAGL inhibitors as anti-inflammatory, antinociceptive and anti-cancer agents. Recent studies suggest that the repeated administration of MAGL inhibitor will lost its activity and produced cross tolerance to cannabinoid receptor (CB1). In this review we discuss about the Biochemical and Physiological role of MAGL inhibitors, and various scaffolds which are discovered as potent for inhibition of MAGL.

1.INTRODUCTION

Endocannabinoids like N-Arachidonoylethanolamine (AEA) and 2-Arachidonoylglycerol (2-AG) are most likely to present in most mammalian tissues, and they stimulate cannabinoid receptors CB1 and CB2. They modulate the physiological responses, including nociception, depression and anxiety. These cannabinoids degradation involves two major enzymes, which are monoacylglycerol lipase (MAGL) and fatty acid amide hydrolase (FAAH). 80% degradation of endocannabinoids by MAGL hydrolase enzyme and the rest of catabolism done by a/þ-hydrolase domain containing serine hydrolase enzymes like ABHD6 and ABHD12 (Korhonen et al., 2014).

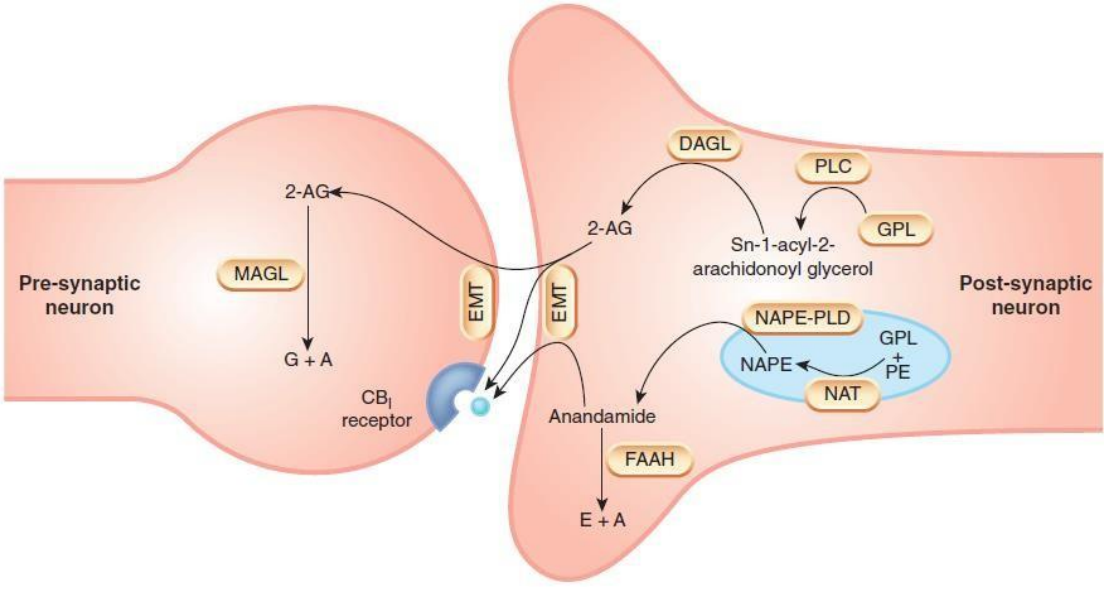
FAAH is primary enzyme for degrading AEA, but it is also hydrolysed other N-acylethanolamines (NEAs), n-palmitoylethanolamine (PEA), N-oleoylethanolamine (OEA) and sleep-inducing lipid oleamide. Inhibition of FAAH considered as potential for treatment of nervous system disorder, anxiety, inflammation, pain and depression (Korhonen et al., 2014).

Endocannabinoids stimulates and activate the cannabinoid receptors CB1 and CB2. Thus, activation of receptor/cannabinoid system have the most therapeutic potential for the treatment of disease like pain, inflammation, obesity, cancer, anxiety. But the use of CB1 receptor agonist is associated with psychotropic effects. This fact leads to search for alternative strategies, so other way to activate the ECS is increasing the level

endogenous cannabinoids (eCBs) anandamide (AEA) and 2-arachidonoylglycerol (2-AG) by inhibiting enzymes involved in their catabolism. AEA hydrolysed by FAAH, and selective inhibition of FAAH enhances level of AEA and leads to analgesia and anxiolytic effect without psychoactivity. However, the irreversible inhibitor of FAAH not give the analgesic effect in phase-II clinical trial. Thus the 2-AG is major agonist for CB1 and CB2 receptor, the enzyme involved in degradation of 2-AG is monoacyl glycerol lipase (MAGL). (Hernández-Torres et al., 2014)

The level of MAGL in cancer cell and primary tumor is very high because it supplies the free fatty acid for synthesis of oncogenic signalling lipids that enhance the migration, invasion of tumor, so MAGL inhibitor will act as potent for cancer treatment (Nomura et al., 2011). MAGL inhibition also leads to reduction in amyloid neuropathology, neuroinflammation and neurodegeneration in mouse model of Alzheimer's disease(Chen et al., 2012).

Some MAGL inhibitors have been studied and they are irreversible inhibitor of MAGL, this permanent blockade of MAGL can impaired the functions of MAGL receptor, and desensitise the receptor that leads to decrease the antinociception properties of inhibitor. So the reversible inhibitors are less likely to develop tolerance, such reversible inhibitors are naturally occurring terpenoids pristimerin and euphol. (Hernández-Torres et al., 2014; Schlosburg et al., 2010)



(Figure 1) biosynthesis of 2-AG and anandamide in endocannabinoid system, and inactivation of endocannabinoids (Rang & Dale, 2012)

In last decade lots of efforts and studies have been made for developing MAGL inhibitors, three types of MAGL inhibitors are reported so far: I) compounds binds covalently and reversible, II) compounds binds covalently and irreversible, III) compounds bind noncovalently (Granchi et al., 2017; Labar et al., 2010).

2.BIOCHEMICAL AND PHYSIOLOGICAL ROLE OF MAGL

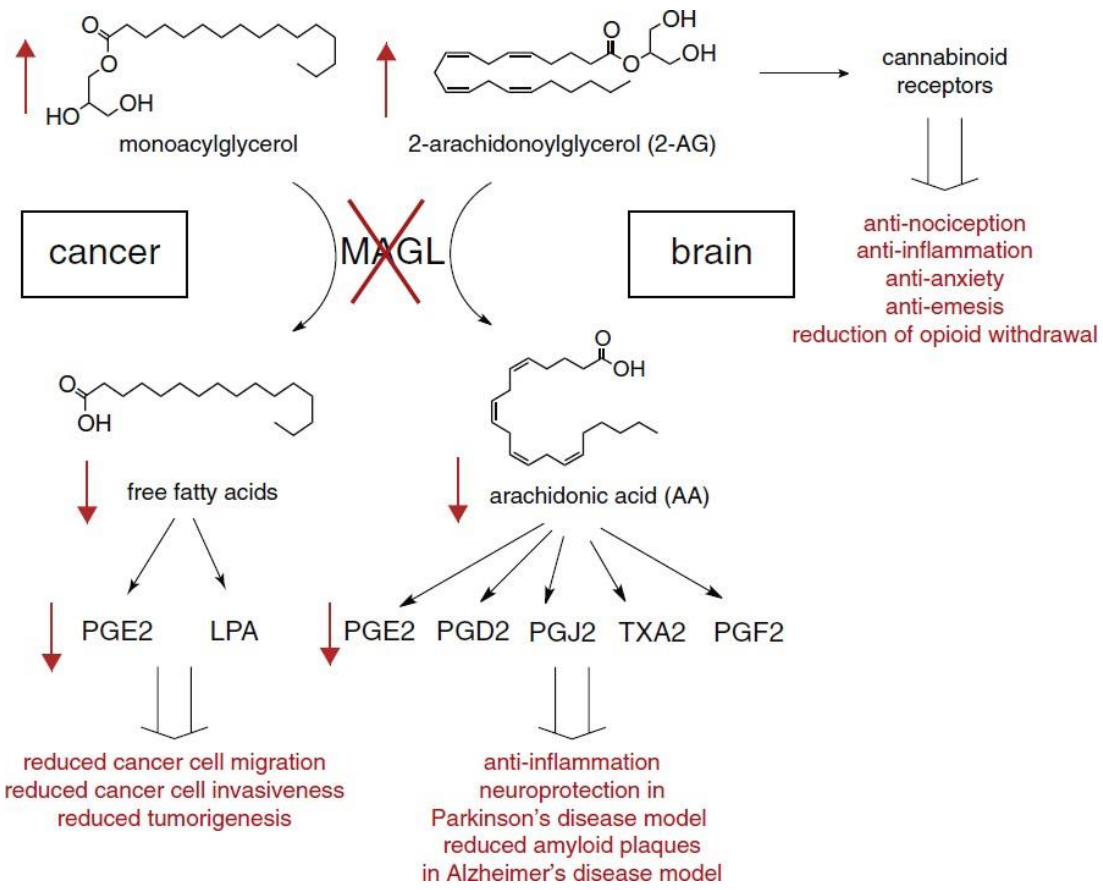
2- AG produced in post-synaptic neuron and binds to CB1 receptor on pre-synaptic neuron to modulate release of neurotransmitters by two retrograde synaptic depression, Depolarization induced suppression of excitation (DSE) and inhibition (DSI). MAGL present at presynaptic neuron to catabolise 2-AG and produce arachidonic acid and glycerol. This arachidonic acid is responsible for the synthesis of proinflammatory agents like prostaglandins and thromboxane. proinflammatory agent leads to development of different physiological disorders like pain, inflammation, anxiety, neuroinflammation. (Mulvihill & Nomura, 2013)

Inhibition of MAGL also indicates accumulation of 2-AG and it enhances the signalling of cannabinoid receptor CB1 and CB2. In brain, lungs, and liver MAGL controls the primary arachidonic acid pool for proinflammatory prostaglandins production. Blockade of MAGL will helpful by either improving endocannabinoid signals or by reducing eicosanoids. MAGL inhibitors controlling the free fatty acids (FFAs) level in cancer cell and such like FFAs are primary agents to produce protumorigenic signalling lipids like (PGE2) and lysophosphatidic acid (LPA). (figure 2) (Mulvihill & Nomura, 2013)

2.1 THE ROLE OF MAGL IN PAIN AND INFLAMMATION

Cannabinoid receptor CB1 and CB2 agonists are presently used as analgesic, anorexia and antiemesis. Also, agonists of CB1 and CB2 receptor used to treat inflammation in different rodent models of neuropathic pain and inflammation. NSAIDs are widely used drugs for treatment of pain, inflammation and fever. NSAIDs acts through blocking cox1 or cox2 enzyme and lowering the level of pro-inflammatory prostaglandins and thromboxane. NSAIDs such as aspirin and ibuprofen also effective for antiplatelet therapy and so it prevents the heart attack, stroke and clot formation in blood.

Congruous to this MAGL inhibitor block the 2-AG catabolism and stop the production of proinflammatory prostaglandins and thromboxane. So, the acute blockade of MAGL gives CB1 dependent antinociceptive, anti-inflammatory and analgesic effect. MAGL barricade decreases mechanical and acetone actuated cold allodynia in mice exposed to interminable choking injury of the sciatic nerve. (Kinsey et al., 2009; Long et al., 2009; Mulvihill & Nomura, 2013)



(figure 2) Biochemical and Physiological role of MAGL inhibitor

2.2 THE ROLE OF MAGL IN NEURODEGENERATIVE DISEASE

Alzheimer's disease (AD), Parkinson's disease, multiple sclerosis and stroke are the major neurodegenerative diseases. To treat or protect neurodegenerative disease cannabinoid receptor agonist and COX inhibitors are used. In 1-methyl- 4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) Parkinson's disease models, all non-selective agonist of CB1/CB2 receptor and selective agonist of CB2 agonist shows enhanced life span of dopaminergic neurons and fibres, reduction of dopamine depletion in substantia nigra, and improved motor function in a CB1 or CB2-dependential manner through

reduction of NAPDH oxidase, reactive oxidative stress, and ace provocative cytokine discharge from initiated microglia(Chung et al., 2011; Mulvihill & Nomura, 2013)

Cannabinoid receptor agonist WIN55,212-2 and JWH-133 supress microglial activation, tumor necrosis activation factor a (TNF a) levels, COX2 behaviour, and amyloid þ plaque levels in transgenic amyloid precursor protein (app+) in Alzheimer's disease (AD) mouse model (Chen et al., 2012; Mulvihill & Nomura, 2013; Ramirez, 2005).

2.3 THE ROLE OF MAGL IN ANXIETY

All cannabinoid receptor agonists and FAAH selective inhibitors will increase the level of anandamide or CB1 signalling in rodent having anxiety (Zanettini, 2011). JZL184 is a MAGL inhibitor shows anti-anxiety effect. Blocking of MAGL by MAGL inhibitor also express the anxiolytic action in an elevated plus maze paradigm for anxiety, which shows elevated percentage of open arm time and number of open arm entries in circumstances of high level environmental aversion (Mulvihill & Nomura, 2013; Sciolino et al., 2011)

2.4 THE ROLE OF MAGL IN CANCER AND CANCER RELATED SYMPTOMS

MAGL inhibitors exerts various effects such as anti-nociceptive, anti-inflammatory, analgesic and anxiolytic, but the blockade of MAGL also exerts anti-cancer effects. MAGL inhibitors act through enhancing apoptosis in vitro and angiogenesis and metastasis in vivo. The extra benefit of cannabinoids is to avoid chemotherapy side effects like nausea, pain, and lack of appetite(Guzmán, 2003). Traditionally prostaglandins produced from COX2 and which has been used by cancer cell and the inhibition of COX by genetically or pharmacologically will leads to limit the cancer malignancy (Mulvihill & Nomura, 2013; Schneider & Pozzi, 2011)

In human cancer cells and primary tumors MAGL level is increased, because it provides the source of lipolysis for free fatty acids (FFAs) to synthesize oncogenic signalling lipids which enhance the growth of cancer. Blockade of MAGL in breast, ovarian, melanoma cancer cells reduces cell migration, invasiveness, and tumorigenicity through decreasing free fatty acids (FFAs) and protumorigenic signalling lipids, such as lysophosphatidic acid and prostaglandins(Kopp et al., 2010).

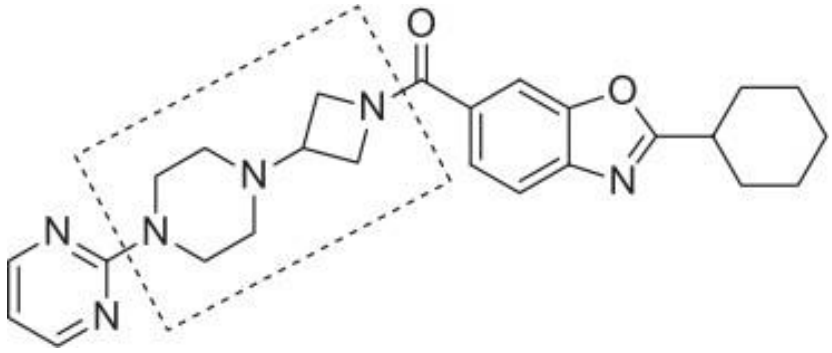
MAGL inhibitors give side effects on chronic administration and causes functional antagonism of cannabinoid receptors in brain(Schlosburg et al., 2010). This also cause the neuropsychiatric effects like anxiety and depression. Irreversible inhibition of MAGL will decrease learning performances and loss of memory (Scalvini et al.,2016)

3. MAGL INHIBITORS

various studies and discoveries have been done on MAGL blocker. Different scaffolds are developed and studied till date like Azetidine derivatives, piperazine derivatives and carbamates.

3.1 AZETIDINE DERIVATIVES

Azetidine containing molecule developed by janssen pharmaceutics in 2010, it is reversible and very potent inhibitor. They explained the binding of inhibitor by X-ray resolution of a crystal structure of human MAGL(fig 3) (Granchi et al., 2017)

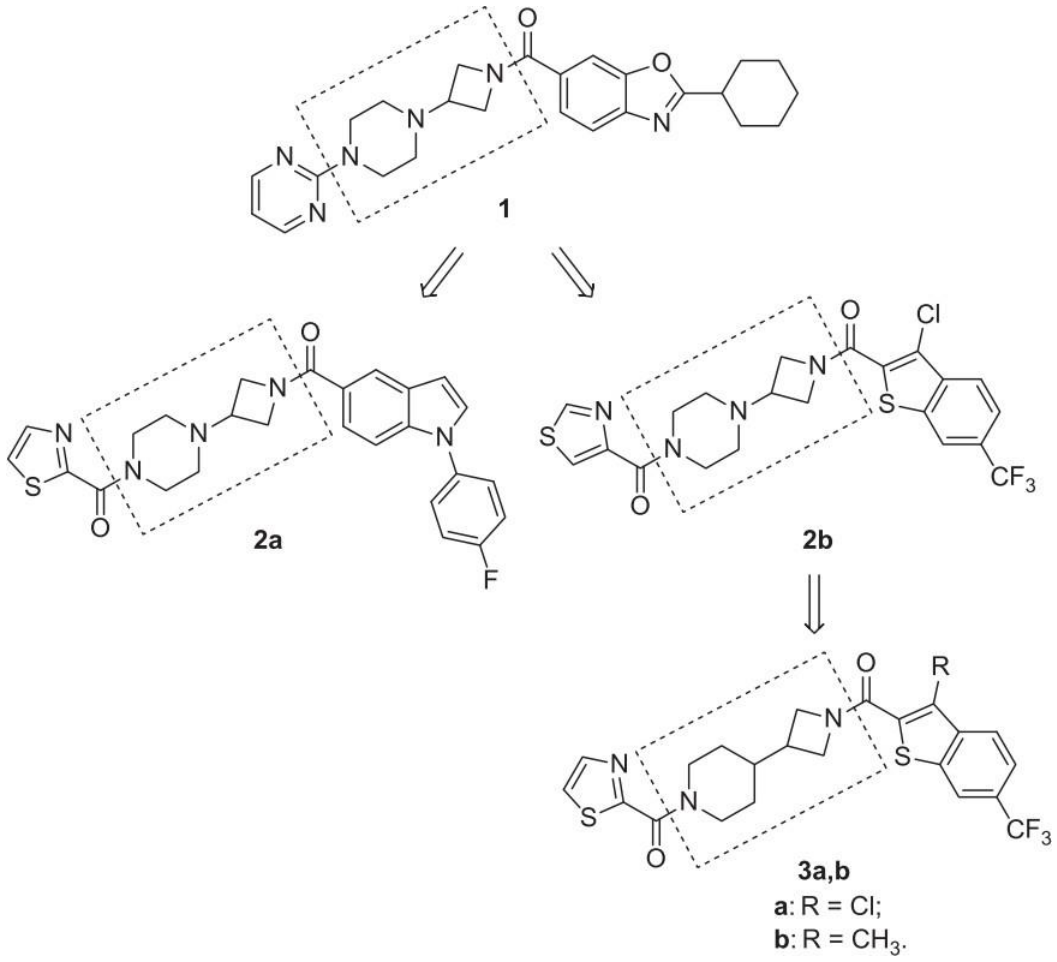


(Figure 3) azetidinyl-piperazine MAGL inhibitor (ZYH) (Granchi et al., 2017)

These Azetidine derivatives still have piperazine and azetidine cycles with carbonyl group which are attached to the nitrogen atom of the piperazinyl azetidine scaffold with other aromatic and heteroaromatic cycle (2a) (fig 4).

The patented compounds have been studied on MAGL enzyme and 4- methylumbellif eryl butyrate has been used as the substrate. Although it is not an endogenous MAGL ligand, it is being used in enzymatic assay as it is fluorogenic lipase substrate, forming fluorescent 4-methylumbelliferonone due to hydrolysis of ester bond. It was stated the activity of compound (2a) and it is expressed as IC_{50} value. The value of IC_{50} for

compound ZYH was lower than 5nM on MAGL. Its MAGL inhibition activity was confirmed after 30 minutes of incubation by measuring the accumulation of 2-AG in rat brain homogenates. Results are represented as compound/vehicle ratio percentage. And it shows the increased 2-AG accumulation in a brain homogenate preparation by 2081% and 463% When assay done at 1and 0.1 μM, respectively.



(Figure 4) azetidinyl-piperazine MAGL inhibitor (Granchi et al., 2017)

Considering that by mobilizing fat stores, MAGL contributes to energy homeostasis, its inactivation can lead to obesity. In addition, obesity is often marked by pathologically elevated levels of endocannabinoids; thus, increased levels of 2-AG caused by MAGL inactivation may have cannabinoid-like effects on feeding behaviour, lipid accumulation, and energy expenditure. (Granchi et al., 2017)

Overexpression of MAGL in mice shows the quickly acquired an obese profile with increase in the body weight and body fat mass, while in mice which have the lower

expression of MAGL showed the decreased in body weight and lower body fat. (Douglass et al., 2015)

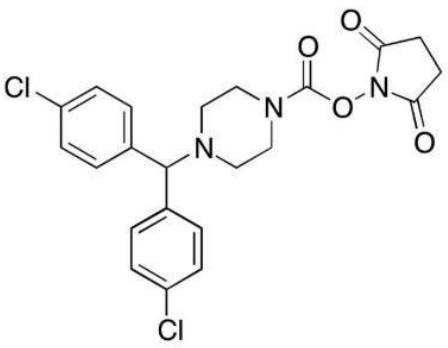
Consequently, the in vivo experiments on azetidine derivatives were aimed at demonstrating that these MAGL inhibitors had a beneficial impact on body weight and metabolism. Treated mice consumed less food (about 2.4-fold less food) than mice control over a 30-minute cycle. In addition, oral administration of these compounds for five consecutive days at doses of 0, 15 and 50 mg / kg / day resulted in a decrease in mean body weight at the highest concentration test. Lastly, in vivo studies were conducted in dogs undergoing similar treatment, with doses of 0, 5, 15 and 45 mg / kg / day for five consecutive days. Treated dogs reported a decline in body weight t and in the intake of food, thus confirming the study data in mice. (Granchi et al., 2017)

The second patented molecule from this class of MAGL inhibitors, the Janssen pharmaceutica gives the further example (2b), which retains the same central scaffold and varies for the place in which the thiazole ring is connected to the piperazinyl azetidine center, and for the bicyclic heteroaromatic ring on the other side of the molecule, which is a substituted benzothiophene ring rather than an indole ring. Compound 2b biologically assayed and shows an IC_{50} value of 12 nM on MAGL, so it is having less potency than 2a.

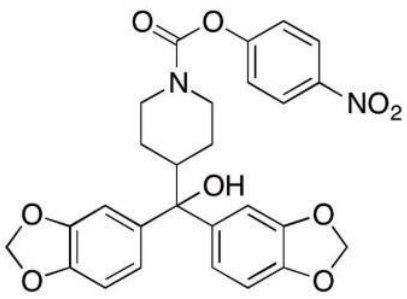
In 2013 a US patent published general compounds Structures close to those of the previous group of MAGL inhibitors, differing mostly in the presence of a piperidine rather than a piperazine ring. They were tested using 4-methylumbelliferyl butyrate as the substrate on MAGL.

The best compounds of this chemical class displayed IC50 values below 5 nM on MAGL without specifying the exact value of the representative compounds 3a and 3b.

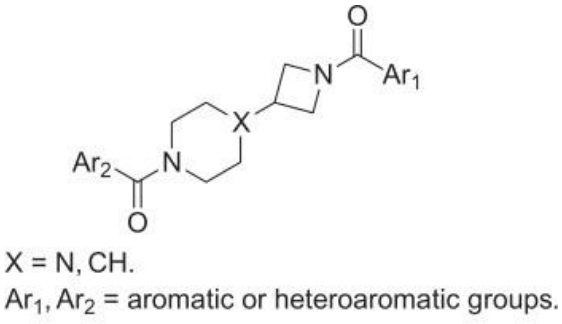
A patent which covers MAGL inhibitors to treat fibrosis, Lotersztajnet al. have published several organs since fibrosis may result in inflammation in inflammatory pathologies involving MAGL. For this purpose MAGL inhibitors patented were the (2,5-dioxopyrrolidin-1-yl 4- (bis(4-chlorophenyl)methyl)piperazine-1-carboxylate) (MJN110) (fig-5), 4-nitrophenyl-4-[bis(1,3-benzodioxol-5-yl)(hydroxy)methyl]piperidine-1-carboxylate(JZL184) (fig 5), as well as azetidinyl-piperidine or -piperazine derivatives of general structure (fig 4).



(4) MJN110



(5) JZL184



(6)

(figure 5) (MJN110 & JZL184)

The beneficial effects of MAGL inhibition were confirmed by reduced liver fibrosis in myeloid cells where the expression of MAGL was demolished. (Granchi et al., 2017)

3.2 PIPERAZINE AND/OR CARBAMATE-BASED DERIVATIVES

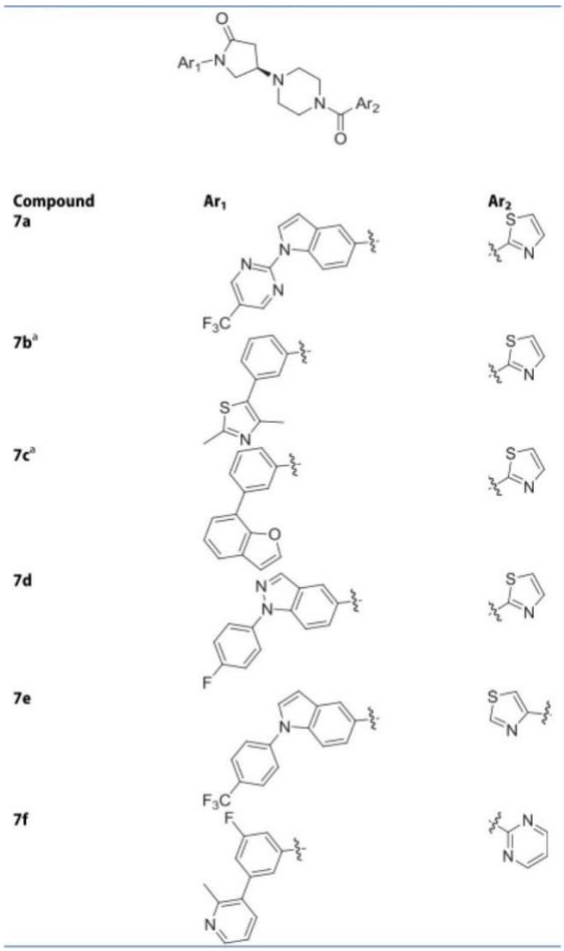
Piperazine moiety is usually found in the structure of MAGL inhibitor. A series of 4-(piperazin-1-yl)-pyrrolidin-2-one compound was reported by Koike et al. as therapeutic agents in the treatment and prevention of Alzheimer's disease (Chen et al., 2012). MAGL inhibition has been shown to lead to the reduction of this pathological condition 's normal neuro-degeneration by suppressing neuroinflammation, nerve cell death, and þ-amyloid plaque accumulation. In addition, a potential use of these MAGL inhibitors was anticipated for other neurodegenerative pathologies, such as Parkinson's disease, amyotrophic lateral sclerosis, Huntington 's disease and multiple sclerosis. Other pathologies listed are traumatic brain injury,

glaucoma, anxiety disorder, inflammatory and nervous pain, epilepsy, depression, migraine, cerebral edema and ischemia, because the authors announced that these diseases could benefit from MAGL inhibition; although, it was shown that the direct intervention of MAGL inhibition was beneficial only for some of them.

Compound 7a was the most potent MAGL inhibitor from reported derivatives, 7a will completely block the MAGL activity by 10 μM. Mass spectrometry then confirmed inhibition activity of 7a, as well as those of other compounds like 7b and 7c. Intracerebral 2-AG and AA measurements were performed for selected compounds (7a, 7d – f) at doses of 10 mg / kg (10 mL gavage) for compounds 7a and 7e–f or 30 mg / kg (10 mL gavage) for compound 7d given to mice. All compound which were tested increased 2-AG accumulation compared to control. Compounds 7a and 7e raised substrate concentration by about sevenfold, and compounds 7d and 7f caused a tripling. when, the MAGL product AA concentration was reduced by the four compounds from 20 to 40 percent, thus confirming the results observed for 2-AG.

In most of patents of these piperazine based MAGL inhibitors, the piperazine ring mostly attached to carbamate group, because carbamate inhibitors are well known MAGL inhibitor, carbamate inhibitor will block MAGL by acylating the catalytic serine present in the site of the enzyme.

Abide Therapeutics, Inc. has developed a second group of piperazine-based MAGL inhibitors, in partnership with the Cravatt et al. research group at the Scripps Research Institute. These compounds were developed as dual inhibitors of MAGL and/or ABHD6, and are based on the presence of a group of hexafluoropropan-2-yl carbamates on one of the piperazinic nitrogen. ABHD6 is a serine hydrolase having a/þ hydrolase domain 6. It is also inhibiting the MAGL by less percentage compare to MAGL in brain.

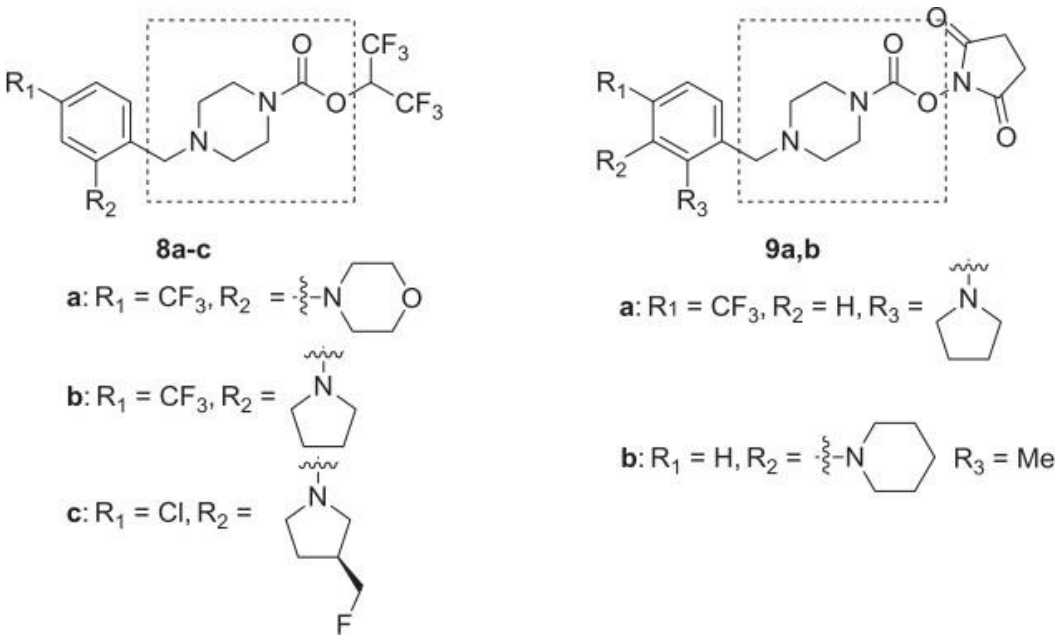


(figure 6) Structures of 4-(piperazin-1-yl)-pyrrolidin-2-one-based MAGL inhibitors.

Such compounds were also tested for fatty acid amide hydrolase (FAAH) inhibition in addition to their MAGL and ABHD6 inhibition activities to assess a potential effect on the enzyme responsible for endocannabinoid anandamide hydrolysis. Mouse brain mem-brane fractions or cell lysates were incubated with the inhibitors and human recombinant MAGL activity was also used to check these compounds' inhibition capacity. JZL184 was used as reference to assay this compound. Compound 8a and8b are selective for MAGL, when 5mg/kg p.o. administered in mice it shows less potency on ABHD5 and FAAH and completely selective for MAGL.

Abide therapeutics, inc., in 2016 report the second patent for subsequent development of the series of 4-benzylpiperazine derivatives, it is still carrying the hexafluoropropane 2-yl carbamate group, but with various substituent in the benzyl group, as dual MAGL and/or ABHD6 inhibitor. All compounds displayed insignificant inhibition activities on FAAH, with IC50 values greater than 1000 nM, whereas the intervals of IC50 values for MAGL and ABHD6 were in the nanomolar range (1000–100 or less than 100 nM),

with general selectivity for MAGL relative to ABHD6. Compound 8c among the most selective MAGL inhibitors in this series confirmed its selective MAGL inhibitory activity, which exceeded 75% in vitro at 1 μM as well as 5 mg / kg in vivo compared to the lower activity of the other two enzymes (less than 25% inhibition).



(figure 7) Piperazine-containing carbamic MAGL inhibitors developed by Abide Therapeutics.

After few months of this patent, a third patent was released in view of previously discovered compound 8b, which is potent and selective inhibitor for MAGL both in vivo and in vitro. The more recent patent outlined its therapeutic application for treating various diseases, which are usually characterized by inflammation or neuropathic pain. Compound 8b display values for IC_{50} ranging from 8 nM (human recombinant MAGL) to 27 nM (murine MAGL). So this 8b acted as a very potent inhibitor of MAGL enzymes derived from mouse brain, rat prefrontal cortex, dog prefrontal cortex and human prefrontal cortex, prostate cancer PC3 cells, and recombinant methodology, as determined by activity-based protein profiling technology (ABPP), this compound inhibit the 2-AG catalysis in AA by 94% and result IC_{50} value of 1.7 nM in human brain and similarly 2.2 nM for PC3 cell. So many studies by PET technique in nonhuman primates and by activity-based protein profiling (ABPP) technology in

rodents and dog were done to check ability of compound 8b to target MAGL in neuron and to elevate 2-AG concentration. The pain efficacy checked on rat model. In last safety, tolerability, pharmacokinetic and pharmacodynamic properties were tested of compound 8b hydrochloride salt and covering the studies which were needed for phase I. Phase II trials for multiple sclerosis pain and spasticity, migraine prophylaxis, and other inflammation-and pain-related disorders in patients affected by Alzheimer's disease or functional chest pain and Phase III studies were planned in detail.

Compound 9a and 9b (fig 7) were also discovered by same team, in fact its structure has the same central scaffold only vary for the group bound to the carbamate oxygen atom. The differing group is succinimide in place of hexafluoropropane moiety, and in the other part of the central piperazinic scaffold variously substituted benzyl groups present. Compound 9b in vivo shows fully blockade of MAGL activity in mouse brain at dose of 5 mg/kg p.o., but also it exerted very potent activity in vitro $IC_{50} \leq 100$ nM, it also gives some action on ABHD6, without affecting FAAH. In vivo 9a compound has the less activity, it only inhibits 75% MAGL but more selective for MAGL $IC_{50} \leq$ 100 nM.

Carbamate group mostly present in the scaffold since this group allow the interaction with the catalytic activated serine 122. Hydroxyl group will attack by nucleophilic reaction to carbamate carbonyl group of inhibitors. So, by this mechanism it leads to blockade of MAGL.

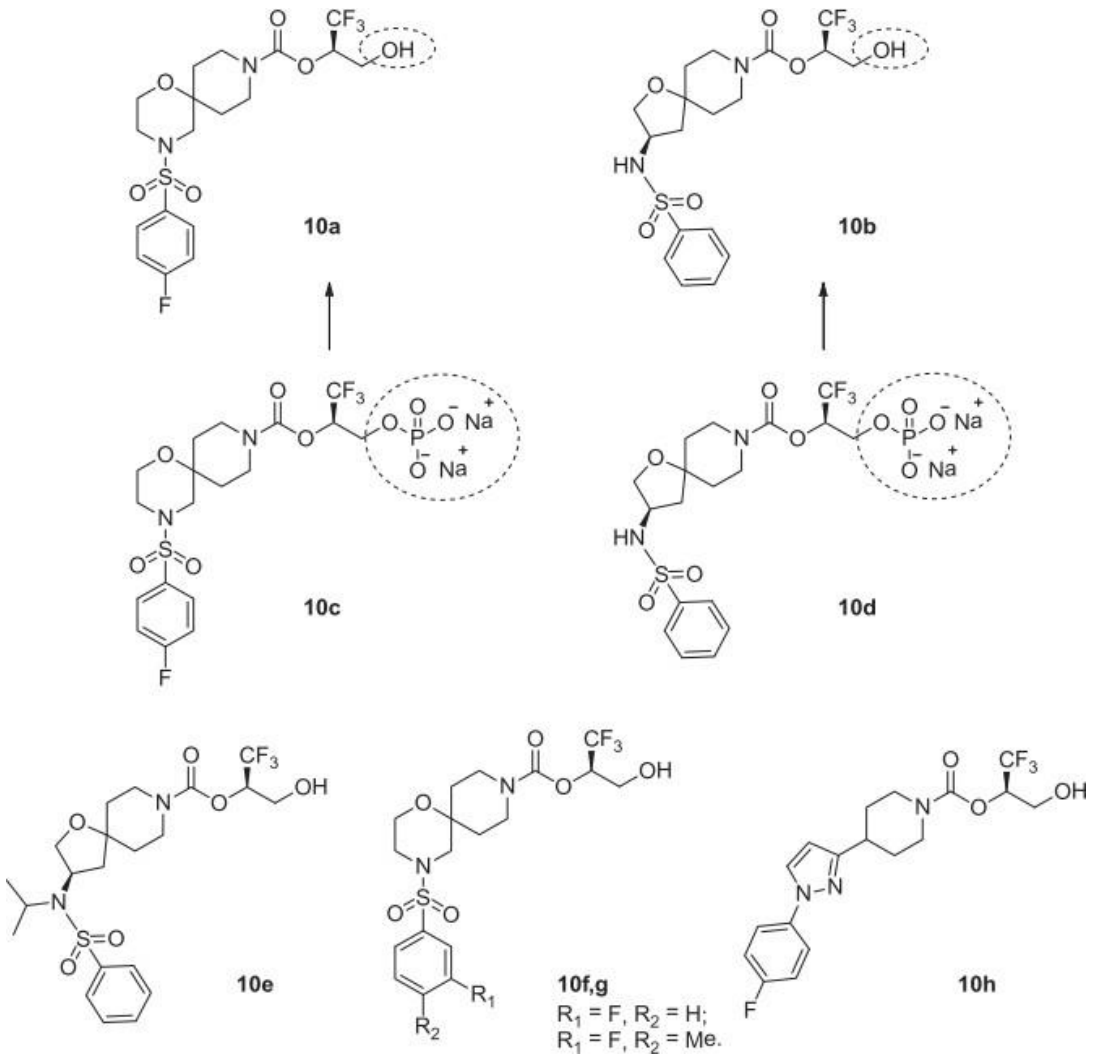
in 2017, Pfizer has patented a carbamate MAGL inhibitor series and the use of discovered compunds are analgesic, anti-inflammatory, traumatic brain injury, anti-depressant, antianxiety, Alzheimer’s disease, cancer. Kinetic experiments have been conducted to prevent K_i and K_{inact} values, as Kinact is a parameter linked to the timed-dependent decline in irreversible inhibition IC50 values.

Pfizer gives the series of compounds which block the MAG. Now 10a and 10b are one of them, they are spiro derivatives (fig 8) and moiety contains trifluoro-3-hydroxypropan-2-yl piperidine-1-carboxylate and terminally sulfonamide group. This moiety are potent inhibitors with IC_{50} of 7 and 4 nM respectively at 30 minutes, and having good selectivity to MAGL compare to FAAH.

10a and 10b are the bioactive form of phosphate disodium salts of 10c and 10d respectively (fig 8). So, the 10c and 10d will act as prodrug for 10a and 10b.

2 mg/kg i.v. dose of 10c and 10d were administered into wistar Hann rat and 1 or 0.7 mg/kg i.v. were given to beagle dog to check their pharmacokinetic factor and blood samples were also analysed at different time to check relative amount of prodrug formed and corresponding active forms.

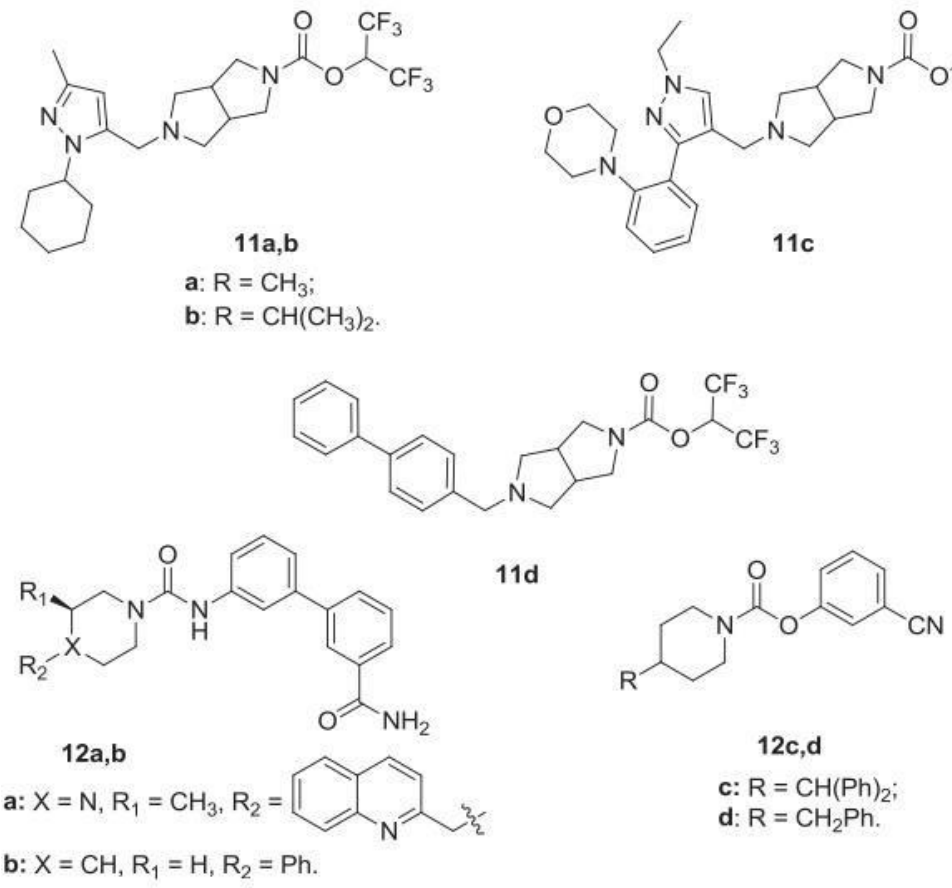
The most potent and selective inhibitor from this class are 10e-g (fig 8). All have shown IC_{50} of 1 nM on MAGL and not having activity on FAAH. Compound 10h have the trifluoro-3-hydroxypropane and piperidine ring but does not contain the spiro and sulfonamide group. This group replaced by a 1-(4-fluorophenyl)-1H-pyra- zole, 10h is the most active FAAH inhibitor from this series with IC_{50} of 1.14 μM, and have the IC_{50} 8 nM for MAGL inhibition.



(figure 8) Carbamate MAGL inhibitors developed by Pfizer

Abide therapeutics lnc. Developed a series having octa- hydropyrrolo[3,4-c] pyrrole-based carbamates which can inhibit MAGL and/or ABHD6. The developed series contains the before mentioned piperazinic compounds, they bearing a succinimide ringor an hexafluoropropane on the carbamate scaffold. When nucleophilic attack occure at enzymatic site then this groups leave the site because it has the good leaving property.

From this developed series most selective MAGL inhibitors were 11a-c (fig 9), with IC_{50} value lower than 100 nM. They posses the less activity against ABHD6 and completely not active against for FAAH. 11d compound exert the same inhibition on every 3 enzymes, Further experiments with Mycobacterium tuberculosis showing a minimum inhibitory concentration of less than 50 μM were chosen.



(figure 9) Other carbamate MAGL inhibitors

In 2016, to increase or maintain the concentration of endocannabinoid by blocking their catabolism dual MAGL and FAAH inhibitor were published. So, these increase in level of endocannabinoid will also lead to active CB1 and CB2 receptor. Which may have

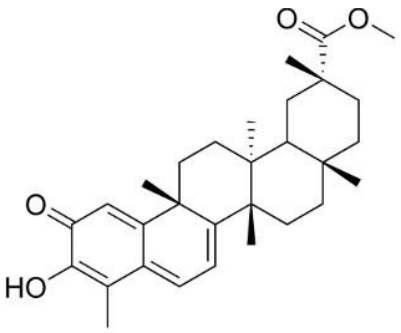
some biological use in diseases like neurodegeneration, nausea and vomiting due to chemotherapy, cancer, pain and inflammation (Mulvihill & Nomura, 2013). This cannabinoid enzyme inhibitor will give beneficial effect like cannabinoid receptor agonist give but no risks resulting from the active stimulation of these receptors, such as addictive and psychotropic effects. It is important to report that dual MAGL / FAAH blockade induced catalepsy and similar responses to O9 tetrahydrocannabinol, as evidenced by the in vivo use of the dual MAGL / FAAH inhibitor JZL195. In fact both enzyme inhibition will show relatively same effect of direct CB1 agonist in contrast to selective blocking of MAGL alone.

MAGL inhibitors class drug having two pharmacophoric part. An 'inhibition subunit' and a 'binding subunit'. Inhibition unit will be containing the carbonyl group that act with enzyme and inhibit. The binding unit will provide the sufficient interaction of the molecule with target. The fluorometric technique is used as assay for MAGL, arachidonoyl 7-hydroxy-6-methoxy-4-methylcoumarin ester (AHMMCE) is used as substrate and hydrolysed product 7-hydroxy-6-methoxy-4-methyl- coumarin was obtained and detected at 460 nm. 12a and 12b are the most studied compound from this class of urea derivatives (fig 9) or carbamate compound (12c and 12d). the IC_{50} value for 12a-c range from 0.01-0.1 µM for human and rat FAAH, but they are less active for human and rat MAGL, although 12d shows the less selectivity for FAAH but it displayed IC_{50} value 0.01 to 0.1 µM for both MAGL and FAAH. 12d have showed the highest brain/plasma ration (0.71) compare to 12a and 12b to cross the blood brain barrier.

3.3 NATURAL TERPENOID AS MAGL INHIBITOR

Looking for new scaffolds that could be used to develop reversible MGL inhibitors, King and Coworkers found two naturally occurring terpendoids, pristimerin and euphol, which showed high potency and selectivity (King et al., 2009). Pristimerine (fig 10) is an example of natural terpenoid, which block the MAGL IC_{50} = 93±8 nM). Pristimerine is a pentacyclic triterpenoid having quinone-methidegroup, this group will act on cysteine residues. Pristimerine inhibition is the reversible inhibition.

Recently a pentacyclic terpenoid studied is similar to pristimerine, þ- amyrin was gives MAGL inhibition by IC_{50} 2.8 µM, þ- amyrin will give rapid, reversible and non-competative inhibition.



Pristimerin

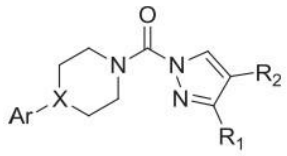
(figure 10) natural terpenoid

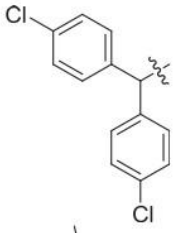
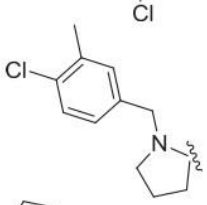
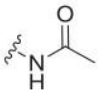
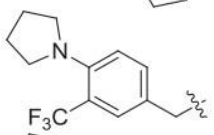
3.4 OTHER HETEROCYCLIC DERIVATIVES

In 2015 patent by abide therapeutics and the scripps research institute give compounds, these compounds will contains the common an ureic scaffold substituted (a) on one nitrogen atom from urea portion with having no substitution or differently substituted pyrazole moiety and (b) on the other nitrogen atom with ring like piperazine, pyrrolidine , 1,4-diazepane. These various compounds are patented for inhibiting MAGL, FAAH and ABHD. But few of them tested as platelet-activating factor (PAF) acetylhydrolase (PLA2G7).

Compounds 13a-c were the most active and selective MAGL inhibitors of these series, as they displayed IC_{50} values in the low nanomolar range for MAGL (less than 100 nM), exhibiting only poor activity (IC_{50} values higher than 1 μM) on FAAH and ABHD6, and these three compounds were characterized by very similar chemical structures, which differed only for the sub-stituent in the pyrazole cycle.

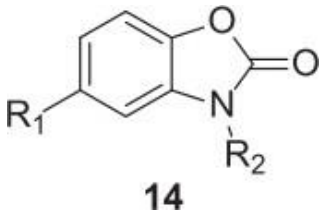
Now, the recent two patents from same company give the development of ureic acid derivatives. In vitro experiments on human and mouse MAGL and mouse FAAH and in vivo tests with oral gavage in mice at 5 mg / kg showed that certain compounds, exemplified by two representative compounds 13d and 13e (fig 11). They were able to selectively inhibit both mouse and human MAGL with IC_{50} values below 100 nM, exerting only a marginal impact on the FAAH mouse (IC_{50} values within the range 1–10 μM), and these compounds were also in vivo powerful MAGL inhibitors (MAGL inhibition as much as 75%). The new structure has maintained the pyrazole ureic scaffold, although it having the substituted piperazine on another part of molecule. Or in some of cases piperazine will replaced by a 1,8-diazaspiro [4.5] decane heterocycle.



Compound	Ar	X	R_1	R_2
13a		N	H	H
13b		N	H	CH_3
13c		N	H	F
13d				H
13e		N		H
13f		N	H	

(figure 11) Structures of pyrazole-based MAGL inhibitors

13f and 13e are mostly same but only vary for methyl sulfonamide on pyrazole ring (R2 in place of R1, fig 11) and instead of pyrrolidine the imidazole on the benzylic part of piperazinic nitrogen. It was a potent MAGL inhibitor compared to FAAH, achieving 75 per cent inhibition of human MAGL at 1 μM, and remaining0–25 per cent inhibition of FAAH at the same concentration.



14

(figure 12) Structures of heterocyclic MAGL inhibitors.

The class of benzoxazolones, the main target of the cannabinoid receptors has been patented for use as dermatologic agents and cosmetics, to be applied topically to inflamed or irritated skin or mucosa, since both CB1 and CB2 are present in keratinocytes and fibroblasts and regulate many pathways of inflammation. These compounds are having more activity because of their more lipophilic property and modulatory activity on skin. So, it can be used for skin irritation, itching, inflammation, pruritis, pain, edema, allergic condition. 14 compound is a summarised common structure for this class of compounds (fig 12).

4.0 conclusion

In the past five years, about 20 patents for com-pound inhibition of MAGL activity have been filed by both academia and pharmaceutical companies. The main attraction of people towards this MAGL inhibitors are due to some main factors like; (a) the basic involvement of MAGL into degradation of endocannabinoid 2-AG. So, the inhibition of MAGL will indirectly attach to increase in cannabinoid or activation of cannabinoid recpetors CB1 and CB2., (b) MAGL maintain the fatty acid secretion or metabolism from lipid store in cancer cell, which will allow them to activate the lipid signalling and that will activate migration, invasion, survival, and tumor growth. Due to this property MAGL inhibitor are the new participants for cancer therapy.

This is interesting to note that, owing to the various functional functions that MAGL performs in the human body, scientists have licensed MAGL inhibitors for a wide variety of medical applications, including, though not limited to, pain and depression, metabolic diseases (such as obesity and diabetes), neurodegenerative pathologies (such as Alzheimer's disease), as well as cancer therapy, anxiety and diabetes. Another significant thing concerns the selectivity properties displayed by the compounds published. Specific hydrolases such as ABHD6, ABHD12, and FAAH show binding site similarity to MAGL, which have distinct endogenous substrates and tissue distribution. Therefore, the experimental selectivity analyzes recorded in many of the patents filed improve the importance of their findings by highlighting the main role of inhibition of MAGL in in vivo tests.

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