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## Perspective

### The Endocannabinoid System: Drug Targets, Lead Compounds, and Potential Therapeutic Applications

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#### 1. Introduction

As far as can be ascertained from archeological sources, preparations of the hemp *Cannabis sativa* L. were widely used by humans for either therapeutic or ritual purposes in ancient times. According to the legend, the Chinese emperor Sheng Nung (3000 B.C.) had a transparent abdomen that allowed to him to see the effects of plants and medicines. He discovered the therapeutic values of three major medicinal plants: ginseng, ephedra, and cannabis. Under the name “ma” or “ta ma” meaning great hemp, cannabis is described in traditional Chinese pharmacopoeia in 200 A.D. Cannabis has been used by different civilizations for a variety of medical applications such as pain, stimulation of appetite, nausea, fever, infections, and gynecological disorders.<sup>1,2</sup> The first evidence of the use of cannabis (and perhaps the testimony of an early medical use) was the discovery of a gray carbonized material containing a derivative of  $\Delta^9$ -tetrahydrocannabinol (**1**),  $\Delta^8$ -tetrahydrocannabinol (**2**) (quoted as  $\Delta^6$ -tetrahydrocannabinol in the original publication, using a different numbering system; see below), lying near the body of a pregnant woman in a burial tomb near Jerusalem. The finding of different bronze coins dating to A.D. 315–392 near the body allowed the dating of this finding.<sup>3</sup> Although  $\Delta^8$ -tetrahydrocannabinol is present at low concentrations in the plant, it is a stable constituent; in fact, it is

the main product resulting from the combustion of  $\Delta^9$ -tetrahydrocannabinol (**1**, Chart 1).

Despite the use of cannabis as a medicine in the U.K. during the 19th century (Sir John Russell Reynolds, Queen Victoria's physician, was a proponent of cannabis as a therapeutic agent<sup>4</sup>), the progress of the knowledge of cannabis pharmacology was very slow. Agents such as cannabidiol (**3**, Chart 1) and cannabinol (**4**, Chart 1) had been isolated and investigated chemically in the early 1940s,<sup>5,6</sup> but they were not responsible for the psychoactive effect of the plant. The correct structure of cannabidiol was reported in 1963.<sup>7</sup>

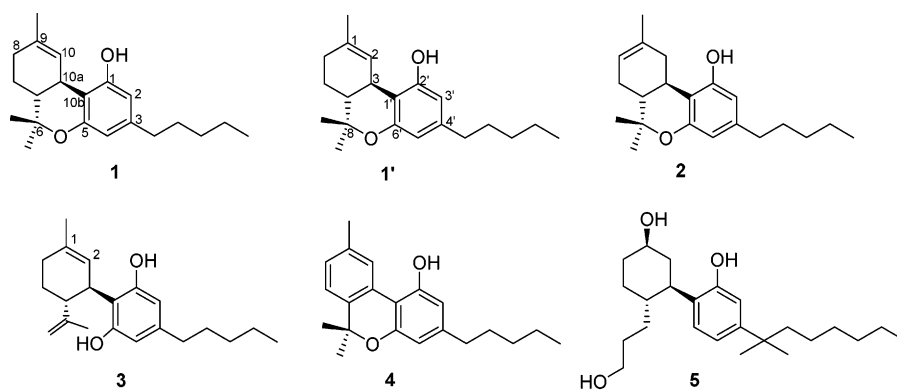
An important milestone was thus the isolation and the elucidation of the structure of the main psychoactive constituent from the leaves of *Cannabis sativa* L. In 1964, Gaoni and Mechoulam<sup>8</sup> identified  $\Delta^9$ -tetrahydrocannabinol (**1**), ending a quest of almost 60 years. Two different numbering systems are used in the benzopyran ring of **1**. Thus, according to the authors, the main psychoactive agent can be termed either  $\Delta^9$ -tetrahydrocannabinol or  $\Delta^1$ -tetrahydrocannabinol, two names for a single molecule depending on the system used for the numbering, respectively, for dibenzopyran and monoterpenoid systems. Its chemical name is (6 $\alpha$ R,10 $\alpha$ R)-6 $\alpha$ ,7,8,10 $\alpha$ -tetrahydro-6,6,9-trimethyl-3-pentyl-6H-dibenzo[b,d]pyran-1-ol. The absolute configuration of **1** was established some years later.<sup>9</sup> The history of the chemical research on cannabinoids has been recently reviewed.<sup>10,11</sup>

Until the 1980s, the term “cannabinoids” represented, by definition, the group of typical diterpene C<sub>21</sub> com-

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**Chart 1.** Natural Cannabinoids and the Derived Synthetic Prototype CP-55,940<sup>a</sup>

<sup>a</sup> The main psychoactive ingredient of *Cannabis sativa* L.,  $\Delta^9$ -tetrahydrocannabinol, is represented with its two numbering systems (**1** and **1'** yielding  $\Delta^9$ -tetrahydrocannabinol and  $\Delta^1$ -tetrahydrocannabinol, respectively).  $\Delta^8$ -Tetrahydrocannabinol<sup>8</sup> (**2**), cannabidiol (**3**),<sup>7</sup> and cannabinol (**4**) are also present in the hemp *Cannabis sativa* L. The synthetic prototype presented here is CP-55,940 (**5**) from Pfizer.

pounds present in *Cannabis sativa* L., their carboxylic acids, analogues, and transformation products. They are now sometimes termed phytocannabinoids. In the plant, the important cannabinoid compounds are  $\Delta^9$ -tetrahydrocannabinol,  $\Delta^8$ -tetrahydrocannabinol (**2**), cannabidiol (**3**), and cannabinol (**4**). The structures of these natural cannabinoids are presented in Chart 1. Cannabidiol is the subject of considerable interest, in view of its anti-inflammatory properties and lack of psychotropic effects.<sup>12,13</sup> This compound, however, does not act at the "classical" cannabinoid receptors (see below) and is consequently outside the scope of the present review.

Despite the identification of  $\Delta^9$ -tetrahydrocannabinol, its cellular target was not identified for more than a quarter of a century, not the least as a consequence of the high lipophilicity of the compound. During this period, the debate between scientists was open. Some authors considered that the stereoselectivity of  $\Delta^9$ -tetrahydrocannabinol analogues was a point arguing for the existence of receptors. Others pointed out the interactions between **1** and biological membranes. In terms of medicinal chemistry, most of the endeavors were concentrated on the study of the structure–activity relationships of **1**. Before the identification of cannabinoid receptors, *in vivo* screening assays for cannabinoid activity were largely behavioral in nature, initially with the dog ataxia test<sup>14</sup> and with the characterization of overt changes in behaviors in rhesus monkeys<sup>15</sup> (for excellent reviews detailing cannabinoid assays used, see refs 16 and 17). Of these behavioral assays, the "cannabinoid tetrad" test<sup>18</sup> is still used to provide valuable quantitative information on the central cannabimimetic activity of test compounds. This assay comprises four different behavioral tests performed mostly in mice: diminution of rectal temperature (hypothermia), immobility in a multiple photoelectric cell chamber (diminution of locomotion), a ring test or bar test (catalepsy), and hot plate or tail flick tests (analgesia). Each test per se is somewhat unspecific (and has long been used to characterize cannabinoid effects pharmacologically<sup>17</sup>), but cannabinoids could be identified as producing a response in all four tests.<sup>18</sup>

An important clue to the existence of cannabinoid receptors was provided by studies showing that incubation of neuroblastoma cells with cannabinoids induced a decrease of cAMP in the cells, suggesting that the putative cannabinoid receptors were negatively coupled

to adenylyl cyclase.<sup>19–21</sup> The existence of cannabinoid receptors was confirmed in 1988 when an open analogue of **1**, the Pfizer compound CP-55,940 (**5**, Chart 1), was made available. This compound was less lipophilic than **1** and upon tritiation was used as the first probe of cannabinoid receptors by competitive binding assays.<sup>22</sup> It still remains a widely used radioligand.

In 1990, the cloning of a cannabinoid receptor from the rat was reported.<sup>23</sup> It was indeed negatively coupled to adenylyl cyclase and a member of the G-protein-coupled-receptor superfamily. Three years later, a second receptor was found by sequence homology.<sup>24</sup> This receptor has been cloned from HL-60 cells and was found to be restricted to the immune system, including macrophages from the spleen. Since the 1990s, the term cannabinoid has been extended to any molecule that binds to one of the cannabinoid receptors. The current NC-IUPHAR nomenclature accepts these two receptor types, termed CB<sub>1</sub> and CB<sub>2</sub> cannabinoid receptors.<sup>17</sup> Although there are some data suggesting the presence of additional receptors (e.g., see refs 25–28 for recent examples), current evidence is not yet considered sufficiently strong to warrant the formal definition of additional CB receptors.

With this discovery of cannabinoid receptors, the quest for an endogenous ligand of these receptors was launched. The long route from the discovery of **1** to the endogenous compound together with the technical and conceptual problems at this time has been nicely reviewed elsewhere.<sup>29</sup> On the basis of the assumption that the endogenous cannabinoid ligand was a lipid-soluble compound, a lipid derivative, arachidonylethanolamide (C20:4), was isolated from chloroform–methanol extracts of porcine brain and christened anandamide (**6**), referring both to the Sanskrit "ananda" meaning bliss and to "amide" for the chemical nature of the compound.<sup>30</sup> The C20:4 shown in parentheses refers to the number of carbon atoms and unsaturated bonds in the acyl side chain. Besides anandamide, numerous endogenous compounds have now been isolated sharing with **6** the ability to bind and activate at least one cannabinoid receptor. These molecules have been termed cannabimimetic fatty acid derivatives<sup>31</sup> and will be described in section 2 below.

Since these pioneering studies, much work has been undertaken to characterize and identify molecules interfering with the sites where endocannabinoids act

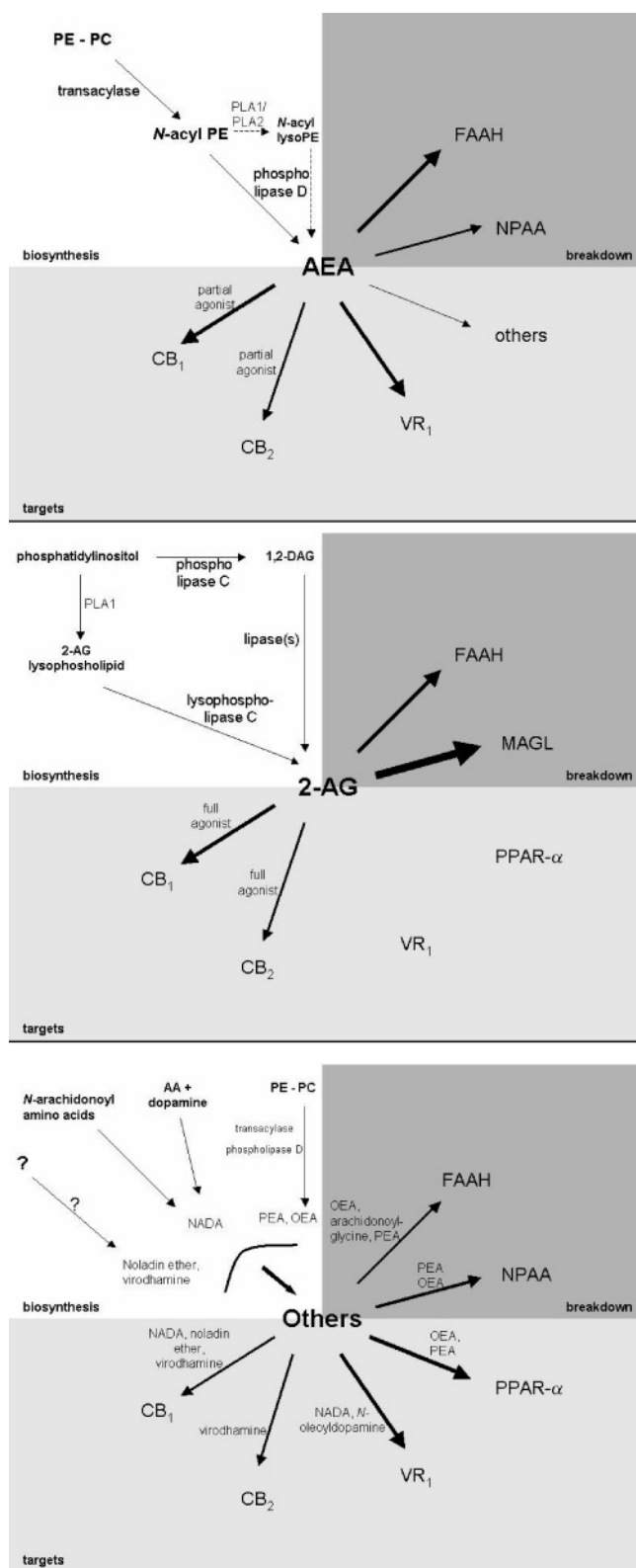
or bind. The endocannabinoid system has, as expected for a signaling system, components responsible for synthesis, action, and inactivation, all of which are legitimate targets for pharmacological intervention (Figure 1). In this Perspective, compounds affecting the major targets of the endocannabinoid system will be described, together with an outline of some of the possible therapeutic benefits of such compounds.

## 2. Endocannabinoids and Related Endogenous Compounds: Structures and Biosynthesis

As mentioned above, the quest for endogenous ligands for the cannabinoid receptors led to the discovery of a family of polyunsaturated compounds derived from fatty acids, mostly from arachidonic acid (Chart 2). At the outset, it is worth discussing the definition of an endocannabinoid. In theory, an endogenous compound capable of interacting with cannabinoid receptors can be considered an endocannabinoid. We have elected here to use a more conservative definition of endocannabinoid, namely, that in addition to it fulfilling this criterion it should additionally (a) have defined synthetic and catabolic pathways and (b) be supported by either direct or indirect evidence demonstrating that it can be released in sufficient concentrations to be considered capable of activating cannabinoid receptors *in vivo*. Similarly, endocannabinoid implies that the interaction with the cannabinoid receptors is paramount and that the ability of the compounds to interact with other molecular targets is a secondary phenomenon. This admittedly stringent definition would limit known endocannabinoids to two: anandamide (**6**) and 2-arachidonoylglycerol (**7**). Indeed, a case has been made<sup>32</sup> that anandamide is as much an endovanilloid as an endocannabinoid (see below).

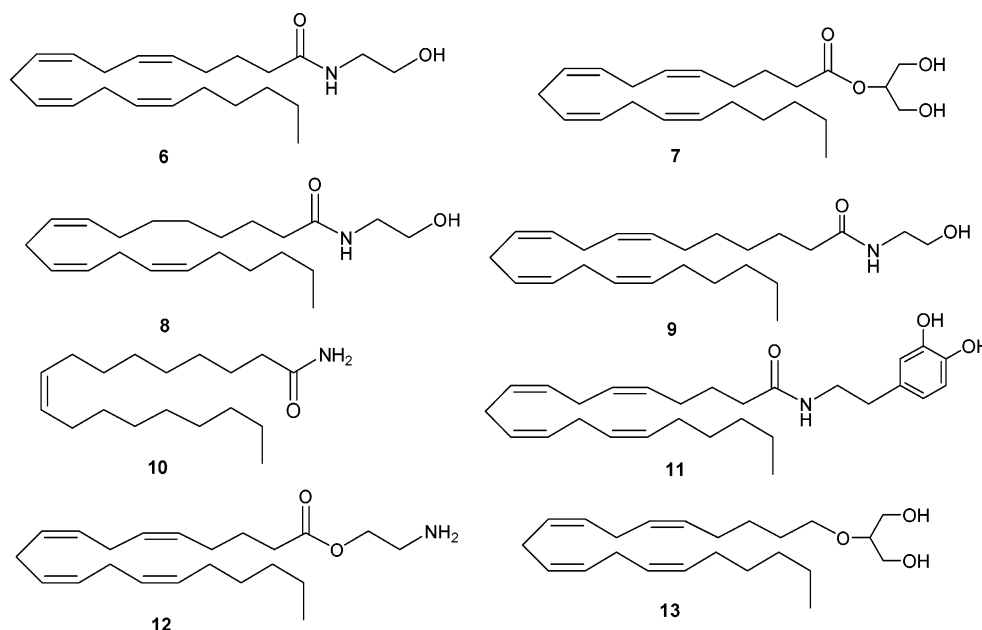
In the discussion below, we have considered the synthesis and properties of these two endocannabinoids, together with compounds that can be considered as putative endocannabinoid candidates: noladin ether, virodhamine, and arachidonoyldopamine. Finally, endogenous compounds that can produce cannabinoid-like effects *in vivo* but do not interact directly with cannabinoid receptors *in vitro* are discussed. Effects on biological targets other than cannabinoid receptors are also discussed below, but these are not the main focus of this review.

**2.1. Endocannabinoid Amides: *N*-Acylethanolamines and Fatty Acid Primary Amides.** Among the *N*-acylethanolamines, anandamide<sup>30</sup> has been the most well studied. Under conditions where its hydrolysis is prevented, **6** interacts with CB<sub>1</sub> and CB<sub>2</sub> receptors with *K<sub>i</sub>* values of 72 and 370 nM, respectively (median values from the three different studies reported in ref 17 and shown in Figure 2). For comparison, the median *K<sub>i</sub>* values for the "standard" cannabinoids (calculated from the data shown in Figure 2; literature citations given in the figure caption) for CB<sub>1</sub> and CB<sub>2</sub> receptors, respectively, are as follows: Δ<sup>9</sup>-tetrahydrocannabinol, 37 and 20 nM (*n* = 8); **5**, 1.4 and 2.6 nM (*n* = 7); WIN 55,212-2, 9.9 and 1.8 nM (*n* = 12). **6** has a very much lower affinity for the two splice forms<sup>33,34</sup> of human CB<sub>1</sub> receptors so far discovered (see section 3.1) (*K<sub>i</sub>* values of 50, 11 000, and 12 500 nM being found for hCB<sub>1</sub>, hCB<sub>1a</sub>, and hCB<sub>1b</sub> receptors, respectively).<sup>34</sup> This is in

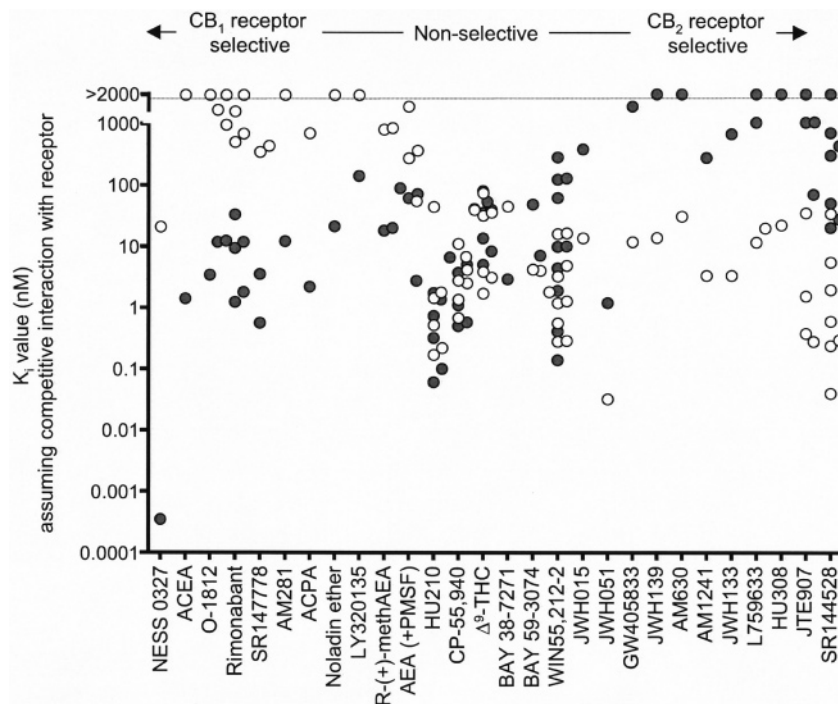


**Figure 1.** Diversity of endocannabinoids: synthetic pathways, targets, and inactivation processes. The top panel presents the data for anandamide (AEA), the middle panel presents the data for 2-arachidonoylglycerol (2-AG), and the bottom panel represents the data for the other putative endocannabinoids and related lipids. The width of the arrow represents the importance of the phenomenon.

contrast to the situation for **1** and the synthetic agonists HU210 and **5**, where the respective affinities are not more than 3-fold different between the receptor forms.<sup>34</sup>

Chart 2. Endocannabinoids<sup>a</sup>

<sup>a</sup> Chemical structures of arachidonoylethanolamide (anandamide, **6**), 2-arachidonoylglycerol (**7**), homo- $\gamma$ -linolenylethanolamide (**8**), docosatetraenoylethanolamide (**9**), oleamide (**10**), arachidonoyldopamine (**11**), *O*-arachidonylethanolamine (virodhamine, **12**), and 2-arachidonoylglyceryl ether (noladin ether, **13**).



**Figure 2.** Selected examples of affinities of a series of cannabinoid CB ligands for CB<sub>1</sub> (filled symbols) and CB<sub>2</sub> (unfilled symbols) receptors in vitro, as assessed in radioligand binding experiments. Multiple points for a given compound indicate data from several different investigations and/or species. The data are taken from Table 2 of ref 17 (and the references therein) together with selected studies published since that review<sup>204,205,212,220,271,425,426</sup> (with apologies to authors of studies not included in this figure). In the case of anandamide, only values in the presence of **47** (to prevent its hydrolysis) have been included. In addition, values in ref 17 given as “> 1000 nM” have been excluded together with their pair value for the other receptor. The wide spread of the data is partly due to species variations in the sensitivity<sup>204,427</sup> and to the lipophilicity of the compounds, a bane of cannabinoid researchers’ existence! A final point that should be remembered is that the in vivo relative potencies are dependent upon additional factors such as bioavailability of the compounds. The difference between the potencies of rimonabant and *N*-piperidinyl-[8-chloro-1-(2,4-dichlorophenyl)-1,4,5,6-tetrahydrobenzo[6,7]cyclohepta[1,2-*c*]pyrazole-3-carboxamide] (NESS 0327) toward CB<sub>1</sub> receptors, for example, is much less marked in vivo than in vitro.<sup>426</sup>

With respect to efficacy, **6** is generally considered to act as a partial agonist at CB<sub>1</sub> and CB<sub>2</sub> cannabinoid receptors in vitro.<sup>35,36</sup> However, recent data may require a slight modification of this view. Modern receptor

theory has moved from the notion that the receptor is in an inactivated or an activated state (the two-state hypothesis) to more complicated models, whereby the activated receptor can couple to different signaling



pathways and the agonist used is an important determinant in this process. This has been termed "agonist-directed trafficking of receptor stimulus"<sup>37</sup> and has been elegantly demonstrated, among others, for 5-HT<sub>2C</sub> receptors expressed in Chinese hamster ovary cells; thus, when efficacy of the receptor was assessed by measuring phosphoinositide breakdown as the response to these G-protein-coupled proteins, 3-trifluoromethylphenyl-piperazine and ( $\pm$ )-1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane acted as full and partial agonists, respectively. However, when arachidonic acid release was used as a measure of efficacy, the pattern was reversed.<sup>38</sup> Recent data investigating the coupling of cannabinoid CB<sub>1</sub> receptors to G<sub>i</sub> proteins have suggested that similar mechanisms are operative. In particular, (*R*)-methanandamide, the nonhydrolyzable analogue of **6**, was suggested to act as an agonist at CB<sub>1</sub> receptors coupled to G<sub>i3</sub> proteins but as an inverse agonist at CB<sub>1</sub> receptors coupled to G<sub>i1</sub> and G<sub>i2</sub> proteins.<sup>39</sup> In other words, the observed efficacy of **6** and other (endo)-cannabinoids in any given cell will depend on the relative expression of the G proteins capable of interacting with the receptor and upon the response being measured.

Anandamide shares in vivo properties with **1**, for instance, in the cannabinoid tetrad test,<sup>40</sup> albeit with a shorter mode of action, as has been well detailed in recent reviews.<sup>41–42</sup> **6** also produces effects on other biological systems, of which the vanilloid receptor (TRPV1) has been the most investigated.<sup>32,43</sup> Although this latter effect has led to the description of anandamide as a "Janus molecule", whereby opposing effects on CB and TRPV1 molecules may be observed at different ambient anandamide concentrations,<sup>44</sup> it does induce analgesia in different pain models including formalin-evoked pain,<sup>45,46</sup> the tail-flick model applied to rhesus monkeys<sup>47</sup> as well as nonarthritic and arthritic rats,<sup>48</sup> the carrageenan model applied to mice<sup>49</sup> and rats,<sup>50</sup> and after turpentine injection into the rat bladder.<sup>45</sup> The effects of **6**, often TRPV1-mediated, on cell proliferation have been widely studied and recently reviewed.<sup>51,52</sup> Other biological actions of **6**, usually (but not exclusively) mediated by CB and/or TRPV1 receptors, include effects upon cell proliferation and apoptosis in a variety of cell lines,<sup>53–59</sup> effects in reproduction<sup>60,61</sup> and memory processes,<sup>62</sup> attenuation of cholera toxin-induced fluid accumulation,<sup>63</sup> modulation of anxiety,<sup>64</sup> increasing of cytochrome P450 content and activity in rat,<sup>65</sup> modulation of epilepsy,<sup>66</sup> and modulation of feeding.<sup>67</sup>

As with other *N*-acylethanolamines, different biosynthetic pathways have been proposed for **6** (Figure 1) (see refs 68–70 for reviews). A one-step pathway might occur through energy-independent condensation of ethanolamine and fatty acid,<sup>71,72</sup> catalyzed by a "synthase". However, nonphysiological concentrations of the two substrates are necessary for this enzyme to work, very probably because the "synthase" is the enzyme that normally catalyzes acylethanolamine hydrolysis and can be forced to work "in reverse".<sup>73</sup> A second, and more physiologically relevant, route occurs through the hydrolysis of a phospholipid precursor, *N*-acylphosphatidylethanolamine (*N*-acyl-PE), catalyzed by a phosphodiesterase of the D type.<sup>74</sup> This phospholipase D has

been widely studied<sup>75,76</sup> and was recently cloned.<sup>77</sup> The *N*-acyl-PEs, in turn, can be produced from the acylation of the amino group of phosphatidylethanolamine by using an acyl moiety stereoselectively donated by the sn-1 position of other phosphoglycerides through the action of a Ca<sup>2+</sup>-dependent *N*-acyltransferase or *trans*-acylase.<sup>74</sup> This latter enzyme, to our knowledge, is not yet cloned.

Recently, the possible presence of an alternative two-step pathway from *N*-acyl-PE has also been reported. The first step is the hydrolysis of *N*-acyl-PE to *N*-acyl-lysoPE by PLA<sub>1</sub>/PLA<sub>2</sub> enzyme(s) followed by the release of NAEs from *N*-acyl-lysoPE by lysoPLD (lysophospholipase D) enzyme(s).<sup>78</sup> Biosynthetic enzymes may constitute attractive targets for the development of inhibitors as drug candidates. The recent progress and in particular the cloning of a specific phospholipase D generating *N*-acylethanolamines including **6** strongly suggest that new and selective inhibitors might be useful as therapeutic agents, with pharmacological properties parallel (but not identical) to those of cannabinoid receptor antagonists.

Different amide congeners have been proposed. Two additional fatty acid ethanolamides that bind to the cannabinoid CB<sub>1</sub> receptor, homo- $\gamma$ -linolenylethanolamide (C18:3, **8**,  $K_i = 53$  nM) and docosatetraenylethanolamide (C22:4, **9**,  $K_i = 34$  nM), were found in the porcine brain.<sup>79</sup> Little is known about the biological properties of these compounds. Nevertheless, their effects on the mouse vas deferens, a CB<sub>1</sub> cannabinoid receptor containing tissue,<sup>80</sup> and on the inhibition of adenylyl cyclase<sup>81,82</sup> have demonstrated their agonist properties. Cannabinomimetic behavioral effects were observed in vivo (motor activity in an open field, hypothermia, catalepsy on a ring, and analgesia on a hot plate) and were found to be similar to those observed with **1** and **6**. Recently, it was reported<sup>83</sup> that mouse astrocytes in culture produce **6** but also other acylethanolamides, including **8** and **9**. However, in contrast with 2-arachidonoylglycerol (discussed below) and **6**, these two acylethanolamides were not implicated in the microglial cell migration during neuroinflammation.<sup>84</sup>

A primary amide of an unsaturated fatty acid, oleamide (**10**), produces effects upon a multiplicity of targets (for a review, see ref 85), including modest effects upon CB<sub>1</sub> cannabinoid receptors in vitro ( $K_i = 1.14$   $\mu$ M vs [<sup>3</sup>H]-CP-55,940 binding to rat brain membranes).<sup>86</sup> Whether or not oleamide can be considered as a member of the endocannabinoid family is a moot point.<sup>87</sup> However, it shares with **6** some pharmacological properties: sleep induction,<sup>88</sup> anticonvulsant effects,<sup>89</sup> and modulation of appetite.<sup>90</sup>

A second family of conjugates of unsaturated long-chain fatty acid derivatives have been recently discovered, the arachidonoyl amino acids and arachidonoyl biogenic amines, which share some similarity with **6**, but most of the members are not able, so far, to interact with CB receptors. The exception is arachidonoyl-dopamine (**11**). In 2000, it was found that **11** (Figure 1) acted as a selective CB<sub>1</sub> agonist ( $K_i$  values for CB<sub>1</sub> and CB<sub>2</sub> receptors, respectively, were 250 nM and 12  $\mu$ M) and inhibited proliferation of human breast cancer cells.<sup>91</sup> Evidence of the endogenous existence of **11** was provided 2 years later by its isolation from rat brains

and bovine dorsal root ganglions.<sup>92</sup> In addition, **11** and its shorter congener *N*-oleoyldopamine were shown to potently activate TRPV<sub>1</sub> receptors.<sup>93</sup> It is possible that these compounds may act as endovanilloids,<sup>32</sup> although further data are needed in this respect.

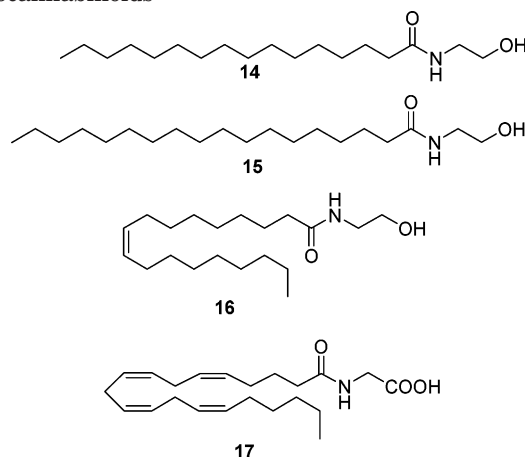
**2.2. Endocannabinoid Esters.** Esters of arachidonic acid have also been found as endocannabinoids. Nowadays, 2-arachidonoylglycerol (**7**) is considered as a major actor of the endocannabinoid system, and an increasing number of publications is devoted to this molecule. More than 20 years ago, Prescott and Majerus<sup>94</sup> reported the transient production of **7** in thrombin-stimulated human platelets, suggesting a role of this molecule as an intermediate in phospholipase C induced arachidonate release. In 1995, **7** was identified as an endocannabinoid in the canine gut<sup>95</sup> and the rat brain.<sup>96</sup> This compound is present in brain in considerably greater amounts than **6**.<sup>97</sup> It should be borne in mind, however, that the involvement of **7** in lipid metabolism means that only a fraction of the total **7** present in the brain is likely to be involved in endocannabinoid signaling. **7** interacts with both cannabinoid CB<sub>1</sub> and CB<sub>2</sub> receptors with apparent potencies lower than those for **6**. The *K<sub>i</sub>* value for **7** toward CB<sub>1</sub> receptors has been found to vary somewhat between studies, but a median value from refs 34, 95, 96, and 98 of 505 nM can be calculated. However, in biological media, **7** is not that stable,<sup>99</sup> so it is likely that this is an underestimate of the potency of this compound. Indeed, it has been argued that under conditions where 2-arachidonoylglycerol degradation is limited, **7** is more potent than **6** in mediating CB<sub>1</sub>-receptor-dependent binding of GTPγS to rat cerebellar membranes.<sup>100</sup> **7** is a full agonist at both CB<sub>1</sub> and CB<sub>2</sub> receptors.<sup>100,101</sup> Pharmacological properties of **7** include inhibition of proliferation of breast and prostate cancer cells,<sup>102</sup> induction of hypotension,<sup>103</sup> contractile action on colon smooth muscle,<sup>104</sup> neuroprotection after brain injury,<sup>105</sup> attenuation of naloxone-precipitated withdrawal signs in morphine-dependent mice,<sup>106</sup> stimulation of nitric oxide release,<sup>107</sup> enhancement of the production of chemokines in HL-60 cells,<sup>108</sup> and stimulation of feeding.<sup>109</sup>

As with other monoglycerides, **7** takes part in several pathways of lipid metabolism, and many biochemical routes may lead to and/or involve **7**. In its role as a signaling molecule, two biosynthetic pathways have been proposed (for a recent review, see ref 110). The first pathway consists of the cleavage of phosphatidylinositol by phospholipase C to give 1,2-diacylglycerol, which is subsequently hydrolyzed at the sn-1 position (the C1 position on the glycerol molecule) by sn-1-diacylglycerol lipases to give **7**. The second pathway also starts from phosphatidylinositol, which is selectively hydrolyzed in the sn-1 position by phospholipase A<sub>1</sub> to give 2-arachidonyllysophosphatidylinositol. This product might be subsequently hydrolyzed by lysophospholipase C to give **7**. Two sn-1-diacylglycerol lipases that can act as the final step of the first pathway have recently been cloned. The two enzymes, 1042 and 672 amino acids in length, show good sequence homology between man and mouse, a discrete expression across the brain, and are potently (IC<sub>50</sub> ≤ 100 nM) inhibited by the statin compound tetrahydrolipstatin.<sup>111</sup>

Compounds affecting endocannabinoid synthesis, such as tetrahydrolipstatin, may be useful in addressing a currently "hot" research area, namely, the ability of endocannabinoids to act as retrograde signaling molecules. In this role, endocannabinoids are released postsynaptically and act presynaptically to reduce transmitter release. Initial studies (all published in March 2001) demonstrated this by following two related electrophysiological phenomena, depolarization-induced suppression of inhibition and depolarization-induced suppression of excitation, and were able by judicious use of CB<sub>1</sub> receptor agonists and antagonists/inverse agonists to demonstrate a key role of endocannabinoids in modulating these processes.<sup>112–114</sup> This has subsequently been extensively characterized in a variety of brain regions (for a recent review, see ref 115), and there is evidence in hippocampal neurons that phospholipase Cβ1 acts as a "coincidence detector" to coordinate release in response to both depolarization and activation of G<sub>q/11</sub>-coupled receptors.<sup>116</sup> While antagonist studies of these processes have conclusively shown a role for endocannabinoids, it is not fully clear as to which endocannabinoid is involved, although a reasonable guess would be that the endocannabinoid involved is likely to vary across the brain. Indeed, studies using tetrahydrolipstatin and conditions where metabolism of endocannabinoids is compromised have indicated the involvement of both **6** and **7** in such processes.<sup>117,118</sup> Hopefully, tetrahydrolipstatin and future compounds targeting either **6** or **7** synthesis will shed further light on this issue.

As its chemical name, i.e., *O*-arachidonylethanolamine, indicates, virodhamine (**12**) (christened from the Sanskrit "virodha" meaning opposed) consists of the ester of arachidonic acid and ethanolamine. It has been recently isolated from the rat brain,<sup>119</sup> but it was also found in several rat peripheral tissues including skin, spleen, kidney, and heart. It can also be produced nonenzymatically from **6** (and vice versa), as a "cautionary note for lipid analyses" has reported.<sup>120</sup> The potency of **12** was similar at both CB receptors (EC<sub>50</sub> values of 1.9 and 1.4 μM, respectively, being found in assays measuring receptor-dependent binding of GTPγS for CB<sub>1</sub> and CB<sub>2</sub> receptors, respectively<sup>119</sup>). It exerts, however, distinct effects: full agonist at the CB<sub>2</sub> cannabinoid receptor and partial agonist at the CB<sub>1</sub> cannabinoid receptor. Its pharmacology is poorly understood. After icv administration, **12** decreased the body temperature in the rat.<sup>119</sup> A recent report mentioned that **12** induced endothelium-dependent relaxation in the rat isolated small mesenteric artery mounted in a myograph and precontracted with methoxamine.<sup>121</sup> Information about its biosynthesis, storage, and release is still lacking.

**2.3. Endocannabinoid Ether.** In 2001, 2-arachidonoylglycerol ether (**13**, termed noladin ether) was identified in porcine brain using a GC–MS with electron ionization detector procedure.<sup>122</sup> Interestingly, it only binds to the cannabinoid CB<sub>1</sub> receptor (*K<sub>i</sub>* = 21 nM<sup>122</sup>), and it exhibits the four typical effects in the mouse cannabinoid tetrad after ip injection. A CB<sub>1</sub>-mediated neuroprotective action in vitro of **13** against neurotoxicity produced by human amyloid-β peptide has also been reported.<sup>123</sup> The regional distribution of **13** in the

**Chart 3.** Endogenous Molecules Related to Endocannabinoids<sup>a</sup>

<sup>a</sup> Structures of palmitoylethanolamide (**14**), stearoylethanolamide (**15**), oleoylethanolamide (**16**), and arachidonoylglycine (**17**).

rat brain was studied using isotope dilution LC-MS. High concentrations were found into the thalamus, the hippocampus, and the striatum.<sup>124</sup> However, another group<sup>125</sup> was not able to find **13** in several mammalian brains (rat, mouse, hamster, guinea pig, and pig), so it is unclear as to whether **13** is present in sufficient amounts in the brain to be considered as an endocannabinoid. In addition, no biosynthetic pathway has so far been proposed. Nonetheless, its metabolic stability<sup>126</sup> and its selectivity for the cannabinoid CB<sub>1</sub> receptor constitute attractive properties for a medicinal chemistry template.<sup>127</sup> Additional molecular targets have been recently proposed for **13** in the rat isolated mesenteric arterial bed.<sup>128</sup>

**2.4. Endogenous Molecules Related to Endocannabinoids.** In addition to the endocannabinoids, related endogenous fatty acid derivatives have been called “endocannabinoid-like” because they exhibited cannabimimetic properties but are devoid of direct affinity for the CB receptors *in vitro*.

Different molecules belong to this category: amides (including palmitoylethanolamide **14**, stearoylethanolamide **15**, oleoylethanolamide **16**, and *N*-arachidonoylglycine **17**) and fatty glycerol esters (the two major ones are 2-linoleoylglycerol and 2-palmitoylglycerol). In addition to producing biological effects on other systems (see below), these compounds can affect cannabinoid function by acting as “entourage compounds”. This term was first coined to explain the ability of 2-linoleoylglycerol and 2-palmitoylglycerol to potentiate the actions of **7** at cannabinoid receptors,<sup>129</sup> but it has also been seen *in vitro* for the potentiation of **6** effects at TRPV<sub>1</sub> receptors by palmitoylethanolamide and oleoylethanolamide.<sup>130,131</sup> Such entourage effects can be brought about by a number of mechanisms, including the modulation of the target receptor,<sup>130</sup> although perhaps the most likely effect is to interfere with the degradation of endocannabinoids and thereby, by enhancing their levels, to strengthen their biological actions mediated by CB<sub>1</sub> and CB<sub>2</sub> receptors.

Four of these compounds have been studied in some detail. Palmitoylethanolamide (**14**, Chart 3), found at much higher concentrations in the brain than **6**,<sup>132</sup> was first thought to be an endogenous CB<sub>2</sub> ligand in light of the report<sup>133</sup> that **14** was able to inhibit binding of [<sup>3</sup>H]-

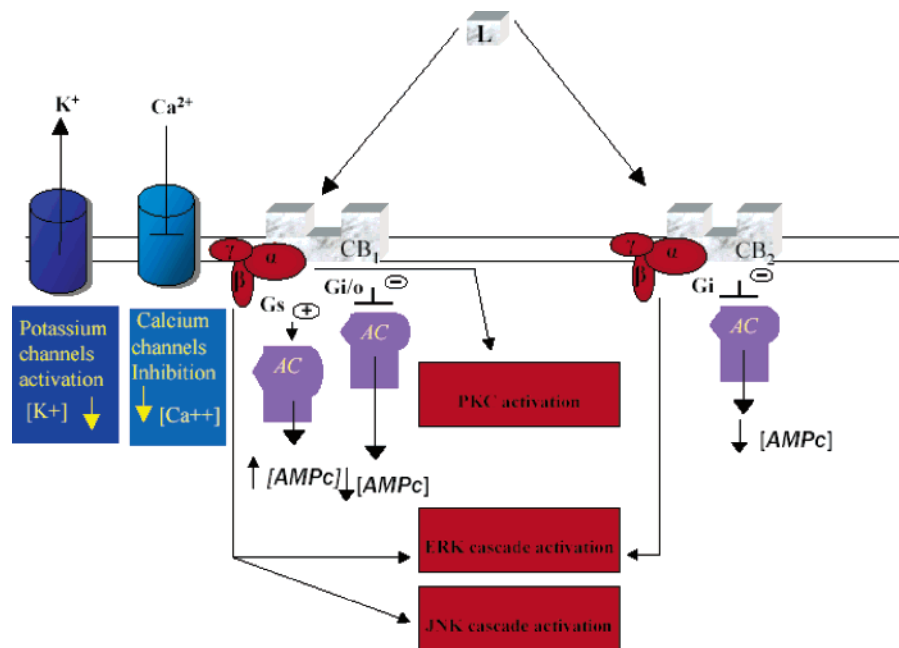
WIN 55,212-2 to RBL-2H3 basophilic leukaemia cell membranes. However, other groups, including ours, were unable to detect an interaction between **14** and CB<sub>2</sub> receptor in other tissues.<sup>134,135</sup> Despite the lack of cannabinoid receptor affinity, **14** exhibits a great number of pharmacological properties<sup>85,136–137</sup> including analgesic,<sup>45,46,138,139</sup> anti-inflammatory<sup>140,141</sup> (including neuroinflammation<sup>84</sup>), anticonvulsant,<sup>142</sup> and antiproliferative<sup>143,144</sup> properties. The analgesic properties of **14** can be blocked by the CB<sub>2</sub> receptor antagonist SR144528.<sup>46,138,139</sup> Whether this reflects an entourage effect to potentiate endocannabinoid actions at this receptor, an ability of the compound to release an endogenous CB<sub>2</sub> receptor agonist, or a non-CB<sub>2</sub>-receptor action of this compound awaits elucidation. Very recently, the nuclear receptor PPAR- $\alpha$  (peroxisome proliferator-activated receptor- $\alpha$ ) was identified as the molecular target responsible for the anti-inflammatory properties of **14**.<sup>145</sup> Indeed, the anti-inflammatory effects of **14**, given *ip* at 10 mg/kg, evaluated in two animal models (i.e., carrageenan-induced paw and phorbol ester induced ear oedemas) were confirmed in wild-type mice but were not seen in mice deficient in PPAR- $\alpha$ .<sup>145</sup>

Stearoylethanolamide (**15**, Chart 3) is found in the brain of different mammals, again at much higher levels than **6**.<sup>132</sup> It shows proapoptotic activity in rat C6 glioma cells,<sup>146</sup> as well as inhibition of lipid peroxidation *in vitro*.<sup>147</sup> *In vivo*, it exerts cannabimimetic activity.<sup>148</sup> Recently, it was reported that the intraperitoneal administration of **15** induced a marked dose-dependent anorexic effect.<sup>149</sup> This effect was accompanied by a reduction in liver stearoyl-CoA desaturase-1 (SCD-1) mRNA expression, without implication of the PPAR- $\alpha$  receptor. Oleoylethanolamide (**16**, Chart 3), in contrast to anandamide which produces overeating, decreases food intake and body weight through a mechanism independent of cannabinoid receptor, both after intraperitoneal<sup>150</sup> and oral administrations.<sup>151,152</sup> This effect is mediated through activation of PPAR- $\alpha$ ,<sup>153</sup> which leads to a stimulation of lipolysis.<sup>154</sup> Arachidonoylglycine (**17**, Chart 3) is a lipoamino acid present in bovine and rat brain as well as in other tissues. It suppresses tonic inflammatory pain.<sup>92</sup> It is metabolized by fatty acid amide hydrolase, the enzyme responsible for the hydrolysis of **6** (see section 4.2 below) and, as such, may serve as an endogenous regulator of tissue anandamide concentrations.<sup>155</sup>

### 3. Cannabinoid Receptors as Molecular Targets of Endocannabinoid Action

**3.1. Cannabinoid Receptor Structure and Function.** The CB<sub>1</sub> cannabinoid receptor,<sup>23,156</sup> which was first visualized by autoradiography and radioligand binding studies using [<sup>3</sup>H]-CP-55,940,<sup>22,157</sup> has been cloned from several species: human,<sup>156</sup> cat,<sup>158</sup> rat,<sup>23</sup> mouse,<sup>159</sup> the bird Zebra finch,<sup>160,161</sup> the amphibian newt,<sup>162</sup> and the puffer fish.<sup>163</sup> The CB<sub>1</sub> cannabinoid receptor is very conserved (for a review, see ref 164). It is expressed in the brain and some peripheral tissues including testis, ileum, urinary bladder, and vas deferens. Alternatively spliced forms of the CB<sub>1</sub> cannabinoid receptor have also been described<sup>33,34</sup> and shown to be expressed at low levels, but so far, no peculiar property in terms of ligand recognition and receptor activation has been shown for these variants other than a lower affinity for **6**.<sup>34</sup> In





**Figure 3.** Transduction pathways of the CB cannabinoid receptors. The cannabinoid CB<sub>1</sub> receptor is presented on the left, and the cannabinoid CB<sub>2</sub> receptor is presented on the right.

man, the CB<sub>1</sub> receptor is responsible for the “high” produced by smoked marijuana.<sup>165</sup>

The CB<sub>2</sub> cannabinoid receptor was discovered by sequence homology.<sup>24</sup> It is predominantly detected in the immune system (spleen, tonsils, immune cells,<sup>24</sup> and brain microglia under inflammatory conditions<sup>84,166</sup>) and was cloned from the human,<sup>24</sup> mouse,<sup>167</sup> and rat.<sup>168,169</sup> The analysis of the amino acid sequences of both cannabinoid receptors reveals the common characteristics of G-protein-coupled receptors including a seven lipophilic transmembrane  $\alpha$  helical structure. Signal transduction associated with cannabinoid receptor stimulation (Figure 3) involves inhibition of cAMP production through inhibition of adenylyl cyclase,<sup>170,171</sup> inhibition of calcium influx (and hence neurotransmitter release),<sup>158,172</sup> activation of potassium channels,<sup>173</sup> and activation of the MAP kinase pathway.<sup>174–177</sup> The agonists of CB<sub>1</sub>–CB<sub>2</sub> cannabinoid receptor responses are sensitive to pertussis toxin, suggesting that both subtypes of cannabinoid receptors are predominantly coupled to G<sub>i/o</sub>-type proteins. However, recent studies suggested that the cannabinoid receptors are also able to activate G<sub>s</sub>-type proteins.<sup>178–180</sup>

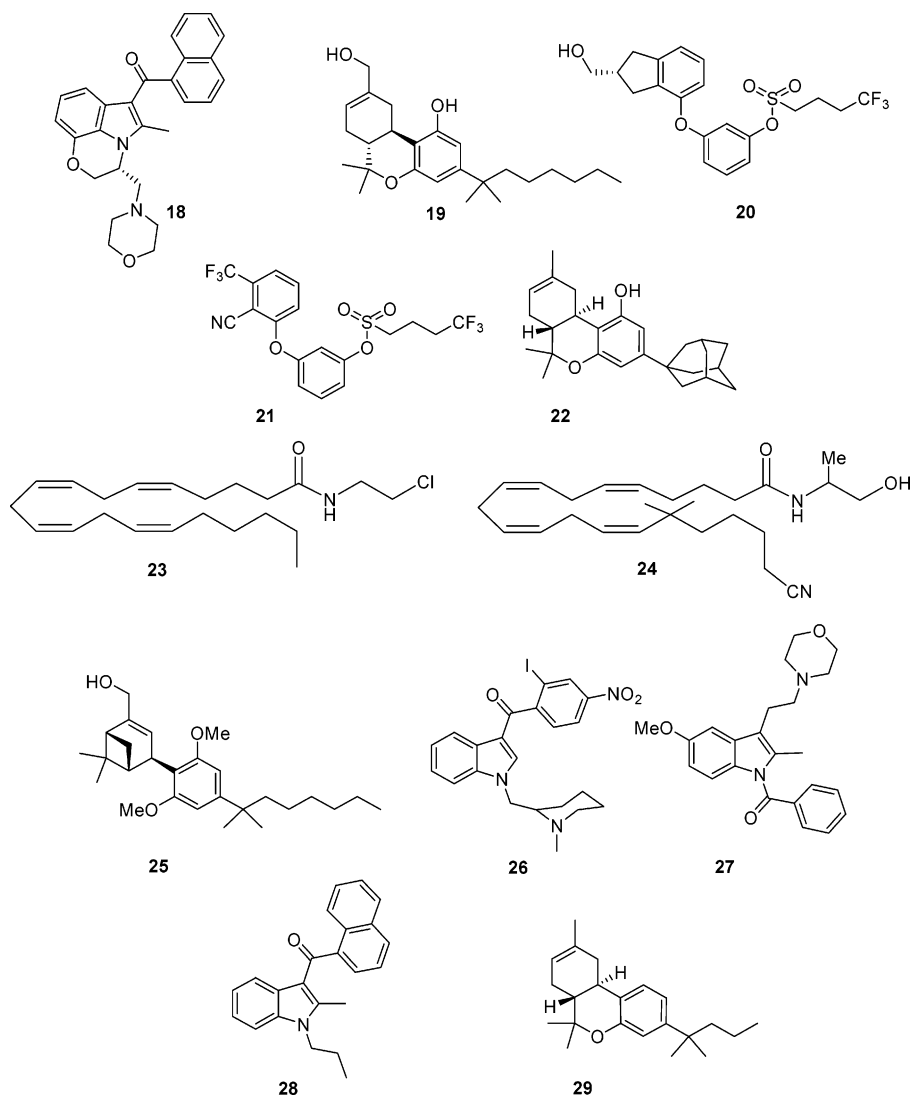
Knockout animals have been successfully obtained for the CB<sub>1</sub> cannabinoid receptor by several groups<sup>181–183</sup> since 1999. Some months later, the development of animals where the CB<sub>2</sub> gene had been deleted was reported. These CB<sub>2</sub><sup>-/-</sup> animals were then successfully crossed with CB<sub>1</sub><sup>-/-</sup> mice to produce double knockout animals.<sup>28</sup> More recently, mice with deletions of CB<sub>1</sub> receptors in the forebrain but not in either cortical GABAergic or cerebellar neurons have been described.<sup>184</sup> Mice lacking CB<sub>1</sub> and/or CB<sub>2</sub> receptors are healthy and fertile, albeit with some disruptions in embryonic development.<sup>185,186</sup> However, CB<sub>1</sub><sup>-/-</sup> mice show a number of behavioral abnormalities that have been ascribed to disrupted endocannabinoid signaling. These include alterations in activity and exploratory behavior,<sup>181,182</sup> memory processing,<sup>183,187,188</sup> food and ethanol intake,<sup>189–191</sup> behavioral and biochemical responses to

addictive drugs,<sup>182,192,193</sup> anxiety-like behavior,<sup>194</sup> and response to anxiolytic drugs.<sup>195</sup> The animals are also more sensitive to the deleterious effects of focal ischemia<sup>196</sup> and excitotoxic compounds such as *N*-methyl-D-aspartate and kainate.<sup>184,196</sup> In vitro, electrically evoked release of tritiated compound has been found to be increased in hippocampal but not cortical or striatal slices preincubated with [<sup>3</sup>H]-choline (i.e. a measure of acetylcholine release) and in vas deferens but not atrial or striatal preparations preincubated with [<sup>3</sup>H]-noradrenaline.<sup>197,198</sup> Depolarization-induced suppression of inhibition in cerebellar Purkinje cells is also deficient in CB<sub>1</sub><sup>-/-</sup> mice.<sup>199</sup> While it should be remembered that compensatory mechanisms in utero can complicate interpretation of data with knockout mice, these animals have provided a valuable tool for the delineation of responses that are mediated by the CB<sub>1</sub> (or CB<sub>2</sub>, as appropriate) receptors, as well as for identification of responses that are not. Thus, for example, the effects of **6** and the CB<sub>1</sub> receptor agonist HU-210 (**19**) upon mean arterial pressure are lost in CB<sub>1</sub><sup>-/-</sup> mice,<sup>28</sup> whereas the ability of WIN 55,212–2 (**18**) to inhibit the release of [<sup>3</sup>H]-glutamate from hippocampal synaptosomes is retained in the knockout mice.<sup>200</sup> Such mice have also been used to investigate antagonist/inverse agonist effects of compounds such as AM251. Thus, the antidepressant-like effects of this compound in the Porsolt forced-swim test are not seen in CB<sub>1</sub><sup>-/-</sup> mice, whereas the effects of the antidepressant agent desipramine are retained in these animals.<sup>201</sup>

**3.2. Synthetic Cannabinoid Receptor Ligands. Examples, Binding Pockets, and Specificity.** The pharmacology of cannabinoid receptors has been reviewed extensively (see refs 17, 202, 203). Rather than repeat these excellent reviews, the present article will restrict itself to providing a general overview and to focus on an important issue, namely, the question of the specificity of the compounds.

**3.2.1. Overview of Available Compounds.** Cannabinoid ligands are characterized by a wide chemical



Chart 4. Cannabinoid Receptors Ligands<sup>a</sup>

<sup>a</sup> Structures of WIN 55,212-2 (**18**), HU210 (**19**), BAY 38-7271 (**20**) (high-efficacy cannabinoid ligands), BAY 59-3074 (**21**), AM411 (**22**) (low-efficacy cannabinoid ligands), ACEA (**23**), O-1812 (**24**) (CB<sub>1</sub> selective agonists), HU308 (**25**), AM1241 (**26**), GW405833 (**27**), JWH-015 (**28**), JWH-133 (**29**) (CB<sub>2</sub> selective agonists).

diversity. This diversity has different consequences. First, it has been rather complicated to provide a common pharmacophore for all cannabinoid agonists. Second, the structure–activity relationships were not the same in the entire family. To rationalize these discrepancies, most authors have classified the cannabinoid receptors agonists in different families: the classical cannabinoids structurally related to **1**, the non-classical cannabinoids related to **5**, the aminoalkylindoles related to the lead compound **18**, and the eicosanoids related to the endocannabinoids.<sup>17,202,203</sup> Additional compounds, such as JTE907 and BAY 38-7271, that do not fall into these standard classes have also been described.

The result of a massive and growing medicinal chemistry effort has been the identification of a spectrum of compounds with different efficacies and affinities for the CB<sub>1</sub> and CB<sub>2</sub> receptors, compounds that have been essential in characterizing the role of cannabinoid receptors in the body. In the 2002 IUPHAR review dealing with the classification of CB receptors,<sup>17</sup> the affinities of a large number of compounds for CB<sub>1</sub> and CB<sub>2</sub> receptors were given. Rather than merely repeat

the data presented there, we have elected to show the values in Figure 2 together with selected data published since this time, to give an easily assimilable overview of a huge research effort. Examples of individual compounds are given below (for original citations, we refer to refs 17, 202, and 203) together with comments highlighting their usefulness and in some cases potential therapeutic utility.

(a) High efficacy compounds with no pronounced selectivity between CB receptors (Chart 4) are CP-55-940 (**5**), WIN 55,212-2 (**18**), HU-210 (**19**), BAY 38-7271 (**20**), and **7**. **18** is a chiral molecule, and the other enantiomer, WIN 55,212-3, is inactive at CB receptors at least at concentrations less than to 1 μM,<sup>204</sup> a useful property when characterizing CB- and non-CB mediated responses (see below). **20** is structurally unrelated to the main classes of agonists.<sup>205</sup>

(b) Partial agonists with no pronounced selectivity between CB receptors (Chart 4) are **1**, **6** and its stable analogue methanandamide, and BAY 59-3074 (**21**). A recent study has found that for the CB<sub>1</sub> receptor at least, there is no absolute correlation between the efficacy of the agonist and its ability to desensitize the receptor.<sup>206</sup>

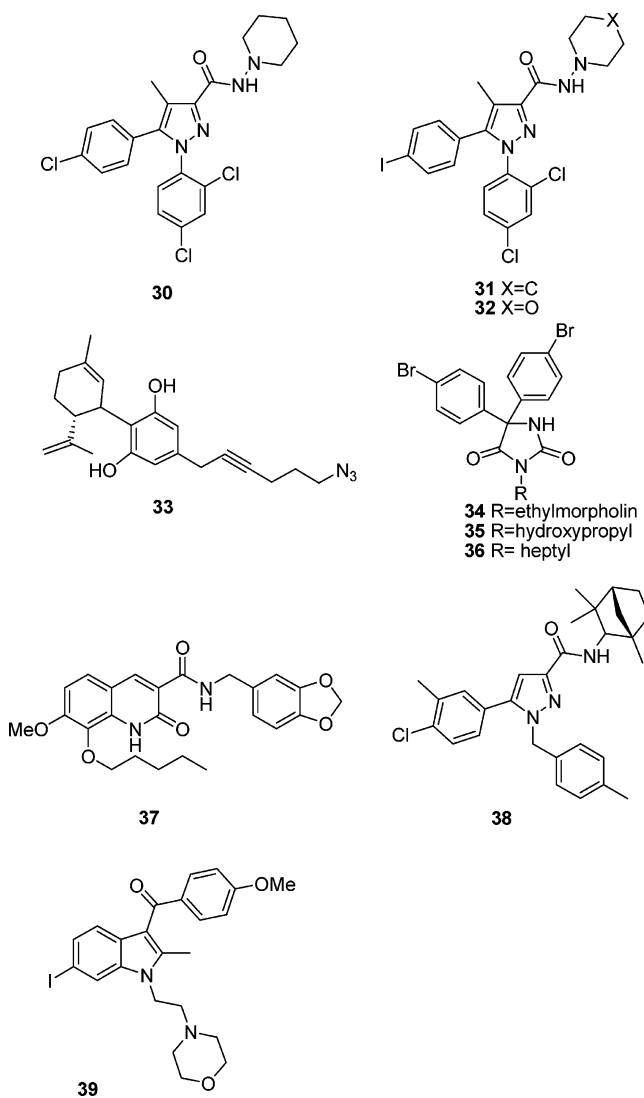
In this respect, the study identified a compound, AM411 (22), that showed “significant selectivity for CB<sub>1</sub> receptors compared to CB<sub>2</sub> receptors” (but unfortunately did not present quantitative data), had a similar efficacy to 18, but desensitized the receptor more slowly than this compound.<sup>206</sup>

(c) CB<sub>1</sub> receptor-selective agonists (Chart 4) are ACEA (23) and O-1812 (24). Both these compounds can be described as anandamide analogues but with greatly improved CB<sub>1</sub>-receptor selectivity over the original compound.<sup>207,208</sup> Plant-derived cannabinoids have already limited clinical use for the treatment of emesis and for weight gain in AIDS patients, and serious attempts are being made to evaluate clinically the usefulness of extracts containing defined amounts of 1 and/or 3 in patients suffering from multiple sclerosis (see refs 209 and 210). At first sight and in contrast to the situation for CB<sub>1</sub> receptor antagonists, the therapeutic usefulness of synthetic CB<sub>1</sub>-selective and nonselective agonists might seem unclear, since a general activation of CB<sub>1</sub> receptors would be expected to produce the same sort of side effect profile that is associated with cannabis ingestion. Certainly, drug discrimination data suggest that the CB<sub>1</sub>-selective agonist 24 can substitute for 1 in a drug discrimination test, although subtle differences between the two drugs can be seen.<sup>211</sup> On the other hand, it has been argued that low-efficacy agonists might be better in this respect, particularly for treatment of conditions associated with locally increased CB<sub>1</sub> receptor sensitivity (see ref 212 and references therein). Other situations can also be envisioned whereby synthetic CB<sub>1</sub>-selective and nonselective agonists could be very useful. Three such examples are acute intervention after neurotrauma<sup>205</sup> (where transient drug effects upon the central nervous system (CNS) function are not likely to place a major obstacle to drug development given the condition of the patient at this time), local treatment of pain,<sup>213,214</sup> and glaucoma<sup>215</sup> (where CNS concentrations will not be sufficient to produce unwanted effects). An alternative strategy in this respect is the development of CB<sub>1</sub> receptor agonists that do not cross the blood–brain barrier.<sup>216</sup>

(d) CB<sub>2</sub>-receptor-selective agonists (Chart 4) are, for example, HU308 (25), AM1241 (26), and GW405833 (27) also called L768242, JWH015 (28), and JWH133 (29), respectively. The predominantly peripheral localization of CB<sub>2</sub> receptors has made them an attractive target for drug development, since psychotropic events following their stimulation would not be expected. Recent data have demonstrated that CB<sub>2</sub> receptor agonists have potentially useful effects in a number of models of inflammatory and neuropathic pain<sup>217–222</sup> possibly involving the release of endogenous opioids (this has been demonstrated for the effects of 26 in thermal nociception; whether it also plays a role in more clinically relevant pain conditions awaits elucidation)<sup>223</sup> and can inhibit growth of CB<sub>2</sub>-receptor-expressing glioma *in vivo*.<sup>224,225</sup>

(e) The main representatives of CB<sub>1</sub> receptor-selective antagonists/inverse agonists (Chart 5) are SR141716A (30, rimonabant), AM251 (31), AM281 (32), O-2654 (33) (6'-azidohept-2'-yne-cannabidiol). Other compounds have been reported in recent comprehensive review articles.<sup>226,227</sup> It is now well-established in animal models

**Chart 5.** Cannabinoid Receptors Ligands<sup>a</sup>



<sup>a</sup> Structures of SR141716A (30), AM251 (31), AM281 (32), O-2654 (33), DML20 (34), DML21 (35), DML23 (36) (CB<sub>1</sub> receptor antagonists), JTE-907 (37), SR144528 (38), AM630 (39) (CB<sub>2</sub> receptor antagonists).

that blockade of CB<sub>1</sub> receptors results in weight loss and a blockade of the motivational effects of nicotine (e.g., see refs 228 and 229). As of the time of writing, rimonabant is at present undergoing phase III clinical trials for the treatment of obesity<sup>230,231</sup> and smoking cessation.<sup>231</sup> Other pharmaceutical companies have also reported novel CB<sub>1</sub> receptor antagonists,<sup>232,233</sup> and it is likely that several novel structures will be published in the coming years (see refs 226 and 227 for an overview of the current patent situation). 31 and 32 were devised initially as close analogues of rimonabant but with an iodine group to allow radioiodination and hence use in binding and single photon emission computerized tomography (SPECT) studies.<sup>234–236</sup> However, these compounds have also been used widely in pharmacological studies characterizing endocannabinoid systems.

In recombinant systems, rimonabant behaves as an inverse agonist rather than as an antagonist,<sup>237</sup> a property shared by other compounds (e.g., see ref 238). In contrast, 33 may act as a neutral antagonist<sup>239</sup> in the mouse *vas deferens*. Further experiments will be necessary to assess definitively the neutral antagonism

behavior of **33**. For instance, 3-alkyl-5-arylimidazolidinediones (**34–36**), weak but selective CB<sub>1</sub> ligands, were first reported as neutral antagonists<sup>240,241</sup> in rat tissue preparations. However, the same derivatives displayed different behavior when they were compared in [<sup>35</sup>S]-GTPγS assays testing the coupling of CB<sub>1</sub> receptors to G proteins and using two sources of cannabinoid receptors: rat cerebella (where they still behaved as neutral antagonists) and human recombinant receptor preparations (where they behaved as inverse agonists).<sup>242</sup> Recently, the biosisosteric replacement of the 2-carbonyl of 3-alkyl-5-arylimidazolidine-2,4-diones by a thiocarbonyl leads to an improvement of affinity.<sup>243</sup>

(f) CB<sub>2</sub> receptor-selective antagonists/inverse agonists (Chart 5) are JTE907 (**37**), SR144528 (**38**), and AM630 (**39**). These compounds have been extremely useful in the characterization of the roles played by CB<sub>2</sub> receptors, but as yet to our knowledge a therapeutic application of CB<sub>2</sub> antagonists has not been followed up in clinical trials.

The above is a short summary of a considerable research effort from many laboratories. Important SAR and QSAR data have been obtained for both CB<sub>1</sub> and CB<sub>2</sub> receptors, which have aided development of pharmacophores (e.g., see refs 203, 244, 245 and references therein). With the emergence of mutagenesis studies in both CB<sub>1</sub> and CB<sub>2</sub> sequences, it appears clearly now that cannabinoid ligands, whatever their function on the receptor, may only partially share common binding sites. Thus, for example, dipeptide insertion into the first extracellular loop of the CB<sub>1</sub> receptor resulted in a loss of affinity for **5**, whereas the affinity for **30** was retained.<sup>246</sup> Conversely, mutations in the structure of the CB<sub>1</sub> receptor have been reported where the affinity for **5** was retained but the affinity for **18** and **30** was reduced.<sup>247</sup> The affinity for **6** was also differentially affected, leading the authors to conclude that “the endocannabinoid binding pocket at CB<sub>1</sub> may only partially overlap the binding pockets of **18** and **30**”. Further studies by this group have given considerable insight into the conformation of the endocannabinoids best suited to interaction with CB receptors<sup>248,249</sup> and the key amino acids involved in CB<sub>1</sub> receptor activation,<sup>250</sup> but these are outside the scope of the present article, as are the important data delineating the structural requirements for CB receptor signal transduction and desensitization (e.g., see refs 251–253).

One issue that is of central importance for the use of receptor-selective agonists and antagonists is their selectivity vs other targets. The old adage that a compound is specific only when no other targets have been investigated certainly rings true in the cannabinoid field. Thus, for example, effects of **30** and/or **31** upon TRPV1 receptors,<sup>254</sup> adenosine A1 receptors,<sup>255</sup> and sodium channels<sup>256</sup> have been reported in vitro albeit at concentrations that are higher than required for blockade of CB<sub>1</sub> receptors. In vivo effects of **30** have also been reported in CB<sub>1</sub><sup>-/-</sup> mice,<sup>257,258</sup> which indicates that the demonstration of a process that can be antagonized by this compound is not absolute proof that the process is CB<sub>1</sub>-receptor-mediated. Among agonists, non-CB receptor-mediated effects of **18**, **23**, methanandamide, and even **1** itself have been reported in the literature.<sup>200,259–269</sup> The picture is hardly made easier

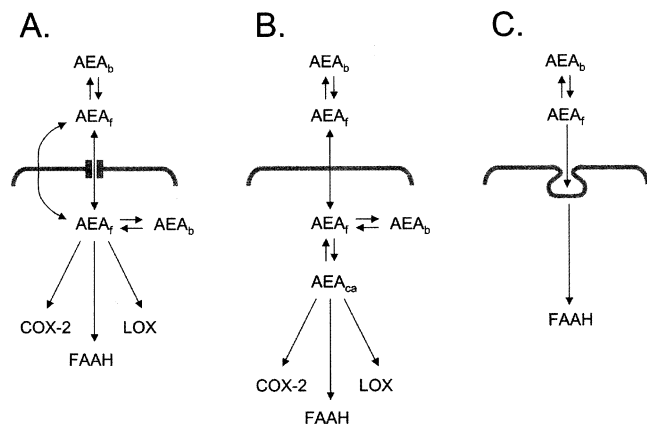
by the fact that bell-shaped dose–response curves can on occasion be found for CB receptor agonists in some assay systems (e.g., see ref 270 for an example of such a curve for the inhibition by 3-(5'-cyano-1',1'-dimethylpentyl)-1-(4-N-morpholinobutyryloxy)-Δ<sup>8</sup>-tetrahydrocannabinol hydrochloride, O-1057, of electrically evoked contractions of the mouse vas deferens). It is of course likely that successive generations of agonists and antagonists will have an increased selectivity compared with the compounds listed above. In this respect, the novel CB<sub>1</sub> receptor antagonist SR147778 was tested against a battery of > 100 other receptors, enzymes, and ion channels and found to produce < 50% inhibition at 1 μM, compared with K<sub>i</sub> values of 3.5 and 442 nM for inhibition of [<sup>3</sup>H]-CP-55,940 binding to human CB<sub>1</sub> and CB<sub>2</sub> receptors, respectively.<sup>271</sup> That of course does not rule out potential effects against as yet untested targets, but that is true for any pharmacologically active agent!

## 4. Proteins Involved in Endocannabinoid Degradation

**4.1. Overview and the Vexed Question of the Anandamide Transporter Protein.** As described in section 2 above, endocannabinoids are synthesized and released upon demand and exert their actions upon cell surface CB receptors. As with all signaling molecules, effective mechanisms for endocannabinoid removal are present. This involves their intracellular accumulation and enzymatic degradation. The enzymatic degradation processes for **6**, **7**, and entourage molecules such as palmitoylethanolamide have been well-characterized and are discussed in sections 4.2–4.5 below. In contrast, the mechanisms whereby endocannabinoids are accumulated is a matter of considerable debate. In this section, we have elected to concentrate on the cellular accumulation of **6**, simply since most is known about this process. As a consequence, the reader is referred to a recent review<sup>272</sup> for a discussion of the uptake of **7** and **14**, together with a recent study suggesting that **6** and **7** may share a common transporter mechanism.<sup>273</sup>

Anandamide uptake from the extracellular medium into cells was first demonstrated in 1993<sup>271</sup> and proposed to occur by an energy-independent process of facilitated diffusion.<sup>274,275</sup> The notion that there is a carrier present upon the plasma membrane that is capable of the transport of **6**, however, has not yet to our knowledge resulted in the cloning of any “anandamide membrane transporter” protein, and much discussion in recent years has related to the extent to which the uptake of **6** is driven by the metabolic enzyme fatty acid amide hydrolase (FAAH, discussed in section 4.2 below).<sup>276–282</sup> In a recent overview of the field, Hillard and Jarrhian<sup>283</sup> attempted to unify the field and proposed an elegant model whereby **6** was accumulated into cells by a process of facilitated diffusion, the intracellular and extracellular concentration gradient being maintained both by anandamide metabolism and by a process of intracellular sequestration. This model is essentially that shown in Figure 4A, the difference being that in the figure (a) the possibility of diffusion of the lipophilic **6** across the membrane is allowed and more importantly (b) extracellular **6** exists as an equilibrium between bound and free forms. **6** binds very readily to albumin,<sup>284</sup>





**Figure 4.** Three possible models for the cellular accumulation of anandamide (AEA). For an explanation of the models, see section 4.1. Abbreviations are as follows: AEA<sub>b</sub> and AEA<sub>f</sub>, bound and free AEA, respectively; AEA<sub>ca</sub>, AEA bound to carrier protein(s); COX-2, cyclooxygenase-2; FAAH, fatty acid amide hydrolase; LOX, lipoxygenases. Models A and C have also been discussed in detail in recent reviews.<sup>283,428</sup>

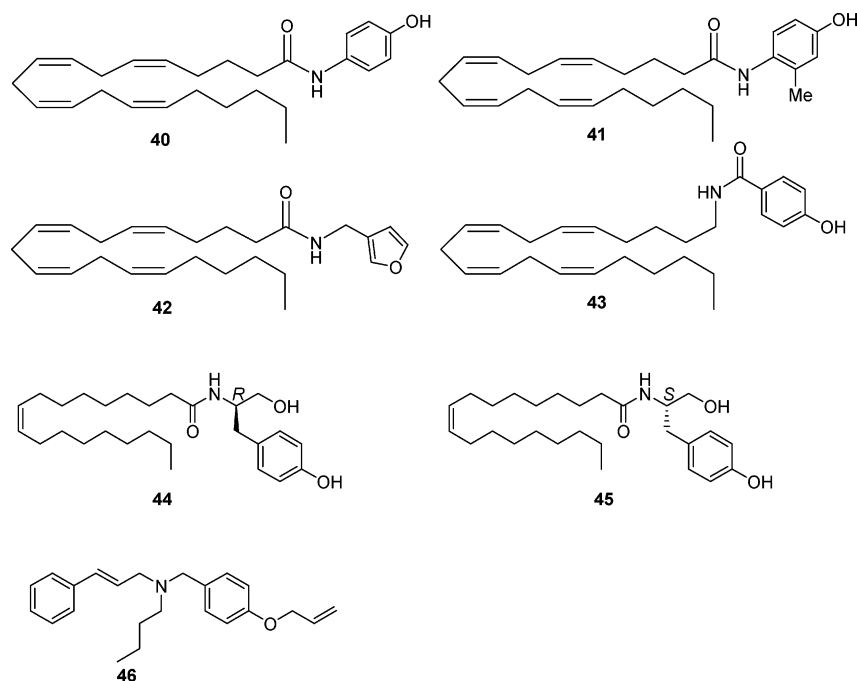
and in our view extracellular **6** is most likely to be attached to extracellular carrier proteins that may, or may not, be specifically designated for this task. Once within the cell, the fate of **6** (attachment to as yet unidentified binding proteins, intracellular sequestration (in the figure, both these possibilities are grouped as “AEA<sub>b</sub>”) and/or metabolism) will be highly dependent upon the relative expression of these molecules and therefore likely to vary from cell to cell.

An alternative scheme is shown in Figure 4B. In this scheme<sup>281,285</sup> the carrier molecule or molecules are not on the plasma membrane but are located intracellularly, but by removal of the free intracellular molecules, **6** can promote the uptake of extracellular **6**, in this case by diffusion across the membrane. A third possibility has been raised by the recent work<sup>282</sup> showing that in RBL-2H3 cells **6** is rapidly delivered to its intracellular target,

FAAH, via a caveolae/lipid raft mediated endocytotic process (Figure 4C). This is certainly consistent with the known ability of *N*-acylethanolamines to bind to cholesterol and the ability of cholesterol depletion to prevent **6**-induced apoptosis.<sup>286–288</sup> Of course, the three schemes illustrated in Figure 4 are not mutually exclusive, and different mechanisms are likely to operate in different cell types. In addition, some of these processes may not even require tailor-made carrier proteins; recent findings that **6** bind to plastic in a manner that can be inhibited by standard anandamide uptake inhibitors<sup>281,285,289</sup> would suggest that the structural requirements for anandamide carriers may not be that extreme.

While the mechanisms responsible for the accumulation of **6** are unresolved, a number of compounds that effectively prevent this process has been described in the literature (Chart 6). The first of these compounds, AM404<sup>290</sup> (**40**), inhibits anandamide uptake with an IC<sub>50</sub> of ~10 μM regardless of the assay or cell line used. Further studies have demonstrated that in addition it is a substrate for FAAH (thereby inhibiting anandamide breakdown by substrate competition)<sup>275</sup> and an agonist at TRPV1 receptors.<sup>291</sup> Additional nonspecific effects of this compound and the structurally related uptake inhibitor VDM11 (**41**)<sup>292</sup> have been reported,<sup>293–295</sup> which somewhat restricts their usefulness. A large number of acyl-based compounds have been investigated with respect to their abilities to inhibit anandamide uptake (e.g., see refs 280 and 296–300) and have led to the identification of the arachidonoyl compounds UCM707 (**42**)<sup>299</sup> and AM1172 (**43**)<sup>280</sup> and the oleoyl-based enantiomers OMDM-1 (**44**) and OMDM-2 (**45**).<sup>300</sup> Of these compounds, the most potent is **42**, with a reported IC<sub>50</sub> value for the inhibition of anandamide uptake into U937 cells of 0.8 μM.<sup>299</sup> However, the potencies of **42**, **44**, and **45** are highly dependent upon the cell line and even the assay used.<sup>285</sup>

#### Chart 6. Inhibitors of the Putative Anandamide Transporter Protein<sup>a</sup>



<sup>a</sup> Chemical structures of AM404 (**40**), VDM11 (**41**), UCM707 (**42**), AM1172 (**43**), OMDM-1 (**44**), OMDM-2 (**45**), and SEP-0200228 (**46**).

The lack of a precisely defined target is of course a difficulty that has to be addressed when considering the actions of uptake inhibitors. Nevertheless, regardless of their precise mechanisms of action, it has been well-established in a large number of *in vivo* studies that the compounds have biological activity. A comprehensive list of *in vivo* actions of anandamide uptake inhibitors has been published elsewhere, and as a consequence, the reader is directed to that review article.<sup>301</sup> However, recent examples include **42** effects upon seizure scores in mice treated with kainic acid<sup>184</sup> and include **44** and **45** effects upon spasticity in mice with chronic relapsing experimental allergic encephalomyelitis.<sup>302</sup>

In terms of a medicinal chemistry perspective, it would be useful to identify potent inhibitors of anandamide uptake that do not have the acyl side chain, since this might allow a more precise target identification. In this respect, Hopkins and Wang<sup>303</sup> recently reported in abstract form a nonarachidonoyl compound, SEP-0200228 (**46**) that inhibited anandamide uptake into human monocytes with an IC<sub>50</sub> of 90 nM and a potency 20-fold greater than **40** with respect to inhibition of anandamide uptake into synaptosomes. A full publication of this study is eagerly awaited!

**4.2. Fatty Acid Amide Hydrolase. 4.2.1. Structure, Distribution, and Substrates.** Although an *N*-acylethanolamine-degrading activity was first reported in 1966,<sup>304</sup> it was not until the mid-1980s before the enzyme was properly characterized.<sup>73,74</sup> The discovery of **6** and oleamide renewed the interest of the study of degradation mechanisms, and in 1996, a rat oleamide hydrolase was successfully purified and its cDNA cloned.<sup>305</sup> This enzyme, recombinantly expressed, has been shown to hydrolyze numerous fatty acid amides including oleamide and **6**. Consequently, it was renamed fatty acid amide hydrolase (FAAH). Recent reviews have detailed the discovery and the recent progress achieved in the characterization of FAAH.<sup>307–312</sup>

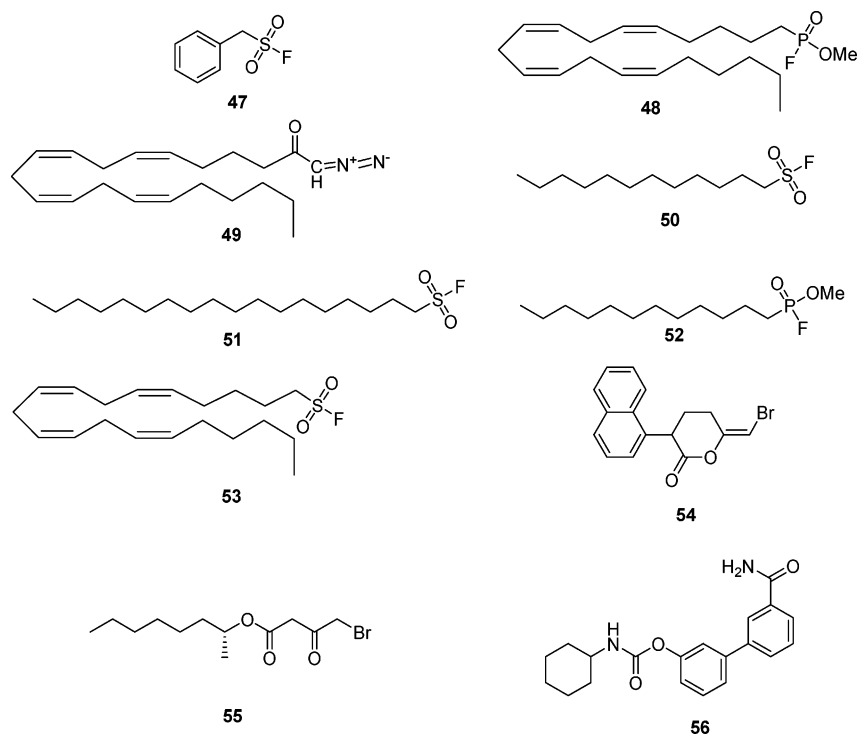
FAAH is a membrane-bound enzyme that belongs to the family of amidase enzymes characterized by a highly conserved region rich in serine, glycine, and alanine residues. This “amidase signature” region is shared by more than 80 amidases, and it corresponds to amino acids 215–257 in mammalian FAAH enzymes. Human,<sup>313</sup> rat,<sup>305</sup> mouse,<sup>313</sup> and porcine<sup>314</sup> FAAH have been cloned and have 73% identity at the overall amino acid sequences and 90% at the amidase signature. In these different species, the FAAH are 579 amino acids in length, but their mRNAs differ in size (for more details on molecular structure of FAAH, see refs 311, 315, and 316). The 2.8 Å resolution X-ray crystal structure of FAAH has been investigated,<sup>317</sup> with results consistent with the hypotheses that (i) FAAH is a dimeric enzyme with a core composed of a serine–serine–lysine triad and (ii) it possesses channels to establish direct access between the bilayer and its active site, to facilitate substrate binding and product release. The distribution of the FAAH varies between species. In rats, FAAH is mainly distributed in the liver, small intestine, brain, testis, uterus, kidney, ocular tissues, and spleen but not in skeletal muscle or heart.<sup>71,318,319</sup> In the rat hippocampus and striatum, the enzyme activity is significantly lower in the dark phase than the light phase of

the day, whereas the reverse was true for anandamide levels in these brain regions.<sup>320</sup> FAAH activity is also found in mouse uterus,<sup>321</sup> and its expression is regulated during pregnancy.<sup>322</sup> In humans, FAAH was mainly detected in the pancreas, brain, kidney, skeletal muscle, placenta, and less abundantly in liver.<sup>313</sup> A single nucleotide polymorphism (resulting in a P129T mutant of the enzyme) has been associated with problem drug use.<sup>323</sup> This mutation results in a low expression level of the protein.<sup>323,324</sup> Human FAAH is also regulated by progesterone, leptin, and palmitoylethanolamide.<sup>143,325,326</sup> The hormonal regulation of the enzyme is mirrored by a variation of lymphocyte FAAH during the menstrual cycle,<sup>327</sup> and a low lymphocyte enzyme activity correlates with spontaneous abortion.<sup>328</sup>

FAAH may hydrolyze a wide variety of substrates (see ref 306 for a comprehensive list of substrate specificities of FAAH from different sources). FAAH cleaves very efficiently several *N*-acylethanolamines and primary fatty acid amides: anandamide, stearoylethanolamide, palmitoylethanolamide, myristoylethanolamide, lauroylethanolamide, oleoylethanolamide, and oleamide. In addition, FAAH also exhibits esterase activity against **7**,<sup>329</sup> methyl arachidonate, and oleoyl methyl ester. Indeed, a study from 1977<sup>330</sup> describing the enzyme responsible for the degradation of 2-oleoylglycerol by rat liver microsomes may in fact be the first purification of FAAH, since the observed molecular weight and pH optimum are entirely consistent with this enzyme rather than with the monoacylglyceride activity (see section 4.3 below) to which it was ascribed.

The importance of the FAAH was clearly demonstrated by the generation of FAAH<sup>-/-</sup> mice in 2001.<sup>331</sup> Those mice showed enhanced endogenous cannabinoid levels and activity together with a very slow hydrolysis of **6** and oleamide in the brain and in the liver. In FAAH<sup>-/-</sup> mice, **6** produces classical behavioral responses in the tetrad (hypomotility, analgesia, catalepsy, and hypothermia) at doses (6.25–50 mg/kg) that are totally inactive in FAAH<sup>+/+</sup> mice. Regarding the phenotype, FAAH<sup>-/-</sup> mice also exhibit reduced pain sensitivity, even in the absence of exogenous administration of a cannabinoid agent. Proconvulsant activity has been recently reported in FAAH<sup>-/-</sup> mice.<sup>332</sup> **6** injected into those mice significantly enhanced the severity of bicuculline- or kainate-induced seizures, while it did not induce the same effect in FAAH<sup>+/+</sup> mice.

A second model of FAAH knockout mice has been generated more recently,<sup>333</sup> where the expression of FAAH has been selectively restricted to the nervous system (FAAH-NS mice). Those mice were found to possess wild-type levels of fatty acid amides (**6**, **10**, and **14** were measured) in the brain and spinal cord but significantly enhanced concentrations of these three lipids in periphery (testis, kidneys, liver). This model provided evidence of a functional dissociation of the central and peripheral fatty acid amide signaling systems: in these animals analgesia seems to be primarily mediated through the interaction of **6** with CB receptors in the nervous system, whereas the anti-inflammatory activity was mediated by peripherally elevated levels of fatty acid amides.<sup>333</sup> Central and peripheral endocannabinoid signaling systems might regulate discrete

**Chart 7.** Chemical Structures of Some Irreversible FAAH Inhibitors Described So Far<sup>a</sup>

<sup>a</sup> Phenylmethylsulfonyl fluoride (**47**), methylarachidonoyl fluorophosphonate (**48**), diazomethylarachidonoyl ketone (**49**), laurylsulfonyl fluoride (**50**), stearylsulfonyl fluoride (**51**), methyl dodecyl fluorophosphonate (**52**), arachidonylsulfonyl fluoride (**53**), (*E*)-6-(bromomethylene)tetrahydro-3-(1-naphthalenyl)-2*H*-pyrano-2-one (**54**), 4-bromo-3-oxobutyric acid 1-methylheptyl ester (**55**), and carbamic acid, cyclohexyl-3'-(aminocarbonyl)[1,1'-biphenyl]-3-yl (**56**, URB-597).

behavioral processes and be targeted for distinct therapeutic purposes.

**4.2.2. FAAH Inhibitors.** Designing FAAH inhibitors constitutes an elegant alternative to CB receptor agonists. Inactivation of FAAH has been shown to have potentially beneficial effects upon pain and anxiety without the side effects (hypomotility, hypothermia, and catalepsy) accompanying activation of CB<sub>1</sub> receptors that are elicited by exogenous cannabinoids such as Δ<sup>9</sup>-tetrahydrocannabinol (for reviews of therapeutic potential of FAAH inhibitors, see refs 334–336). To preserve this lack of “cannabinoid side effects”, the inhibitors must be devoid of affinity for the cannabinoid receptors, especially for the CB<sub>1</sub> receptor, which is involved in most of the unwanted effects of exogenous cannabinoids. Here, the main FAAH inhibitors are described depending on their mechanism of action.

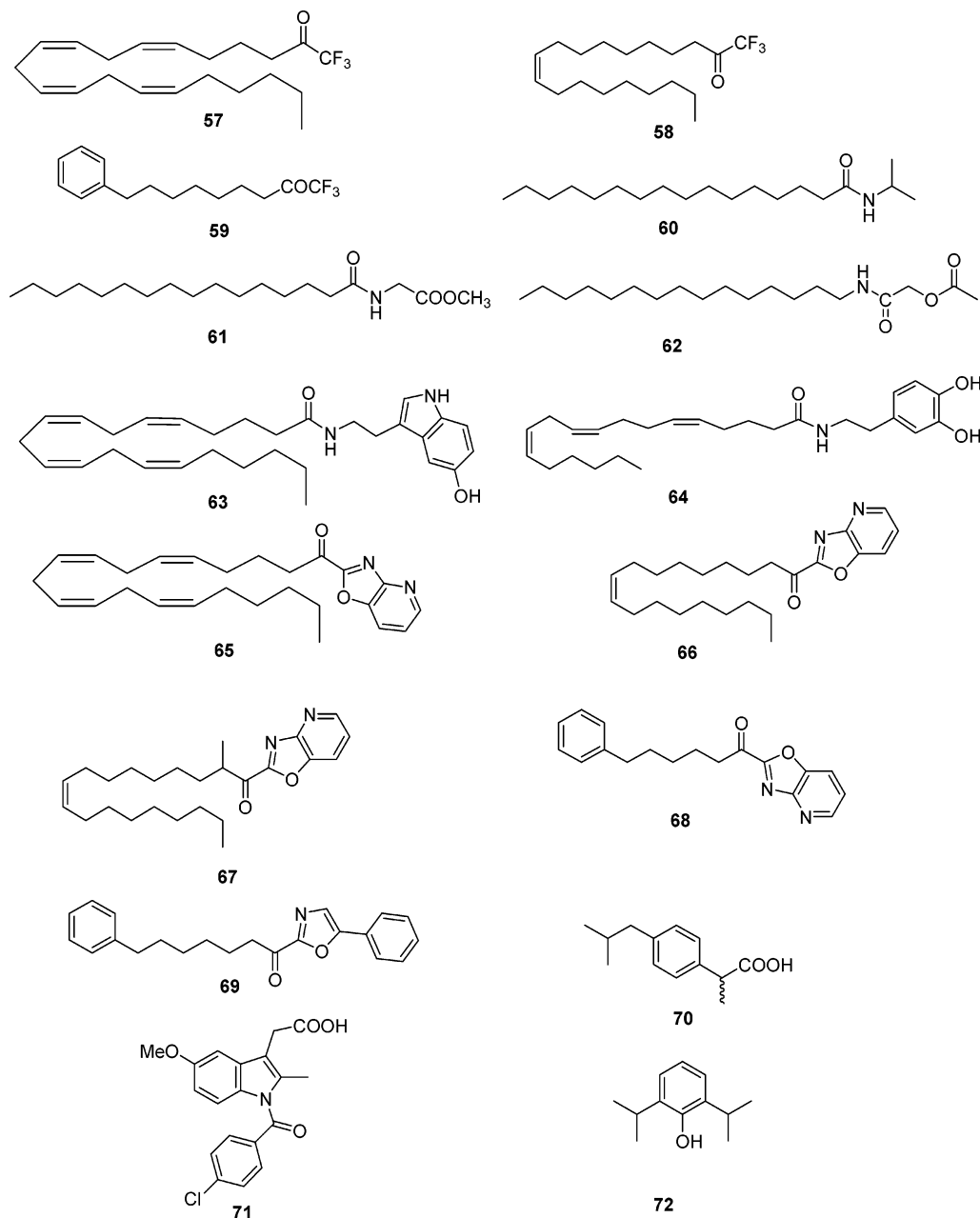
**4.2.2.1 Irreversible Inhibitors of FAAH (Chart 7).** Albeit not selective, phenylmethylsulfonyl fluoride (**47**), a well-known serine protease, was the first (IC<sub>50</sub> = 13 μM) FAAH inhibitor described.<sup>71,318,337</sup> More selective inhibitors have been obtained, based on the structure of a fatty acid: methylarachidonoyl fluorophosphonate (**48**, IC<sub>50</sub> = 1–3 nM),<sup>338,339</sup> diazomethylarachidonoyl ketone (**49**, IC<sub>50</sub> = 0.5–6 μM),<sup>339,340</sup> laurylsulfonyl fluoride (**50**, IC<sub>50</sub> = 3 nM),<sup>341</sup> stearylsulfonyl fluoride (**51**, IC<sub>50</sub> = 4 nM),<sup>341</sup> methyl dodecyl fluorophosphonate (**52**, IC<sub>50</sub> = 3 nM),<sup>342</sup> and arachidonylsulfonyl fluoride (**53**, IC<sub>50</sub> = 0.1 nM).<sup>343</sup> These compounds have significant affinity for the CB<sub>1</sub> receptor, which limits their usefulness, although there may in some cases be a “window of opportunity”. **53**, for example, inhibits [<sup>3</sup>H]-CP-55,940 binding to mouse CB<sub>1</sub> receptors with an IC<sub>50</sub> of 304 nM.<sup>343</sup> This compound also shows greater selec-

tivity than **48** toward other targets (neuropathy target esterase, acetylcholinesterase, and butyrylcholinesterase).<sup>343</sup>

(*E*)-6-(Bromomethylene)tetrahydro-3-(1-naphthalenyl)-2*H*-pyrano-2-one (**54**)<sup>344</sup> and 4-bromo-3-oxobutyric acid 1-methylheptyl ester (**55**) (quoted as 2-octyl γ-bromoacetate in the original publication<sup>345</sup>), proposed as an endogenous compound because it was originally isolated from human cerebrospinal fluid, have been reported as irreversible inhibitors of FAAH (IC<sub>50</sub> values of 0.8 and 2.6 μM, respectively). In the case of **55**, however, only 20–30% of the inhibition appeared to be irreversible following a 2 h preincubation phase between enzyme and inhibitor, the reversible component showing a competitive mode of action (*K<sub>i</sub>* = 0.8 μM).

A new class of potent carbamate inhibitors has been recently described. URB597 (carbamic acid, cyclohexyl-3'-(aminocarbonyl)[1,1'-biphenyl]-3-yl ester, **56**), the most potent in this series, does not exhibit affinity for the cannabinoid receptors.<sup>64</sup> Thus, at doses that inhibit FAAH and substantially raise brain anandamide (but not **7**) levels, this compound does not evoke catalepsy or hypothermia.<sup>64,346</sup> However, **56** exhibits anxiolytic-like actions that are prevented by the CB<sub>1</sub> receptor antagonist **30**.<sup>64</sup> Structure–activity relationships among alkylcarbamic acid aryl esters were reported in 2003.<sup>347</sup> The most potent members of this family are *N*-cyclohexylcarbamic acid biphenyl-3-yl ester and *N*-butylcarbamic acid 4-(phenylmethoxy)phenyl ester, which are devoid of affinity for cannabinoid receptors. The docking of this class of compounds inside the catalytic site of FAAH has been suggested on the basis of molecular modeling data.<sup>348</sup> Whether or not **56** inhibits FAAH irreversibly is yet to be established; a covalent interac-



**Chart 8.** Chemical Structures of Other FAAH Inhibitors Described<sup>a</sup>

<sup>a</sup> Arachidonoyl trifluoromethyl ketone (**57**), oleoyl trifluoromethyl ketone (**58**), 1,1,1-trifluoro-9-phenyl-2-nonanone (**59**), palmitoylisopropylamide (**60**), *N*-(1-oxohexadecyl)glycine methyl ester (**61**), *N*-(2-acetoxyacetyl)pentadecylamine (**62**), arachidonoylserotonin (**63**), pinolenoyldopamine (**64**), 1-oxazolo[4,5-*b*]pyridin-2-yl-eicosa-5*Z*,8*Z*,11*Z*,14*Z*-tetraen-1-one (**65**), 1-oxazolo[4,5-*b*]pyridin-2-yl-9*Z*-octadecen-1-one (**66**) and its methylated derivative in position 2 (**67**), 1-oxazolo[4,5-*b*]pyridin-2-yl-6-phenylhexan-1-one (**68**), OL-135 (**69**), ibuprofen (**70**), indomethacin (**71**), and propofol (**72**).

tion with critical serine groups can be envisaged,<sup>349</sup> but the FAAH activity in the rat brain measured *ex vivo* after a dose of 0.3 mg/kg *ip* returns to normal within 24 h.<sup>346</sup>

**4.2.2.2. Other Inhibitors of FAAH (Chart 8).** Replacing the carbonyl of a fatty acid chain or a fatty acid mimic by a trifluoromethyl group has been widely used; arachidonoyl trifluoromethyl ketone (**57**;  $IC_{50} = 0.23\text{--}3\ \mu\text{M}$ ),<sup>350</sup> oleoyl trifluoromethyl ketone (**58**,  $K_{iapp} = 1.2\ \text{nM}$ ),<sup>351</sup> 1,1,1-trifluoro-8-(4-heptylphenyl)-2-octanone ( $K_i = 96\ \text{nM}$ ), and 1,1,1-trifluoro-9-phenyl-2-nonanone (**59**,  $K_i = 25\ \text{nM}$ )<sup>352</sup> are transition-state inhibitors of FAAH.

Another approach has been the synthesis of derivatives of palmitoylethanolamide (**14**). The rationale of

this approach has been that **14**, by acting as an alternative FAAH substrate, is able to prevent the FAAH-catalyzed metabolism of **6**.<sup>353</sup> Exposure of cells to **14** can also inhibit the expression of FAAH.<sup>143,350</sup> Palmitoylisopropylamide (**60**),<sup>353</sup> *N*-(1-oxohexadecyl)glycine methyl ester (**61**), and *N*-(2-acetoxyacetyl)pentadecylamine (**62**) exhibit inhibitory potential against FAAH<sup>354</sup> ( $IC_{50}$  values of 13, 10, and 8  $\mu\text{M}$  for **60**, **61**, and **62**, respectively). These compounds are not as potent as other FAAH inhibitors. However, **60** inhibits the production of arachidonic acid from exogenous **6** in intact cells (a process requiring the combination of anandamide uptake and metabolism) with a potency at least as good as the prototypical uptake inhibitor **40**.<sup>353</sup>

Amino acid conjugates of arachidonic acid and related compounds also provide FAAH inhibitors, possibly by acting as competing substrates: arachidonoylglycine (**17**,  $IC_{50} = 4.1\text{--}7\ \mu\text{M}$ ),<sup>92</sup> arachidonoylserotonin (**63**,  $IC_{50} = 12\ \mu\text{M}$ ),<sup>355</sup> arachidonoyldopamine (**10**,  $IC_{50} = 22\text{--}68\ \mu\text{M}$ ),<sup>355,356</sup> and pinolenoyldopamine (**64**,  $IC_{50} = 19\ \mu\text{M}$ ).<sup>356</sup>

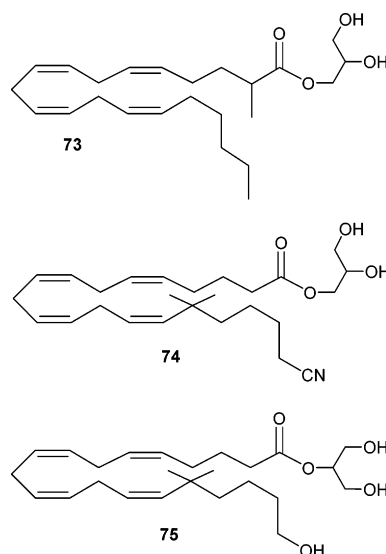
Substituting the polar head of **6** by the  $\alpha$ -keto heterocycles has been investigated in detail by Boger and colleagues. Among the very potent inhibitors are 1-oxazolo[4,5-*b*]pyridin-2-yl-eicosa-5*Z*,8*Z*,11*Z*,14*Z*-tetraen-1-one (**65**,  $K_i = 1\ \text{nM}$ ),<sup>357</sup> 1-oxazolo[4,5-*b*]pyridin-2-yl-9*Z*-octadecen-1-one (**66**,  $K_i = 2.3\ \text{nM}$ ), and its methylated derivative in position 2 (**67**,  $K_i = 9.1\ \text{nM}$ ).<sup>358</sup> The most potent inhibitor of the  $\alpha$ -keto heterocycle family is 1-oxazolo[4,5-*b*]pyridin-2-yl-6-phenylhexan-1-one (**68**), characterized by a  $K_i$  of 200 pM on rat FAAH and of 94 pM on human FAAH.<sup>357</sup> The question of the selectivity of FAAH inhibitors, in particular  $\alpha$ -keto heterocycle inhibitors, relative to other mammalian hydrolases has been addressed recently using a proteomic approach.<sup>359–361</sup> These authors reported, among other findings, that OL-135 (**69**) was a very potent and selective FAAH inhibitor ( $IC_{50} = 2.1\ \text{nM}$ ) that enhanced endocannabinoid signaling and produced analgesia in vivo.<sup>359</sup> Compounds **56** and **58**, on the other hand, were equally potent as inhibitors of FAAH as of triacylglycerol hydrolase, one of the other serine hydrolases tested by these authors.<sup>359,361</sup> However, it is unclear as to the importance of this effect in vivo.

**4.2.2.3 Compounds with Inhibitory Potential but Not Designed as Inhibitors of FAAH (Chart 8).** Phytocannabinoids such as cannabidiol (**3**), cannabinol (**4**), and **1** inhibit FAAH activity in mouse brain microsomes. In the presence of 58  $\mu\text{M}$  anandamide, the inhibitory potency decreased from cannabidiol (160  $\mu\text{M}$ , 66% inhibition) to cannabinol (160  $\mu\text{M}$ , 46% inhibition) to **1** (160  $\mu\text{M}$ , 31% inhibition)<sup>362–364</sup> via a competitive inhibition.<sup>365</sup> Nonsteroidal anti-inflammatory drugs ibuprofen (**70**),<sup>366</sup> suprofen,<sup>366</sup> ketorolac, flurbiprofen,<sup>367</sup> and indomethacin (**71**)<sup>368</sup> also produce inhibition of FAAH with potencies in the low- to high-micromolar range, depending upon the assay pH used (see section 4.4 below).

Propofol (**72**), a general anesthetic, is a competitive inhibitor of FAAH ( $IC_{50} = 52\ \mu\text{M}$ ).<sup>369</sup>

**4.3. Monoacylglycerol Lipase.** Although **7** can act as a substrate for FAAH and although in some cells inhibition of FAAH leads to an increased level of this endocannabinoid,<sup>370</sup> there is good data to demonstrate that in the CNS, this enzyme does not constitute a major metabolic pathway for **7**. Thus, treatment of rats with 0.3 mg/kg ip of **56**, a dose causing an almost complete inhibition of FAAH, increased by ~3- to 6-fold the levels of **6**, **14**, and **16** in the brain measured 2 h after drug administration but did not change the levels of **7**.<sup>64</sup> Consistent with this, the rate of hydrolysis of the related compound 2-oleoylglycerol was not affected in the brain and liver of FAAH<sup>-/-</sup> mice.<sup>371</sup> The enzyme responsible for brain metabolism of **7**, monoacylglycerol lipase (MAGL), was originally investigated with respect to its role in triglyceride metabolism (e.g., see refs 372 and 373) and has been identified as a serine hydrolase enzyme with a close homology between rat, mouse, and

**Chart 9.** MAG Lipase Inhibitors<sup>a</sup>



<sup>a</sup> Three MAG lipase inhibitors are represented:  $\alpha$ -methyl-1-AG (**73**), O-2203 (**74**), and O-2204 (**75**).

man.<sup>374,375</sup> In the brain, MAGL has a more restricted distribution than FAAH,<sup>376</sup> and a recent detailed study has suggested that MAGL is primarily located in axons whereas FAAH is localized to the somatodendritic compartment.<sup>377</sup> These authors speculated that the two enzymes have different functional roles whereby FAAH may be involved in the regulation of resting levels of **6**, whereas MAGL may be involved in the inactivation of **7** in the vicinity of its locus of action.<sup>377</sup> Further studies by this group have shown that the postnatal development of hippocampal FAAH and CB<sub>1</sub> receptors is different.<sup>378</sup>

Dissecting out the physiological roles of **6** and **7** would be greatly aided by the identification of selective MAGL inhibitors (and the further study of possible MAGL heterogeneity).<sup>379</sup> The pharmacology of MAGL is less well-studied than that of FAAH, although it is known that MAGL can be inhibited by nonspecific compounds such as *N*-ethylmaleimide, diisopropyl fluorophosphate, and *p*-chloromercuribenzoate.<sup>372,373</sup> Compounds such as arachidonoyl-, oleoyl-, and palmitoyltrifluoromethyl ketone can inhibit MAGL<sup>98,377,380</sup> (and have been used to demonstrate that ATP-induced production of **7** by astrocytes is potentiated by MAGL inhibition<sup>381</sup>), albeit with a lower potency than toward FAAH, whereas the FAAH inhibitors **56**, **63**, and **68** do not produce significant inhibition of MAGL.<sup>64,380,381</sup> However,  $\alpha$ -methyl-1-arachidonoylglycerol (**73**, Chart 9) and two arachidonoylglycerol analogues, O-2203 (**74**, Chart 9) and O-2204 (**75**, Chart 9), inhibit MAGL and FAAH with roughly equal potencies (~11  $\mu\text{M}$  in the case of  $\alpha$ -methyl-1-arachidonoylglycerol) and do not activate central CB<sub>1</sub> receptors.<sup>98</sup> It might be possible to compare the effects of such compounds with those of FAAH-selective compounds as an initial approach to dissecting out the separate roles of the two enzymes. However, identification of MAGL-selective compounds may only be a question of time; a recent abstract has identified arachidonoyl ethylene glycol as a compound able to inhibit MAGL with an  $IC_{50}$  of 25  $\mu\text{M}$ , whereas the  $IC_{50}$  value toward FAAH is >25  $\mu\text{M}$ .<sup>382</sup>

**4.4. Cyclooxygenase and Lipoxygenase-Catalyzed Metabolism of Anandamide and 2-Arachidonoylglycerol.** There is increasing evidence that additional metabolic pathways may be physiologically important both in terminating the effects of endocannabinoids and in the production of biologically active endocannabinoid metabolites. It is now well established that both cyclooxygenase-2 (COX-2) and lipoxygenases can utilize anandamide and 2-arachidonoylglycerol as substrates *in vitro*<sup>383–389</sup> and that the oxygenated products produced by the lipoxygenase pathway can produce biological effects *in vitro* on cannabinoid receptors and other systems (TRPV1 receptors and peroxisome-activated proliferator protein- $\alpha$ ).<sup>383,387,390–392</sup> In peripheral tissues of the mouse, levels of the COX-2 derived products of anandamide metabolism, prostaglandin ethanolamides (prostamides), were reported to be below detectable limits unless the animals were treated with **6**, particularly so in FAAH<sup>-/-</sup> mice.<sup>393</sup> However, another study reported, in an abstract, the detection of prostamide F<sub>2</sub> $\alpha$  in both brain and peripheral tissues from FAAH<sup>+/+</sup> and FAAH<sup>-/-</sup> mice at levels ranging from 8 to 100 pmol/g wet weight of tissue.<sup>394</sup> The finding that the COX-2 selective inhibitors nimesulide, but not the FAAH inhibitor **56**, potentiates endocannabinoid-mediated depolarization-induced suppression of inhibition in hippocampal slices<sup>395</sup> would point to a role of COX-2 in hippocampal endocannabinoid metabolism.

The prostamides have weak effects at CB receptors, TRPV1 receptors, FAAH, and prostanoid receptors<sup>396–398</sup> but do produce a contraction of the cat iris.<sup>396</sup> COX-derived products of endocannabinoids have also been suggested to be involved in the contraction of isolated rat aorta by **7**<sup>399</sup> and in the peroxisome-activated proliferator protein- $\gamma$ -mediated inhibition of interleukin-2 secretion from mouse splenocytes treated with **6**.<sup>400</sup> There is thus evidence to suggest that the endocannabinoids not only are active biological molecules but can act as precursors to other active agents in the body. This may also be true for cytochrome P450 derived metabolites of **6**,<sup>401</sup> but little has been done in this area.

Given the interaction of endocannabinoids and the COX-2 pathway, and indeed the fact that arachidonic acid bound to COX-2 was used as a basis for the design of non-arachidonoyl inhibitors of anandamide uptake,<sup>303</sup> it should perhaps not be that surprising to note that the reverse situation applies, namely, that the COX inhibitors indomethacin and flurbiprofen exert effects on the endocannabinoid system. Thus, spinal administration of these compounds were found to produce beneficial effects in the formalin model of inflammatory pain that were blocked by CB<sub>1</sub> receptor antagonists.<sup>402,403</sup> The effects of indomethacin were, in addition, not seen in CB<sub>1</sub> receptor knockout mice. The authors suggested that the non-steroidal anti-inflammatory drugs (NSAIDs), by preventing the removal of arachidonic acid, allowed for *de novo* anandamide synthesis.<sup>402,403</sup> The ability of these agents to inhibit FAAH, particularly under acidic conditions,<sup>404–406</sup> would be expected to contribute to this effect by preventing the breakdown of the newly synthesized anandamide. An alternative suggestion has been made,<sup>407</sup> namely, that the ability of the NSAIDs to inhibit both COX-2 (which is constitutively expressed

in the spinal cord) and FAAH prevents two routes of anandamide metabolism, thereby potentiating its effect. Systemically administered indomethacin is also highly effective in the carrageenan model of inflammation in the mouse, and this effect can be prevented by the CB<sub>2</sub> inverse agonist **38** pretreatment,<sup>408</sup> which may also implicate the endocannabinoid system in the mode of action of this drug.

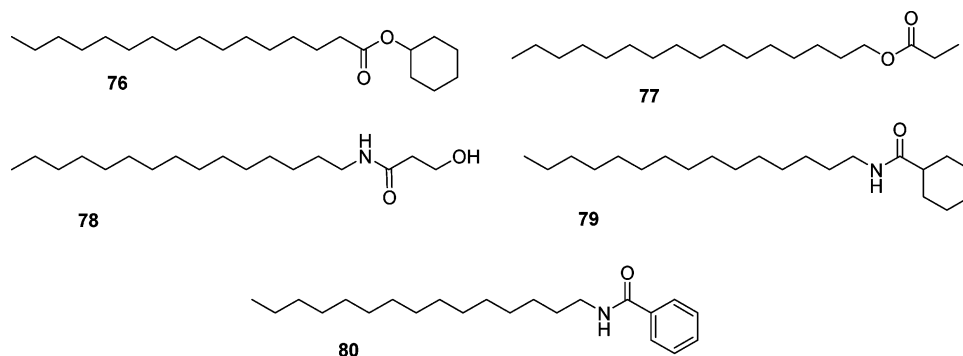
**4.5. N-Palmitoylethanolamine Selective Acid Amidase.** Although not strictly speaking an endocannabinoid metabolizing enzyme, the identification of an additional enzyme able to hydrolyze palmitoylethanolamide is mentioned here because, by affecting the metabolism of an “entourage” compound, it has potential effects upon endocannabinoid actions. The enzyme differs from FAAH in terms of pH optimum (~5 compared with ~9 for FAAH) activity, tissue distribution, substrate specificity, and inhibitor sensitivity.<sup>409–411</sup> Thus, the enzyme, which is widely distributed throughout the body, with the highest activity detected in lung, followed by spleen, small intestine, thymus, and caecum, has a substrate specificity with relative rates of hydrolysis as follows: palmitoylethanolamide, 100%; myristoylethanolamide, 48%; stearoylethanolamide, 21%; oleoylethanolamide, 20%; linoleoylethanolamide, 13%; anandamide, 8%. The enzyme has been termed either *N*-palmitoylethanolamine acid amidase (NPAA) or *N*-acyloylethanolamine hydrolyzing acid amidase (NAAA). Its molecular characterization has very recently been reported.<sup>412</sup> It was cloned from human, rat, and mouse. The deduced primary structure does not present any homology with fatty acid amide hydrolase. However, it has structural and functional similarity to acid ceramidase.

Elucidation of the physiological roles of NPAA would be aided by the identification of NPAA-selective inhibitors. In this respect, the properties of a number of palmitoylethanolamide analogues have been investigated<sup>413</sup> (Chart 10). Among them, cyclohexyl hexadecanoate (**76**), hexadecyl propionate (**77**), and *N*-(3-hydroxypropionyl)pentadecanamide (**78**) were the most potent inhibitors with IC<sub>50</sub> values of 19, 32, and 54  $\mu$ M, respectively. In addition, these compounds showed no or lower inhibitory activity toward FAAH. **75** was then considered as a template for further pharmacomodulations, and various compounds with cyclohexyl or phenyl groups have been synthesized.<sup>413</sup> Among them, two “retroamide” compounds *N*-cyclohexanecarbonylpentadecylamine (**79**)<sup>414</sup> and *N*-benzoylpentadecylamine (**80**, Chart 10)<sup>414</sup> were found to be more potent inhibitors than **76**, resulting in 66% and 61% inhibition at 10  $\mu$ M, respectively. Compound **79**, a noncompetitive inhibitor, exhibited an IC<sub>50</sub> of 4.5  $\mu$ M without any inhibitory effects on FAAH up to concentrations of 100  $\mu$ M. This compound has been used as a pharmacological tool to distinguish the acid amidase from FAAH in cells containing both of the enzymes.<sup>414</sup> Hopefully, these compounds will be useful in the elucidation of the physiological and pathophysiological roles of NPAA and in the investigation of this enzyme as a potential drug target.

## 5. Conclusions

During the relatively short period since the discovery of the endocannabinoids, our knowledge of the endocan-



**Chart 10.** Inhibitors of the *N*-palmitoylethanolamine selective acid amidase<sup>a</sup>

<sup>a</sup> Cyclohexyl hexadecanoate (**76**), hexadecyl propionate (**77**), *N*-(3-hydroxypropionyl)pentadecanamide (**78**), *N*-cyclohexanecarbonylpentadecylamine (**79**), and *N*-benzoylpentadecylamine (**80**).

nabinoid system has increased massively, and there are now compounds available for most of the individual components of the endocannabinoid pathways. On a basic science level, these will be useful in delineating the roles played by the individual endocannabinoids, but of course, it is to be hoped that they will pave the way to a number of novel treatment strategies. Given the variety of physiological processes mediated by CB receptors, many therapeutic areas are possible. In addition to compounds already undergoing phase III trials (in the case of **1** for the treatment of pain<sup>415,416</sup> and multiple sclerosis<sup>417</sup> and in the case of rimonabant for the treatment of obesity and as an aid to smoking cessation<sup>230,231</sup>), potential therapies include the treatment of cancer,<sup>418–419</sup> anxiety,<sup>420</sup> trauma, stroke, motor, and other neurodegenerative disorders<sup>335,421</sup> (the cited references refer to recent reviews on these subjects, with apologies to authors of other excellent reviews for our not citing their work). Our view is that under pathological conditions where there is an increased local endocannabinoid synthesis and where that synthesis is beneficial rather than damaging, compounds that interfere with endocannabinoid metabolism are potentially more useful than compounds acting directly upon CB<sub>1</sub> receptors, since they will potentiate an existing signal in the damaged area rather than overlay a new signal everywhere. Thus, the psychotropic effects seen with CB<sub>1</sub> receptor agonists are not seen with, for example, FAAH inhibitors (e.g., see ref 64). An example of this approach is seen in an experimental model of multiple sclerosis, where there is an increased synthesis of endocannabinoids in affected animals and where FAAH inhibition and anandamide transport inhibition result in an alleviation of the spasticity seen in these animals.<sup>422</sup> The recent finding that these types of compounds blocked growth in vivo on tumor xenografts induced in athymic mice<sup>423</sup> further exemplifies the potential value of this approach. Of course, optimism is a prerequisite for preclinical research, and many are the conceptually interesting ideas that have not resulted in clinically viable treatments. A number of potential problems can be envisaged. For example, if an increased endocannabinoid activity is a beneficial event in a disease state, would not the use of an antagonist negate the benefit or even increase the risk of an adverse event? In this respect, there is a single case report of an individual who developed multiple sclerosis following CB<sub>1</sub> receptor antagonist treatment for obesity,<sup>424</sup> but this of course could very well be a unconnected event.

Similarly, it can be assumed, given the role of **6** in reproduction, that FAAH inhibitors should be avoided in pregnancy. But these are potential rather than real problems, and potential problems can be considered for any case of drug development. It is an exciting time for cannabinoid research!

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## Biographies

**Didier M. Lambert**, born in Brussels, Belgium, completed his pharmaceutical training courses in 1989 and obtained a Ph.D. in Pharmaceutical Sciences (orientation: medicinal chemistry) at Université Catholique de Louvain, Belgium. After a postdoctoral period at the University of Minneapolis, he returned to the Université Catholique de Louvain and is now Professor of Medicinal Chemistry and head of the research unit of Pharmaceutical Chemistry and Radiopharmacy. His research interests are devoted to the medicinal chemistry of the cannabinoid system. His current research team consists of five Ph.D. students having either a chemistry or a pharmacology background. He was awarded the Royal Belgian Academy of Medicine Prize in 1996 and recently the 2004 Vande Velde Prize for progress in the knowledge of new therapeutic targets.

**Christopher J. Fowler**, born in Stockport, U.K., completed his B.A. in 1975 and thereafter obtained his Ph.D. (pharmacology) in 1978 at Cambridge University, U.K. After postdoctoral posts in Sweden, Ireland, and France, he joined AstraZeneca (then Astra Läkemedel) in Södertälje, Sweden, in 1982, where he remained until 1994. Since 1995 he has been Professor of Pharmacology at Umeå University in Northern Sweden. His research is concerned with the pharmacology of endocannabinoids and their metabolism. His research team currently consists of one senior lecturer, two postdocs, two Ph.D. students, and two laboratory assistants. He is a coauthor of a paper published in 1992 that won the Kabi Pharmacia Award for the "best research paper in the field of the brain's aging and its diseases".

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