Classical and Nonclassical Cannabinoids: Mechanism of Action—Brain Binding

Allyn C. Howlett, M. Ross Johnson, and Lawrence S. Melvin

INTRODUCTION

Following characterization and publication by Gaoni and Mechoulam (1964) of the structure of delta-9-tetrahydrocannabinol (delta-9-THC), researchers enthusiastically pursued new therapeutants for 17 years, based on this active principle from marijuana. These research efforts culminated with the marketing of delta-9-THC (Marinol) by Unimed and nabilone (Cesamet) by Lilly and clinical investigation of levonantradol from Pfizer and nabitan from SISA (figure 1). Delta-9-THC and nabilone are approved as an antiemetic for cancer patients being treated with chemotherapy. Levonantradol was studied both as an antiemetic and an analgetic, and nabitan was studied as an analgetic. This chapter will briefly review the research at Pfizer, Inc. related to discovery of the synthetic cannabinoid CP-55,940 as an analgetic in rodents and continuing research at St. Louis University Medical School responsible for the discovery of a mechanism of action and brain-binding site for this compound.

DISCUSSION

At the outset of these research efforts, two key points of information in the cannabinoid literature provided a basis for the discovery of a unique class of nonclassical cannabinoids. First, 9-nor-9-beta-hydroxyhexahydrocannabinol (HHC) was prepared (Wilson and May 1975) and found to exhibit analgetic activity in rodents, with equal potency to morphine (Wilson et al. 1976; Bloom et al. 1977). These results provided a novel chemical target from which potent, nonopiate analgetics could be discovered. Second, published and presented research supported a contention that significant biological activity was present in naturally occurring and synthetic cannabinoids only if they contained the rigid benzopyran ring system present in

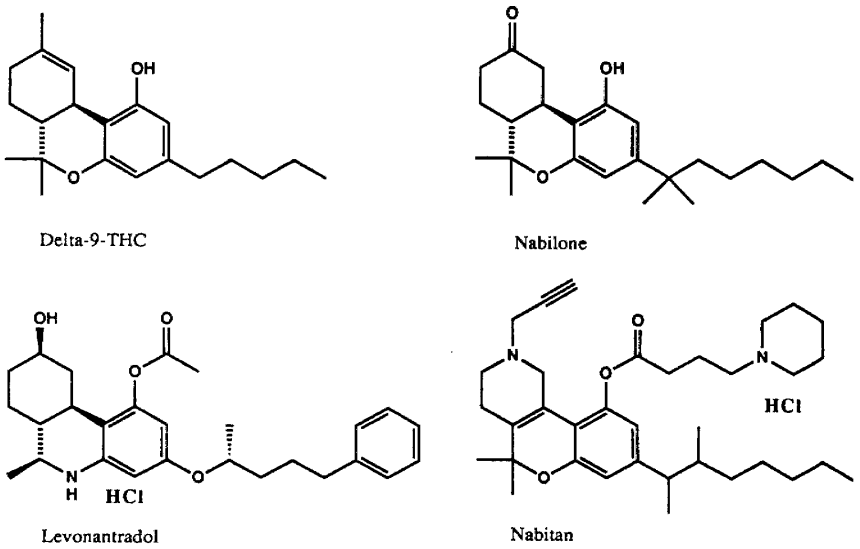


FIGURE 1. *Delta-9-tetrahydrocannabinol and derived drugs*

delta-9-THC (Mechoulam and Edery 1973; Razdan 1987; Mechoulam et al. 1987). This second point was inconsistent with several hypotheses that we had formulated to pursue a mechanism-based approach to the development of drugs based on HHC. Therefore, an early research objective was to directly challenge the need for incorporation of a benzopyran ring in cannabinoid-based drugs. An additional benefit from this structure-activity relationship (SAR) test was that it allowed synthesis of a greater diversity of structurally novel compounds than may have been anticipated from previous studies. This point was also necessarily important for the successful discovery and development of a drug based on HHC. Delta-9-THC and structurally related biologically active cannabinoids present a broad spectrum of activities in animals and, where tested, in man. Such a plethora of effects by a compound would usually contraindicate its use as a drug. The ultimate objective of this research was to structurally isolate the various pharmacophores present in delta-9-THC and HHC and then rebuild compounds with high specificity and potency of action. To achieve progress on this major objective, it was not fortuitous that we hypothesized that the cannabinoids must produce their biological effects on subtypes of an as-yet-unknown specific receptor system.

Initial research leading to the discovery of levonantradol provided substantial understanding of the SAR in the pyran ring portion of HHC (Johnson et al. 1981; Johnson and Melvin 1986). Using analgesia as an endpoint, it was shown that the pyran oxygen atom in HHC could be replaced by carbon,

favored, but varied substitution was also found to be allowable and included just hydrogen. These results indicated the gem-dimethyl pyran ring of HHC was not, in itself, a pharmacophore for analgetic activity in HHC. Nonetheless, it was still possible that the rigidity of the benzopyran ring was responsible for a favorable three-dimensional interaction of more distal functionality (alcohol, phenol, C-3 chain) with a receptor. This possibility was tested with the synthesis of structure *1*, lacking the pyran ring of HHC (Melvin et al. 1984a) (figure 2). Compound *1* also incorporated the 1,1-dimethylheptyl C-3 chain, previously used in nabilone (Archer et al. 1977; Archer et al. 1986) and developed in early work (Adams et al. 1949; Loev et al. 1973), which was expected to provide optimal potency. The phenylpentyloxy C-3 chain of levonantradol and similar side chains were equally suitable for use in structures such as *1*. Analgetically, *1* was more potent than HHC and equipotent with morphine. In fact, molecular modeling had predicted a low-energy conformation of *1* in which the spatial arrangement of the alcohol, phenol, and C-3 chain of HHC and *1* were superimposable. This initial result confirmed the original proposal that the pyran ring of HHC was not a necessary pharmacophore in cannabinoids and that any molecular rigidifying effects it provided were surmountable.

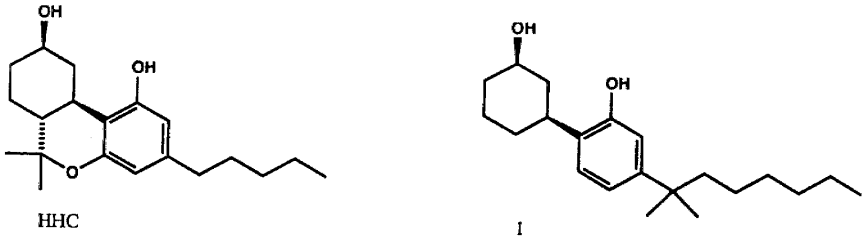


FIGURE 2. *Comparison of HHC and compound* 1

Broad SAR studies of *1* guided in part by an earlier and now less attractive proposal of a prostaglandin mechanism of action (Milne and Johnson 1981), led to the synthesis and extensive study of *2* (CP-55,940), a single enantiomer (Johnson and Melvin 1986) (figure 3). Structure *2* differs from *1* and HHC by having a hydroxypropyl residue in the space previously occupied by the pyran ring of HHC. Nuclear magnetic resonance (NMR) data confirmed that both the hydroxypropyl group and aryl-cyclohexyl bond are freely rotating. Compound *2* was shown to have significantly enhanced analgetic potency with respect to morphine (6 to 37 times as potent) and to be equipotent with levonantradol. Similar to levonantradol and desacetyllevonantradol, the analgetic activity of *2* was not blocked by naloxone

(McIlhenny et al. 1981; Yaksh 1981). The nonclassical cannabinoids, including *2*, exhibit varying degrees of cannabimimetic activity, in addition to analgetic and antiemetic effects (Weissman et al. 1982; Koe et al. 1985; Little et al. 1988). The enhanced analgetic effect of *2* over *1* was attributed to the introduction of a potential new receptor binding element (hydroxypropyl), which interacted with a previously unutilized site at the proposed cannabinoid analgetic receptor. To gain support for the identification of a new binding element, compound *2* was modified in various ways to induce stable conformations of the hydroxypropyl unit. It was argued that such a conformationally restricted binding element would provide maximum biological potency when matched to its binding site at the receptor.

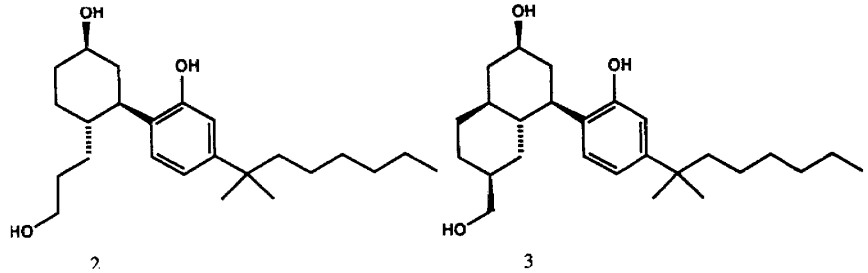
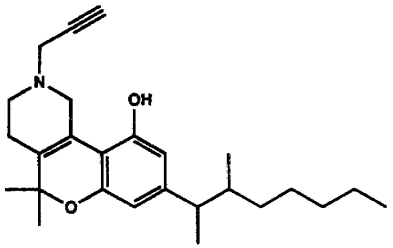


FIGURE 3. *Compound* 2 *(CP-55,940) and the rigid derivative* 3 *(CP-55,244)*

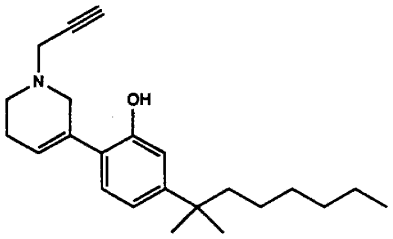
Ultimately, a conformationally restricted derivative of *2* was synthesized with a hydroxymethyl-containing decalin ring (Johnson and Melvin 1986). Compound *3* (CP-55,244), a single enantiomer, showed enhanced analgetic potency (2 to 13 times) over *2* and a strong stereochemical preference for an axial vs. equatorial hydroxymethyl group (31 to 53 times) (figure 3). The enantiospecificity of analgetic activity for *3* was dramatically increased to greater than 5000 times vs. 209 times for *2* and 3 times for *1*, implying a very specific receptor fit. These findings supported the conclusion that the hydroxypropyl functionality on *2* was a significant new contributor to receptor binding.

The less rigid bicyclic cannabinoid prototype illustrated by compound *1* was extended to two other interesting modifications. SP-1, the active form of the prodrug nabitan (Razdan et al 1976; Razdan and Howes 1983), possesses a unique N-propargyl group in place of the alcohol in 6a, 10a-dehydro HHC and utilizes a 1,2-dimethylheptyl C-3 chain. Removal of the pyran ring in SP-1 provided compound *4*, which was still equipotent with HHC as an analgetic but lost potency on some endpoints vs. SP-1 (1 to 8 times) (figure 4). Optimum timing for drug administration was not taken into

consideration. A similar relationship also held true for the fully saturated piperidine ring derivative of *4* (Melvin et al. 1984b).



SP-1

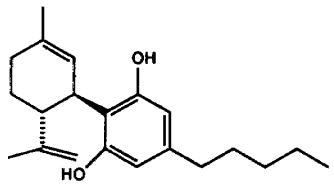


4

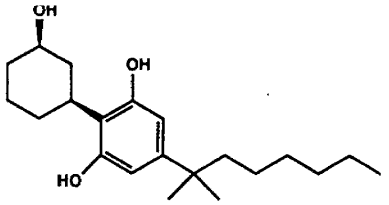
FIGURE 4. *Relationship of SP-1 and compound* 4

Cannabidiol (CBD) is the natural pyran-ring-opened derivative of delta-9-THC (Mechoulam and Shvo 1963). The pharmacology of CBD has been broadly studied, showing that it lacks analgetic activity and is a relatively weak cannabimimetic (Mechoulam et al. 1970; Dewey 1986; Razdan 1986; Consroe and Snider 1986). We speculated that the inactivity of cannabidiol was a result of an unfavorable steric interaction between the bulky isopropenyl group and a phenolic hydroxyl. Unlike *1*, *2*, or *3*, cannabidiol cannot readily adopt a conformation that would overlap its vinyl methyl (possibly oxidized to hydroxymethyl), phenol, and C-3 chain with those of delta-9-THC. Since it was already shown that the pyran ring was not necessary for biological activity, it seemed unlikely that the simple presence of a second phenol in cannabidiol would be so deleterious. Syntheses of compound *5* would test this idea, since *5* contains both phenols of cannabidiol but lacks the isopropenyl group (figure 5). As predicted, compound *5* and its axial alcohol isomer exhibited analgetic activity comparable to that of *1* and its isomer (Melvin et al. 1984a).

With support and direction from accumulating research data, biological studies were initiated to investigate the mechanism of action for the analgetic synthetic cannabinoids. Earlier studies had demonstrated that active cannabinoids, in particular delta-9-THC and desacetyllevonantradol, were reversible inhibitors of adenylate cyclase activity in membranes from neuroblastoma cells (N18TG2) (Howlett 1984; Howlett 1985; Howlett 1987).



Cannabidiol



5

FIGURE 5. *Relationship of cannanidiol and diphenol 5*

Table 1 demonstrates the potent inhibition of adenylate cyclase and the high degree of enantiospecificity of the nonclassical cannabinoids and levonantradol (Howlett et al. 1988). The inhibition of neuroblastoma adenylate cyclase is exhibited by all analgetically active cannabinoids with a significant degree of correlation for potency, enantiospecificity and stereospecificity. Further work supports a conclusion that actual enzyme inhibition by cannabinoids occurs via interaction with a Gi protein complex (Howlett et al. 1986). The receptor mechanism whereby cannabinoids inhibit adenylate cyclase is distinct from that by which the opiates act (Devane et al. 1986). It is tentatively concluded that the mechanism whereby cannabinoids exhibit *in vivo* analgetic activity is identical to their *in vitro* inhibition of adenylate cyclase (Howlett et al. 1988).

TABLE 1. *Inhibition of adenylate cyclase activity* in vitro

Compound	K_{inh} (nM)* (-) Enantiomer**	(+) Enantiomer**
1	79	135
2	25	>5,000
3	5	>10,000
Levonantradol	100	>5,000
Desacetyllevonantradol	7	—
Delta-9-THC	430	—

*Inhibition of secretin-stimulated activity. Data is from a mean of at least three determinations.
**In all cases studied to date, the (-) rotation has been exhibited by the enantiomer with absolute configuration 6aR,10aR (using HHC as an example) and this is the absolute configuration in which all structures in this manuscript are drawn.

The original premise driving research to segregate the various biological activities of cannabinoids, i.e. that the effects of cannabinoids are the result of direct interaction with a discrete receptor(s), was initially pursued in rat brain (Devane et al. 1988). A potent, stable cannabinoid radioligand was needed for this research. Compound *2* (CP-55,940) was chosen and tritium labeled in the C-3 chain by catalytic reduction of the olefinic precursor *6* (figure 6) (Devane et al. 1987).

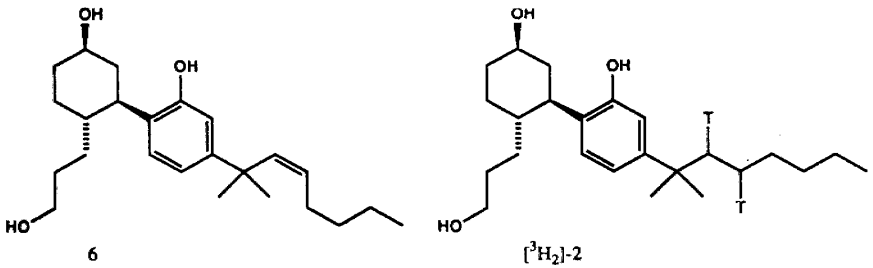


FIGURE 6. *Precursor 6 and tritium-labeled 2*

Specific binding in rat brain to cortical P2 membranes and synaptosomes from hippocampus plus prefrontal cortex was characterized with tritiated *2*. Compound *2* binds to the rate P2 membrane preparation with a Ki=133±11 pM and a Bmax of 1.85 pmol/mg of protein. Desacetyllevonantradol exhibits a binding Ki=123±34 pM, while delta-9-THC is approximately tenfold less potent as a ligand with Ki=1.6±0.56 nM. Conversely, the analgetically inactive cannabidiol gave less than 50 percent displacement of tritiated *2* at 1 μM.

The analgetically less active enantiomer of 2 showed a fiftyfold weaker affinity with Ki=3.4±1.9 nM. Together with unreported data and the continuing accumulation of new data, a significant correlation between analgetic potency *in vivo* and binding to the rat brain receptor *in vitro* defined by tritiated *2* has been shown. As was the case for inhibition of neuroblastoma adenylate cyclase, evidence has been obtained suggesting that the brain-receptor site is linked to a Gi protein complex (Devane et al. 1988). Table 2 summarizes the data that corroborate our conclusion that a specific brain receptor has been identified that is responsible for at least the analgetic activity of cannabinoids.

CANNABINOIDS AND THE IMMUNE SYSTEM

With respect to the possible effects of cannabinoids on the immune system, these lipophilic substances can now be evaluated more specifically for their cell receptor effects. This is important because the literature has not clearly

defined an effect of cannabinoids, particularly delta-9-THC, on the immu system of mammals. Several caveats should be considered when reviewi the reported immunological effects of marijuana or delta-9-THC. First, those effects ascribed to the inhalation route of delivery of delta-9-THC from marijuana are not usually controlled for effects resulting from the simple act of smoking a marijuana or generic cigarette. Second, when e uating a user population of marijuana, it is difficult to control for the use other potentially immuno-affecting substances such as tobacco, alcohol, ar other drugs of abuse. Finally *in vitro* effects on cells are difficult to ass because measurements are frequently conducted under nonphysiological c ditions of drug and protein concentrations. Several recent reviews summa ize research on the potential effects of marijuana on the immune system (Friedman et al. 1988; Yahya and Watson 1987; Kawakami et al. 1988; Murison et al. 1987; Mishkin and Cabral 1987; Pross et al. 1987).

TABLE 2. *Fulfilled requirements for a cannabinoid drug-receptor site interaction*

Receptor Binding of *2* (CP-55,940) to Rat Brain*	
High Affinity	K_d=133 pM
Rapid Equilibrium	<45 min
Reversible/Saturable	100%/100%
Enantioselective	(-)/(+)=0.02
Functional Activity and Correlation with Analgesia	
High Potency	μg/kg
Enantioselective	(-)/(+)=0.004
SAR Correlatable With Binding	R=0.9

*Assay uses [^{3}H]-2 (CP-55,940) of specific activity ≥50Ci/mmole.

CONCLUSION

Utilizing a nonclassical cannabinoid analgetic as a radioligand, a specific r ceptor was identified in rat brain. *In vitro* binding of cannabinoids to this receptor and *in vivo* rodent analgetic potency correlate significantly. Addi tionally, analgetic cannabinoids inhibit neuroblastoma adenylate cyclase activity *in vitro* with a significant correlation to *in vivo* potency. Prelimin ary data support a further conclusion that inhibition of adenylate cyclase a brain receptor binding both occur via interaction with a single Gi protein complex.

Biological and chemical probes are now available to allow an indepth exploration of the pharmacology and mechanisms of action of the cannabi-

noids. Physiologically relevant receptor subtypes can be searched for, including perhaps peripheral receptors that are associated with pain. Antagonists and mixed agonists–antagonists for these receptors can be pursued, and endogenous binding substances can now be looked for. It is anticipated that worthwhile exploitation of these results will follow in the form of action-specific drugs based on cannabinoids.

REFERENCES

Adams, R.; Harfenst, M.; and Lowe, S. New analogs of tetrahydrocannabinol. XIX. *J Am Chem Soc* 71:1624-1628, 1949.

Archer, R.A.; Blanchard, W.B.; Day, W.A.; Johnson, D.W.; Lavagnino, E.R.; Ryan, C.W.; and Baldwin, J.E. Cannabinoids. 3. Synthetic approaches to 9-ketocannabinoids. Total synthesis of nabilone. *J Org Chem* 42:2277-2284, 1977.

Archer, R.A.; Stark, P.; and Lemberger, L. Nabilone. In: Mechoulam, R., ed. *Cannabinoids as Therapeutic Agents*. Boca Raton: CRC Press, 1986. pp. 85-103.

Bloom, A.S.; Dewey, W.L.; Harris, L.S.; and Brosius, K.K. 9-Nor-9β-hydorxyhexahydrocannabinol, a cannabinoid with potent antinociceptive activity: Comparisons with morphine. *J Pharmacol Exp Ther* 200:263-270, 1977.

Consroe, P., and Snider, S.R. Therapeutic potential of cannabinoids in neurological disorders. In: Mechoulam, R. ed. *Cannabinoids as Therapeutic Agents*. Boca Raton: CRC Press, 1986. pp. 21-49.

Devane, W.A.; Dysarz, F.A. III; Johnson, M.R.; Melvin, L.S.; and Howlett, A.C. Determination and characterization of a cannabinoid receptor in rat brain. *Mol Pharmacol* 34:605-613, 1988.

Devane, W.A.; Howlett, A.C.; Johnson, M.R.; Melvin, L.S.; and Milne, G.M. Structural studies leading to the discovery of a physiologically relevant cannabinoid receptor site in rat brain. In: *Abstracts From 194th National Meeting, American Chemical Society*. Washington, DC: American Chemical Society, 1987.

Devane, W.A.; Spain, J.W.; Coscia, C.J.; and Howlett, A.C. An assessment of the role of opioid receptors in the response to cannabimimetic drugs. *J Neurochem* 46:1929-1935, 1986.

Dewey, W.L. Cannabinoid pharmacology. *Pharmacol Rev* 38:151-178, 1986.

Friedman, H.; Klein, T.; Specter, S.; Pross, S.; Newton, C.; Blanchard, D.K.; and Widen, R. Drugs of abuse and virus susceptibility. In: Bridge, T.P., ed. *Psychological, Neuropsychiatric, and Substance Abuse Aspects of AIDS*. New York: Raven Press, 1988. pp. 125-137.

Gaoni, Y., and Mechoulam, R. The isolation, structure, and partial synthesis of an active constituent of hashish. *J Am Chem Soc* 86:1646-1647, 1964.

Howlett, A.C. Inhibition of neuroblastoma adenylate cyclase by cannabinoid and nantradol compounds. *Life Sci* 35:1803-1810, 1984.

Howlett, A.C. Cannabinoid inhibition of adenylate cyclase. Biochemistry of the response in neuroblastoma cell membranes. *Mol Pharmacol* 27:429-436, 1985.

Howlett, A.C. Cannabinoid inhibition of adenylate cyclase: Relative activity of constituents and metabolites of marijuana. *Neuropharmacology* 26:507-512, 1987.

Howlett, A.C.; Johnson, M.R.; Melvin, L.S.; and Milne, G.M. Nonclassical cannabinoid analgetics inhibit adenylate cyclase: Development of a cannabinoid receptor model. *Mol Pharmacol* 33:297-302, 1988.

Howlett, A.C.; Qualy, J.M.; and Khachatrian, L.L. Involvement of Gi in the inhibition of adenylate cyclase by cannabimimetic drugs. *Mol Pharmacol* 297:307-313, 1986.

Johnson, M.R., and Melvin, L.S. The discovery of nonclassical cannabinoid analgetics. In: Mechoulam, R., ed. *Cannabinoids as Therapeutic Agents*. Boca Raton: CRC Press, 1986. pp. 121-145.

Johnson, M.R.; Melvin, L.S.; Althuis, T.H.; Bindra, J.S.; Harbert, C.A.; Milne, G.M.; and Weissman, A. Selective and potent analgetics derived from cannabinoids. *J Clin Pharmacol* 21:271S-282S, 1981.

Kawakami, Y.; Klein, T.W.; Newton, C.; Djeu J.Y.; Dennert, G.; Specter, S.; and Friedman, H. Suppression by cannabinoids of a cloned cell line with natural killer cell activity (42676). *Proc Soc Exp Biol Med* 187:355-359, 1988.

Koe, K.; Milne, G.M.; Weissman, A.; Johnson, M.R.; and Melvin, L.S. Enhancement of brain [3H]flunitrazepam binding and analgesic activity of synthetic cannabimimetics. *Eur J Pharmacol* 109:201-212, 1985.

Little, P.J.; Compton, D.R.; Johnson, M.R.; Melvin, L.S.; and Martin, B.R. Pharmacology and stereoselectivity of structurally novel cannabinoids in mice. *J Pharmacol Exp Ther* 247:1046-1051, 1988.

Loev, B.; Bender, P.E.; Dowalo, F.; Macko, E.; and Fowler, P.J. Cannabinoids. Structure-activity studies related to 1,2-dimethylheptyl derivatives. *J Med Chem* 16:1200-1206, 1973.

McIlhenny, H.M.; Mast, R.W.; Johnson, M.R.; and Milne, G.M. Nantradol hydrochloride: Pharmacokinetic and behavioral effects after acute and chronic treatment. *J Pharmacol Exp Ther* 219:363-369, 1981.

Mechoulam, R., and Edery, H. Structure activity relationship in the cannabinoid series. In: Mechoulam, R., ed. *Marijuana Chemistry, Metabolism, Pharmacology and Clinical Effects*. New York: Academic Press, 1973. pp. 101-136.

Mechoulam, R.; Lander, N.; Srebnik, M.; Breuer, A.; Segal, M.; Feigenbaum, J.J.; Jarbe, T.U.C.; and Consroe, P. Stereochemical requirements for cannabimimetic activity. In: Rapaka, R.S., and Makriyannis, A., eds. *Structure-Activity Relationships of the Cannabinoids*. National Institute on Drug Abuse Research Monograph 79. DHHS Pub. No. (ADM)87-1534. Washington, DC: Supt. of Docs., U.S. Govt. Print. Off., 1987. pp. 15-30.

Mechoulam, R.; Sharvi, A.; Edery, H.; and Grunfeld, Y. Chemical basis of hashish activity. *Science* 169:611-612, 1970.

Mechoulam, R., and Shvo, Y. The structure of cannabidiol. Tetrahedron 19:2073-2078, 1963.

Melvin, L.S.; Johnson, M.R.; Harbert, C.A.; Milne, G.M.; and Weissman, A. A cannabinoid derived prototypical analgetic. *J Med Chem* 27:67-71, 1984a.

Melvin, L.S.; Johnson, M.R.; and Milne, G.M. Cannabinoid receptor site models. In: *Abstracts From 14th Northeast Regional Meeting of the American Chemical Society.* Washington, DC: American Chemical Society, 1984b.

Milne, G.M., and Johnson, M.R. Levonantradol: A role for central prostanoid mechanisms. *J Clin Pharmacol* 21:367S-374S, 1981.

Mishkin, E.M., and Cabral, G.A. Inhibition of cell-associated herpes simplex virus type 2 glycoproteins by delta-9-tetrahydrocannabinol (42514). *Proc Soc Exp Biol Med* 185:41-48, 1987.

Murison, G.; Chubb, C.G.H.; Maeda, S.; Gemmell, M.A.; and Huberman, E. Cannabinoids induce incomplete maturation of cultured human leukemia cells. *Proc Natl Acad Sci USA* 84:5414-5418, 1987.

Pross, S.; Klein, T.; Newton, C.; and Friedman, H. Enhanced suppression of blastogenic responses by weanling mouse lymphoid cells treated with tetrahydrocannabinol *in vitro* (42577). *Proc Soc Exp Biol Med* 186:17-20, 1987.

Razdan, R.K. Structure-activity relationships in cannabinoids. *Pharmacol Rev* 38:75-159,1986.

Razdan, R.K. Structure-activity relationships in cannabinoids: An overview. In: Rapaka, R.S., and Makriyannis, A., eds. *Structure-Activity Relationships of the Cannabinoids.* National Institute on Drug Abuse Research Monograph 79. DHHS Pub No. (ADM)87-1534. Washington, DC: Supt of Docs., U.S. Govt. Print. Off., 1987. pp. 3-14.

Razdan, R.K., and Howes, J.F. Drugs related to tetrahydrocannabinol. *Med Res Rev* 3:119-146, 1983.

Razdan, R.K.; Zilko-Terris, B.; Pars, H.G.; Plotnikoff, N.P.; Dodge, P.W.; Dren, A.T.; Kynol, J.; and Somani, P. Drugs derived from cannabinoids. II. Basic esters of nitrogen and carboxylic analogues. *J Med Chem* 19:454-461, 1976.

Weissman, A.; Milne, G.M.; and Melvin Jr., L.S. Cannabimimetic activity from CP-47,497, a derivative of 3-phenylcyclohexanol. *J Pharmacol Exp Ther* 223:516-523, 1982.

Wilson, R.S., and May, E.L. Analgesic properties of the tetrahydrocannabinols, their metabolites, and analogs. *J Med Chem* 18:700-703, 1975.

Wilson, R.S.; May, E.L.; Martin, B.R.; and Dewey, W.K. 9-Nor-9β-hydroxyhexahydrocannabinols. Synthesis, some behavioral and analgesic properties, and comparison with tetrahydrocannabinols. *J Med Chem* 19:1165-1167, 1976.

Yahya, M.D., and Watson, R.R. Minireview. Immunomodulation by morphine and marijuana. *Life Sci* 41:2503-2510, 1987.

Yaksh, T.L. The antinociceptive effects of intrathecally administered levonantradol and desacetyllevonantradol in the rat. *J Clin Pharmacol* 21:334S-340S, 1981.

AUTHORS

Allyn C. Howlett, Ph.D
Department of Pharmacology
St. Louis University School of Medicine
1402 South Grand Boulevard
St. Louis, MO 63104

M. Ross Johnson, Ph.D
Glaxo Inc.
5 Moore Drive
Research Triangle Park, NC 27709

Lawrence S. Melvin, Ph.D
Central Research
Pfizer Inc.
Groton, CT 06340