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"Redundancy" of Endocannabinoid Inactivation: New Challenges and Opportunities for Pain Control

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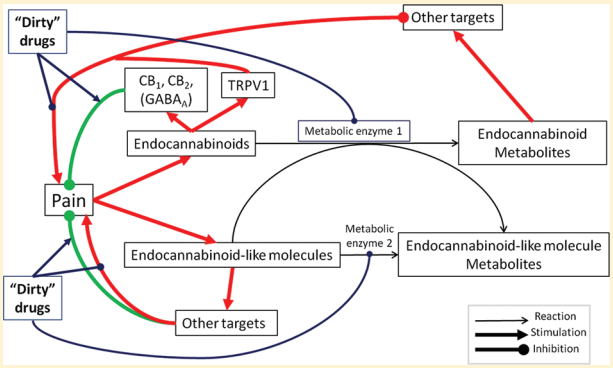
ABSTRACT: Redundancy of metabolic pathways and molecular targets is a typical feature of all lipid mediators, and endocannabinoids, which were originally defined as endogenous agonists at cannabinoid CB_1 and CB_2 receptors, are no exception. In particular, the two most studied endocannabinoids, anandamide and 2-arachidonoylglycerol, are inactivated through alternative biochemical routes, including hydrolysis and oxidation, and more than one enzyme might be used even for the same type of inactivating reaction. These enzymes also recognize as substrates other concurrent lipid mediators, whereas, in turn, endocannabinoids might interact with noncannabinoid receptors with subcellular distribution and ultimate biological actions either similar to or completely different from those of cannabinoid receptors. Even splicing variants of endocannabinoid hydrolyzing enzymes, such as FAAH-1, might play distinct roles in endocannabinoid inactivation. Finally, the products of endocannabinoid catabolism may have their own targets, with biological roles different from those of cannabinoid receptors. These peculiarities of endocannabinoid signaling have complicated the use of inhibitors of its inactivation mechanisms as a safer and more efficacious alternative to the direct targeting of cannabinoid receptors for the treatment of several pathological conditions, including pain. However, new strategies, including the rediscovery of "dirty drugs", and the use of certain natural products (including non-THC cannabis constituents), are emerging that might allow us to make a virtue of necessity and exploit endocannabinoid redundancy to develop new analgesics.



KEYWORDS: *cannabinoid, receptor, FAAH-1, MAGL, COX-2, TRPV1, TRPA1*

That *Cannabis* preparations, such as marijuana, can alleviate pain in humans has been known for millennia. However, the realization that plant cannabinoids, and Δ^9-tetraydrocannabinol (THC) in particular, strongly reduce nociception in animal models of acute, visceral, inflammatory, and chronic pain, is relatively more recent.[1] The cloning in the 1990s of G-protein-coupled receptors for THC, the cannabinoid type-1 and -2 receptors (CB_1 and CB_2),[2,3] strongly expressed in neurons and immune cells (including microglia), respectively, provided a molecular mechanism for these therapeutically promising effects and raised expectations that they could be soon translated into new analgesic and anti-inflammatory medicines. Yet, the possibility arose that direct activation of CB_1 and CB_2 with either natural of synthetic agonists might also produce unwanted effects in terms of psychotropic and immunosuppressive actions,[1,4] respectively. Therefore, the subsequent discovery of endogenous agonists of cannabinoid receptors, known as "endocannabinoids",[5] and of the molecular mechanisms and enzymes controlling their biosynthesis and inactivation, appeared to provide new solutions to this dilemma. In fact, it became soon clear that, unlike neurotransmitters, hormones, and other mediators, and similar to other derivatives of arachidonic acid (eicosanoids) often involved in pain and inflammation, the two most studied endocannabinoids, *N*-arachidonoyl-ethanolamine (anandamide)[6] and 2-arachidonoylglycerol (2-AG)[7,8] are not biosynthesized and stored in secretory vesicles to be released following cell stimulation, but are instead produced and then immediately released to activate CB_1 and CB_2 receptors only locally.[9] Furthermore, it has emerged that endocannabinoid levels might become altered during chronic or inflammatory pain only in those tissues (e.g., the skin, dorsal root ganglia, spinal cord or supraspinal and cortical brain areas) affected by these pathological conditions.[10−15] Thus, devising selective inhibitors of endocannabinoid inactivation would represent a selective and possibly safer way to indirectly modulate cannabinoid receptor activity only when and where needed to counteract pain and inflammation. Several efforts have been made by medicinal chemists and pharmacologists in this direction over the past decade, culminating with the first phase I and II trials on some of such compounds, and, alas, with the realization that even such strategy may be problematic,[16] possibly also due to the high degree of redundancy that

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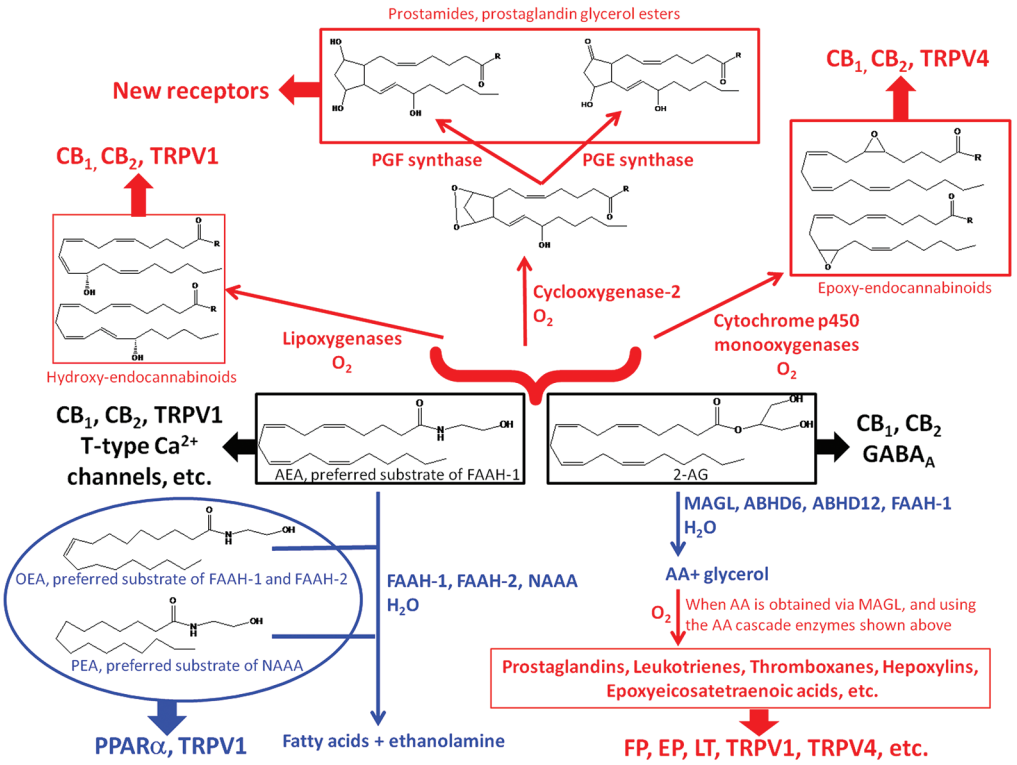

Figure 1. Redundancy of endocannabinoid receptors, catabolic pathways, and enzymes. Hydrolytic (in blue) and oxidative (in red) metabolism of the two most studied endocannabinoids, anandamide (AEA) and 2-arachidonoylglycerol (2-AG). Fatty acid amide hydrolase-1 (FAAH-1) and -2 (FAAH-2), as well as *N*-acylethanolamine hydrolyzing acid amidase (NAAA) catalyze, with varying specificity, the hydrolysis to ethanolamine and the corresponding fatty acids of various *N*-acylethanolamines, including *N*-oleoyl-ethanolamine (OEA) and *N*-palmitoyl-ethanolamine (PEA), which are only weakly active per se on cannabinoid receptors but can stimulate transient receptor potential vanilloid type-1 (TRPV1) and/or peroxisome proliferator-activated receptor-α (PPAR-α). Also AEA and 2-AG have been shown to interact with noncannabinoid receptors. Monoacylglycerol lipase (MAGL), and to a minor extent, α,β-hydrolase-6 (ABHD6) and α,β-hydrolase-12 (ABHD12), as well as FAAH-1, catalyze the hydrolysis of 2-AG. MAGL-catalyzed formation of arachidonic acid (AA) can be utilized as an alternative to phospholipase A_2-mediated pathways to lead, using enzymes of the AA cascade, to the formation of eicosanoids, which then act at their own specific receptors. These same enzymes can be used to oxidize endocannabinoids to the corresponding hydroxy (in the case of lipoxygenases) and epoxy (in the case of cytochrome p450 monooxidases)-derivatives, or to prostamides and prostaglandin glycerol esters (in the case of cycloxygenase-2 and prostaglandin synthases). The formation of hydroxyl and epoxy derivatives, which still exhibit activity at cannabinoid receptors and TRPV1 (or other TRP) channels, has been so far shown to occur only in vitro. Prostamide $F_{2\alpha}$ and prostaglandin E_2-glycerol ester, instead, were recently reported to occur in the spinal cord of mice with knee inflammation and paw skin of rats with local inflammation, respectively, and are inactive at cannabinoid, FP or EP receptors. They were suggested to act at new receptors. Legend: R, ethanolamine or glycerol group.

characterizes most aspects of the biochemistry and pharmacology of the endocannabinoids.

■ ENDOCANNABINOIDS: REDUNDANCY IS EVERYWHERE

We now know that the biochemical routes and enzymes through which endocannabinoids are produced and inactivated are redundant, and the same applies to their receptors (Figure 1). Anandamide, for example, is produced together with other *N*-acylethanolamines, which can be hydrolyzed by the same enzymes as the endocannabinoid, although with varying selectivity for each of the members of this family of lipids. Fatty acid amide hydrolase-1 (FAAH-1)[17] recognizes as substrate, and efficiently hydrolyzes, anandamide, but also *N*-oleoyl-ethanolamine (OEA), an anorexigenic and neuroprotective mediator[18] exhibiting little activity at cannabinoid receptors, but capable of activating two other targets involved in pain and inflammation, that is, the peroxisome proliferator-activated receptor-α (PPAR-α)[19] and the transient receptor potential vanilloid type-1 (TRPV1) channel.[20] While anandamide itself activates and desensitizes TRPV1 channels more efficaciously than OEA,[20,21] OEA, and much less so anandamide, can be hydrolyzed also by FAAH-2, a FAAH-1 isoform not expressed in rodents,[22] whereas a third widely investigated and potent anti-inflammatory *N*-acylethanolamine, *N*-palmitoyl-ethanolamine (PEA),[23] is a good substrate for *N*-acylethanolamine hydrolyzing acid amidase (NAAA).[24] On the other hand, 2-AG, by many regarded as the true CB_1 agonist in the CNS, was recently found to activate $GABA_A$ receptors via interaction with their δ-subunits.[25] This endocannabinoid is mostly inactivated by monoacylglycerol lipase (MAGL),[26] but also other hydrolases, such as α,β-hydrolases 6 and 12, and FAAH-1, may play a role in its degradation.[27] Interestingly, 2-AG has been known for decades to act also as an alternative precursor for arachidonic acid release and eicosanoid production in platelets and sensory neurons,[28] and a recent study confirmed that, among the several 2-AG hydrolytic enzymes, MAGL might be the one responsible for this function in the brain.[29]

Although the enzymatic hydrolysis of endocannabinoids has been most thoroughly investigated and is clearly implicated in the regulation of the tissue levels of these mediators in vivo,

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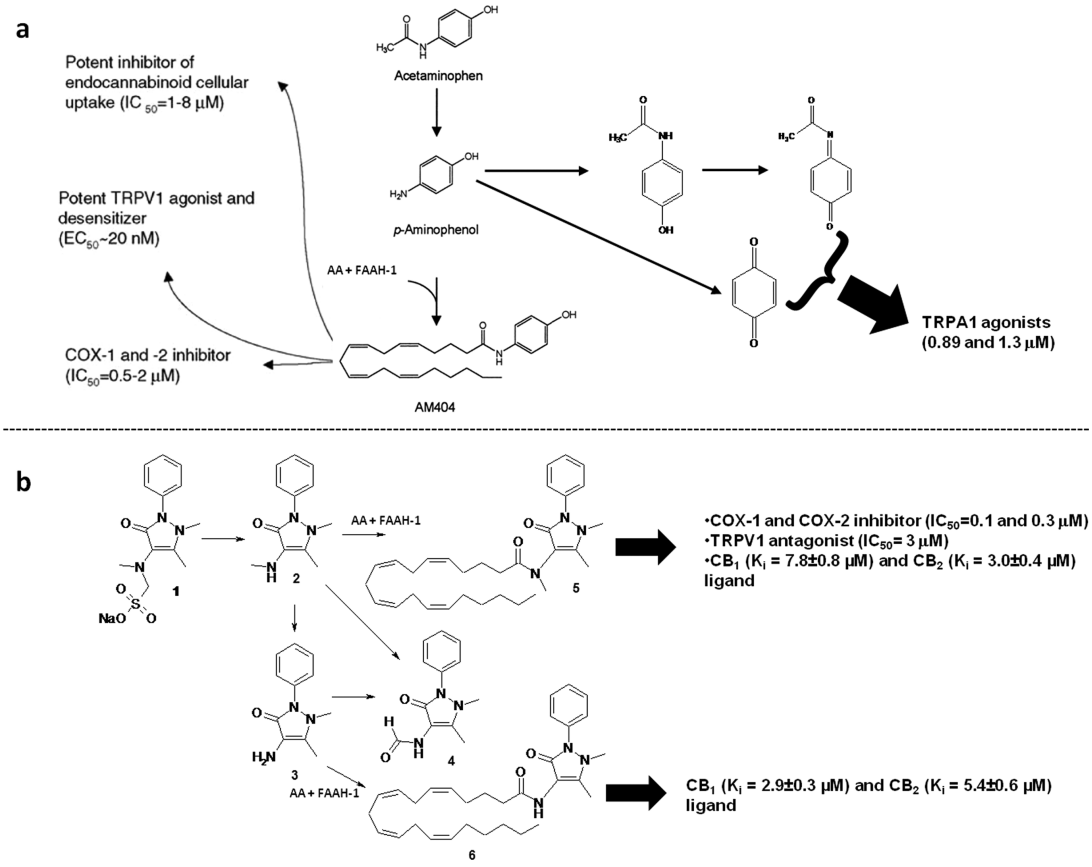

Figure 2. Paracetamol and metamizol are metabolized in vivo to compounds with multitarget analgesic action. Fatty acid amide hydrolase-1 (FAAH-1) catalyzes the condensation of arachidonic acid (AA) with amines produced in vivo from the metabolism of acetaminophen (a) or dipyrone (b) (i.e., *p*-aminophenol and compounds **2** and **3**, respectively). The resulting metabolites, i.e., AM404 and the arachidonoylamides of 4-methylaminoantipyrine (**5**) and 4-aminoantipyrine (**6**), may have different targets involved in pain control. They may still inhibit cycloxygenase-1 (COX-1) and -2 (COX-2) (as in the case of AM404 and compound **5**), and interact with transient receptor potential vanilloid type-1 (TRPV1) channels (by activating and desensitizing them, as in the case of AM404, or by antagonizing them, as in the case of compound **5**). Compounds **5** and **6** also weakly bind to cannabinoid CB_1 and CB_2 receptors. AM404 was also described to weakly inhibit FAAH-1 and bind to CB_1 receptors (not shown), and to counteract endocannabinoid cellular uptake. Finally, other in vivo metabolites of acetaminophen potently activate transient receptor potential ankyrin type-1 (TRPA1) channels and were suggested to cause analgesia through the subsequent inactivation of voltage-activated calcioum channels in spinal neurons.

both anandamide and 2-AG were shown to be good substrates in vitro for several enzymes that oxidize polyunsaturated fatty acids, including cytochrome p450 monooxygenases, lipoxygenases, and cycloxygenase-2 (COX-2).[30,31] Through the COX-2-catalyzed formation of the corresponding endoperoxides, endocannabinoids may serve as precursors for several potential novel lipid mediators,[32] of which the ones known as prostaglandin-ethanolamides (prostamides) and prostaglandin-glycerol esters (PG-GEs) have been most studied[33] (Figure 1). However, unlike most hydroxyl and epoxy derivatives of endocannabinoids, which are still active at cannabinoid and/or TRPV1 receptors,[31,34−36] prostamides and PG-GEs lack activity at both these targets and instead were proposed to act via new, and yet to be fully characterized, receptors,[33,37,38] through which they exert, among others, a stimulatory function on inflammation.[39]

In view of these emerging data, one cannot help wondering what really happens in a cell when inhibiting MAGL or FAAH-1. Does FAAH-1 inhibition result in the "indirect" and site- and time-selective activation not only of CB_1 and CB_2 but also of PPAR-α and TRPV1, as well as in the elevation of prostamide tissue levels, and does all this interfere with the beneficial effects of "indirect" cannabinoid receptor activation? Does MAGL inhibition lead to reduction of eicosanoid production and strengthening of $GABA_A$ activity as extra "bonuses" reinforcing the inhibition of pain and inflammation exerted by indirect CB_1 and CB_2 activation, or does it cause elevation of PG-GE levels as a "collateral event", thereby counteracting the beneficial effects of cannabinoid receptor stimulation? If redundancy in endocannabinoid catabolic pathways and molecular targets hinders the development of new analgesics from FAAH-1 and MAGL inhibitors, is there a way to circumvent this problem? Several studies recently appeared in the literature that might provide the first ways out of this conundrum and will be discussed in the following sections.

"DIRTY" DRUGS VERSUS "MAGIC BULLETS": A WAY TO DEAL WITH ENDOCANNABINOID "PROMISCUITY"

Two widely used nonsteroidal anti-inflammatory drugs (NSAIDs), acetaminophen (paracetamol) and dipyrone (metamizol), have been subjected to several studies investigating their somehow mysterious mechanism of action, and are emerging as "multitarget" drugs. A typical metabolite of acetaminophen, *p*-aminophenol, can be transformed in vivo

into *N*-(4-hydroxyphenyl)-5*Z*,8*Z*,11*Z*,14*Z*-eicosatetraenamide (AM404), by reacting with arachidonic acid in a reaction catalyzed by FAAH-1[40] (Figure 2), an enzyme that under certain conditions can also facilitate the condensation between amides and fatty acids.[41] Interestingly, before its link with paracetamol was discovered, AM404 had been developed as an inhibitor of the putative membrane transporter for anandamide, an as-yet not fully characterized protein or process responsible for anandamide cellular reuptake and release (see below).[42] However, AM404 exhibits several other properties that might be relevant to pain control, including (1) COX inhibition; (2) weak activation of CB_1 receptors; (3) FAAH inhibition; and (4) activation and desensitization of TRPV1.[4043−45] Accordingly, the analgesic effects of paracetamol in rodent models of acute and chronic pain were shown to be mediated by CB_1 or TRPV1 receptors, depending on whether the drug was administered locally, systemically, or centrally.[46−49] Acetaminophen, however, can also be converted into *N*-acetyl-*p*-benzoquinoneimine and *p*-benzoquinone, which, by activating another rapidly emerging target for analgesic drugs, the transient receptor potential ankyrin-type-1 (TRPA1) channel, cause inactivation of voltage-gated calcium channels in spinal neurons involved in ascending pain pathways, and this results in analgesia.[50] Accordingly, the analgesic effects of intrathecal paracetamol were absent in $TRPA1^{-/-}$ mice.[50] Like acetaminophen, also dipyrone is metabolized in vivo to two compounds, 4-methylaminoantipyrine and 4-aminoantipyrine, which can condensate with arachidonic acid in a reaction catalyzed again by FAAH-1[51] (Figure 2). The two resulting arachidonoylamides exhibit some affinity for CB_1 and CB_2 receptors,[51] and the one obtained from 4-methylaminoantipyrine, in particular, also inhibits COX more potently than dipyrone[51] and antagonizes TRPV1 channels.[52] Thus, any of these proteins might mediate the analgesic effects of metamizol, and indeed this widely used drug was recently reported to stimulate the descending pathway of antinociception in rats by a mechanism mediated by CB_1 receptors.[53]

The endocannabinoid-COX link has been strengthened even further by recent discoveries on "old" drugs and the redundancy of endocannabinoid inactivation mechanisms. A study by Duggan and colleagues,[54] by exploiting a multidisciplinary approach consisting of X-ray crystallography, computational modeling, pharmacology, biochemistry, and analytical chemistry techniques, provided a possible explanation as to why a particular class of NSAIDs, the (*R*)-profens, are efficacious anti-inflammatory agents even though they are only weak inhibitors of COX-catalyzed formation of prostanoids. In fact, from the elegant experiments carried out in this study, it turns out that some (*R*)-profens, that is, the *R* enantiomers of ibuprofen, naproxen, and flurbiprofen (Figure 3), despite their relatively weak inhibitory effect on COX-2-mediated oxidation of arachidonic acid, are potent inhibitors of the oxidation of endocannabinoids by this enzyme. Therefore, (*R*)-profens not only prolong the lifespan of anandamide and 2-AG, but also prevent the formation of prostamides and PG-GEs, as shown by the authors using isolated dorsal root ganglia.[54] The selectivity of (*R*)-profens is based on the cooperativity recently described to occur between the two subunits of COX-2, and potentially allows the administration of doses of these compounds that will inhibit the inactivation of endocannabinoids without impairing the production of other COX metabolites, thus avoiding the side effects typical of COX inhibitors. Indeed, the potential benefit of the use of these compounds emerges even more

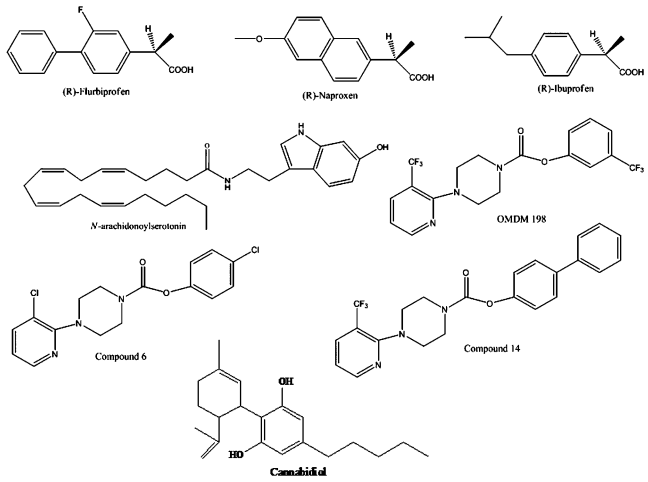

Figure 3. Chemical structures of some synthetic or natural multitarget analgesics discussed in this review.

convincingly if one considers very recent results showing that prostamide $F_{2\alpha}$ is indeed produced in vivo, in the spinal cord of mice with knee inflammation induced by injection of kaolin and λ-carrageenan, and exhibits strong pro-algesic actions.[55] It is tempting to speculate that the failure of a selective FAAH-1 inhibitor at reducing pain in human osteoarthritis, described at the 2010 IASP meeting in Montreal, might have been due also to the concomitant elevation of the levels of pro-inflammatory prostamides, thus perhaps suggesting that coadministration of FAAH-1 and (*R*)-profens might have instead done the trick.

Also the emerging concept that MAGL inhibitors, especially if used at submaximal doses, might replace COX-2 inhibitors, since they appear to inhibit 2-AG/MAGL-driven prostaglandin production in the CNS but not in the GI tract,[29] might be revisited if other preliminary data, suggesting instead that MAGL inhibition, at least in the brain, may open the way to the formation of pro-inflammatory PG-EGs, are confirmed.[56] In view of these complications, the development and use of drug combinations, and, even better, of molecules with more than one target, such as, for example, dual COX-FAAH-1 or COX-MAGL inhibitors, is to be fostered. It is interesting to note that inhibition of endocannabinoid inactivation produces gastroprotection against NSAID-induced gastric hemorrhages in animal models,[57] thus raising the possibility that dual inhibitors of COX and endocannabinoid hydrolytic enzymes might be not only more efficacious but also safer than corresponding "magic bullets" with only one target.

A recent example of the potential successful application of such strategy is represented by *N*-arachidonoylserotonin, a compound originally developed as moderate inhibitor of anandamide hydrolysis,[58] and later found to also potently antagonize TRPV1.[59] *N*-Arachidonoylserotonin (Figure 3) was shown to be more efficacious at counteracting inflammatory and chronic pain than more potent and selective FAAH-1 inhibitors or TRPV1 antagonists.[14,59] More recent attempts at producing more "druggable" dual FAAH-1-TRPV1 blockers starting from *N*-arachidonoylserotonin or other FAAH-1 inhibitors have produced thus far either serotonin derivatives of other unsaturated fatty acids, which are not too dissimilar from the starting compound,[60] or compounds with weaker activity at both targets, such as OMDM198 (compound **10** in ref 61) (Figure 3), the pharmacokinetic profile of which in vivo might nevertheless be more favorable than that of *N*-

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arachidonoylserotonin and is currently being tested. Interestingly, these attempts also led to the development of dual FAAH-1 inhibitors-TRPA1 agonists, such as compounds **6** and **14** in ref 61 (Figure 3), which, based on the aforementioned emerging role of TRPA1 agonists in the control of pain, might also prove to be interesting leads for the development of new analgesics. Nevertheless, *N*-arachidonoylserotonin was recently reported, together with other *N*-acylserotonins, as an endogenous compound in porcine and murine gastrointestinal tracts, and is still the most potent dual FAAH-1-TRPV1 blocker identified to date, thus confirming, in some ways, the "old-fashioned" view that it is difficult to imitate or surpass Nature. Indeed, another natural compound and *Cannabis* constituent, cannabidiol (Figure 3), which belongs to the same chemical class as THC but is devoid of potent direct actions at CB_1 and CB_2 receptors, has proven to be very efficacious against inflammation and pain. Cannabidiol was suggested to exert these effects via several targets, including stimulation/desensitization of TRPV1 and TRPA1,[62] and inhibition of endocannabinoid[62] and adenosine[63] inactivation.[64−66]

In summary, both natural and (old and new) synthetic compounds with more than one mechanism of action seem to have revived the importance of "dirty" drugs, which may now provide us with new strategies to deal with endocannabinoid redundancy and treat pain and inflammation .

ANALGESIA THROUGH INHIBITION OF ENDOCANNABINOID CELLULAR UPTAKE: THE "FLAT" TRUTH?

Another strategy to treat pain that has been so far explored only in preclinical studies is the one based on the inhibition of the putative "endocannabinoid membrane transporter" (EMT), the existence of which has been dwelled upon for over 15 years.[9] In fact, hydrolysis or oxidation of endocannabinoids is effected at the intracellular level, and although these mediators are lipophilic and can cross the plasma membrane by simple passive diffusion, there is indirect evidence for the existence of a membrane transporter-like process facilitating this passage according to the gradient of concentrations, and in a manner subject to regulation by other mediators.[9] However, the fact that no protein has been identified so far to specifically facilitate endocannabinoid transport across the membrane, has cast doubts over the existence of the EMT and hindered the development of truly selective as well as potent inhibitors of this process. Furthermore, the observation that several intracellular proteins bind anandamide with varying selectivity toward other fatty acid derivatives, in order to allow for its trafficking to intracellular enzymes,[67] and thus contribute to anandamide cellular uptake, represents a further complication when it comes to set up a specific assay to assess the role of plasma membrane proteins in this process independently from other mechanisms. This effort, however, would certainly be worthwhile, since inhibitors of the EMT, if such specific protein or process does exist, would have two advantages over MAGL and FAAH-1 inhibitors, inasmuch as they should (1) interfere more selectively with anandamide and 2-AG inactivation with respect to other bioactive substrates (such as other *N*-acylethanolamines) of the two hydrolytic enzymes; and (2) block at the same time the interaction of endocannabinoids with intracellular binding sites (as in the case of TRPV1) or other metabolic enzymes (including COX-2), while leading to a more selective prolongation of their activity at their most important extracellular targets, that is, CB_1 and CB_2 receptors, without producing metabolites with activity per se (e.g., prostamides, and PG-GEs). A series of EMT inhibitors with chemical structures usually related to those of anandamide or OEA (Figure 4) has been developed and shown to be more or less selective toward FAAH-1 and various endocannabinoid targets.[68] Some of these compounds clearly exhibit different in vivo pharmacology, for example, in a test of acute pain, from FAAH-1 inhibitors.[69]

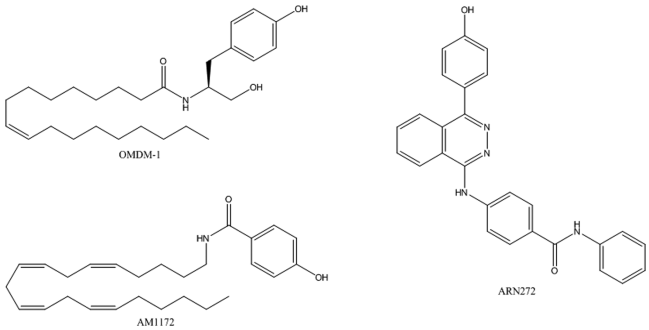

Figure 4. Chemical structures of some synthetic inhibitors of anandamide cellular uptake discussed in this review.

A recent study by Fu et al.[70] sheds light on the possibility of developing new drugs in this direction. The authors identified an intracellular protein that specifically facilitates anandamide cellular reuptake in neurons and astrocytes, and named it "FAAH-like anandamide transporter" (FLAT). FLAT is a further example of redundancy in endocannabinoid inactivation, as it is a splicing variant of FAAH-1 that is catalytically inactive, that is, unable to catalyze the hydrolysis of anandamide, 2-AG, or other *N*-acylethanolamines. Nevertheless, this protein binds anandamide, but not 2-AG or other *N*-acylethanolamines, with high affinity, and acts as an intracellular sink for anandamide transport across the membrane. FLAT is certainly not the long-sought EMT, primarily because of its cytosolic location and of it not being able to recognize 2-AG.[70] Furthermore, other intracellular proteins had been previously suggested to participate in intracellular anandamide trafficking.[67] Nevertheless, the discovery of FLAT is potentially very important from the therapeutic point of view, since this protein, unlike others previously suggested to participate in the intracellular trafficking of anandamide,[67] is selective for anandamide and, as a consequence, its pharmacological inhibition will block the cellular uptake of anandamide, but not 2-AG or other lipid mediators. As perhaps exemplified by data obtained with a dual FAAH-1/MAGL inhibitor, which exhibits some of the same central side effects observed with "direct" CB_1 agonists,[71] inhibiting FLAT might offer some advantages over the inhibition of the inactivation of both endocannabinoids, which instead is achieved with inhibitors of the putative EMT (although, perhaps, less dramatically than with dual FAAH-1/MAGL inhibitors).[71,72] A selective FLAT inhibitor would retard only anandamide delivery to FAAH-1, thus producing similar therapeutic effects as FAAH-1 inhibitors, but without affecting the levels of other FAAH-1 substrates. Furthermore, as observed with inhibitors of less specific proteins mediating *N*-acylethanolamine intracellular trafficking,[73,74] such a compound would still prevent intracellular targets or enzymes from being overexposed to undegraded anandamide, thus avoiding one of the complications of FAAH-1 inhibitors. Indeed, Fu and

colleagues did screen a large library of synthetic compounds for their activity to bind to FLAT and inhibit anandamide cellular uptake, and came up with a novel compound, ARN272 (Figure 4), which competitively inhibits FLAT but not FAAH-1, and exerts potent antihyperalgesic actions in models of acute and inflammatory pain, in a manner attenuated by a CB_1 receptor antagonist.[70]

Despite its potential relevance to the development of future analgesics, does the discovery of FLAT really negate the existence of an EMT process? The fact that FLAT is inhibited by some of those compounds that were previously suggested to be selective for the EMT versus FAAH-1 (i.e., OMDM-1 and AM1172)[70] (Figure 4) might support this possibility. However, these compounds were later suggested to recognize also other, less specific intracellular proteins involved in anandamide and *N*-acylethanolamine trafficking (i.e., some fatty acid binding proteins [FABPs]) (75), while, instead, they are nearly inactive at inhibiting the cellular uptake of anandamide when this endocannabinoid is incorporated into nanoparticles.[74] This indicates that long chain fatty acid-based inhibitors of the putative EMT are too promiscuous toward proteins with fatty acid binding domains (but obviously not toward plasma membrane proteins mediating nanoparticle endocytosis[74]) to be used to draw any conclusion as to whether or not such a process exists, a conclusion that, clearly, will now only be reached through the use of other, possibly more "molecular" approaches. Nevertheless, these compounds, by virtue of the multiple mechanisms which they employ to inhibit endocannabinoid cellular uptake (i.e., by inhibiting FAAH-1 and/or FLAT and/or FABPs), might still be useful lead compounds to develop novel drugs against pain.

CONCLUSIONS: WHEN LIFE GIVES YOU LEMONS, MAKE LEMONADE

In conclusion, redundancy in endocannabinoid inactivation, of which the capability of anandamide to be oxidized by COX-2 in vivo and the existence of alternative splicing variants of FAAH are just recent examples, may complicate the development of safe and efficacious analgesics from inhibitors of this process, but also offer exciting new challenges for the design, on the one hand, of "multi-target" therapies and, on the other hand, of selective inhibitors of anandamide intracellular trafficking. Thus, we might be able to make the best of this peculiar feature of endocannabinoid signaling, and the last word on the future possibility of obtaining new painkillers through the targeting of endocannabinoid inactivation is far from being set.

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Notes

The authors declare no competing financial interest.

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