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Research Perspective

Drug Design: Hiding in Full View

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Strategy, Management and Health Policy				
Enabling Technology, Genomics, Proteomics	Preclinical Research	Preclinical Development Toxicology, Formulation Drug Delivery, Pharmacokinetics	Clinical Development Phases I-III Regulatory, Quality, Manufacturing	Postmarketing Phase IV

ABSTRACT Compounds that can produce potent biological effects in cells encompass a variety of structural motifs. Many of these compounds share a structural feature that has rarely been noted. It is an allylic cluster of atoms, a 3-carbon chain with a double bond between two of the atoms and an oxygen atom at the other end. The oxygen can be in a hydroxyl group, or in an ether or ketal or ester linkage, or simply a carbonyl form. In the latter case, the linkage is an allylic ketone (ene-one) structure. Nitrogen is often seen in equivalent forms. Inclusion of at least one allylic moiety appears to be able to turn a modestly active or inert compound into an effective drug or toxin. Some compounds lack the allylic moiety but develop one by enzymatic action, usually via cytochrome P-450 enzymes. These metabolites probably represent the active drug forms. The above concepts seem to be radically simplistic and improbable, but the evidence supporting them and the explanations for the biological activities are hidden ''in plain view.'' Comparisons with the pleiotropic activities of the allylic sphingolipid, ceramide, indicate that many allylic drugs operate by controlling the state of protein phosphorylation, by activating proteases, by generating reactive oxygen species, by slowing mitochondrial electron transport, or by lowering cellular glutathione concentrations. Drug Dev Res 69:15–25, 2008

Key words: mechanisms of drug action; protein phosphorylation; protein thiol binding

INTRODUCTION

Medicinal chemists have discovered or designed a huge number of drugs that while differing greatly in structure, yet display similar biological activities. In a few cases, a particular structural fragment appears to be the primary source of efficacy, but in most cases there seems to be no rationale for the particular structure chosen. In many cases a single drug has been found to attack a variety of disorders or targets, making the puzzle of design analysis even more curious. The result, a huge indigestible mass of drug data, cries for a simplifying integration that may offer a new research direction.

A few researchers have noted that allylic ketones are potent inhibitors of cell growth, and thus potential anticancer drugs. The value of incorporating the cyclopentenone or cyclopentenedione moiety (Fig. 1) into drugs has been pointed out [Honn and Marnett, 1985; Santoro et al., 1987; Conti, 2006]. The structural essence of these allylic ketone fragments (–C=C–C(=O)–) is seen in many drugs and in some compounds endogenous to the body. A direct comparison of a non-allylic ketocyclopentane-containing plant hormone, methyl jasmonate, with the same compound made into an allylic ketone by incorporating a double bond in the Δ^2 position, showed 29 times the growth inhibitory potency in the latter [Ishi et al., 2004]. In a comparison of benzylacetone, a non-allylic ketone, with benzalacetone (which has the required double bond), the latter exhibited much more anti-tumor activity [Motohashi

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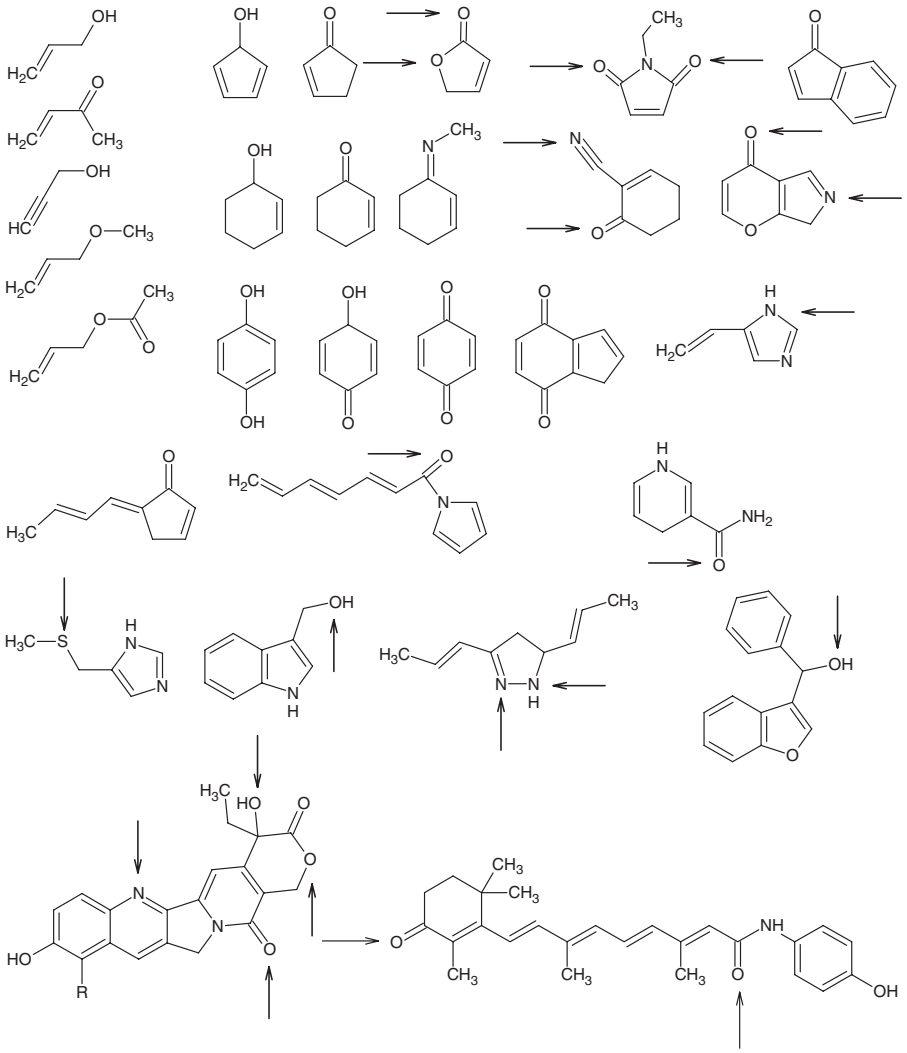

Fig. 1. Different types of allylic moieties. In some fragments, the arrows point to the polar allylic atom. Bottom left: a camptothecin variant has four allylic residues. Bottom right: an allylic ketone metabolite of fenretinide has two (see the cyclohexene ring).

et al., 1998]. A triple bond was even more effective than the double bond.

Similar comparisons of curcumin analogues have also shown the importance of simple allylic ketones, allylic alcohols, and amines. Curcumin, in its main tautomeric form, contains an allylic alcohol and an allylic ketone near each other, each based on a different double bond. Replacement of the two O's with the two N's of a pyrazole ring increases the water solubility and also produces a more active drug [Shim, 2004].

Numerous researchers have reported that allylic ketones bind the major cellular thiol, glutathione (GSH), by the Michael condensation, producing a thio ether and eliminating the allylic double bond. This produces a decrease in cellular GSH, a controller of the activity of key enzymes. Recently it has been recognized that the drugs can also bind to the CySH thiol groups in specific proteins, also a substantial effect [Shiraki at al., 2005; Hamel et al., 2000]. A detailed study of the reaction with a natural allylic ketone, 15-deoxy-$\Delta^{12,14}$-prostaglandin J_2, showed that only specific proteins (such as c-Jun) undergo the Michael condensation [Sánchez-Gómez et al., 2004]. The double bond in the cyclopentenone ring apparently represents the preferred condensation site; however, reduction of this bond still allows condensation with the protein, apparently with the other allylic double bond at $\Delta^{12,13}$, but not to the same extent.

Rapamycin, an anticancer drug containing an allylic alcohol and an allylic methoxyl moiety, owes its activity to the allylic 7-methoxy group [Luengo et al., 1995]. Variants of oleanolic acid, a non-allylic rather inert triterpenoid, show that adding an allylic N atom (via a nitrile) and an allylic carbonyl (sharing the same

double bond in ring A) yields active suppressors of inflammation and inducers of phase 2 cytoprotective enzymes [Sporn et al., 2007]. Replacement of the nitrile by an allylic methoxycarbonyl or an extra allylic ketone, resulted in potent apoptotic activity.

A more general hypothesis has been proposed, based on the properties of ceramide [Radin, 2003]. This simple, widely distributed sphingolipid amide is composed of two building blocks, a fatty acid and a lipoidal amine, the most common being sphingosine (Fig. 2, left top). The hydroxyl on C-3 of sphingosine is an allylic alcohol, due to the *trans*-Δ^4-double bond. Ceramide (Cer) controls cell growth and proliferation, generates reactive oxygen species (ROS) in mitochondria and releases mitochondrial proteins, controls insulin and dendritic cell function, and activates protein kinases, protein phosphatases [Chalfant et al., 1999; Pettus et al., 2002], and some proteases. It inhibits mitochondrial electron flow, modulating operation of Coenzyme Q activity (ubiquinone, a double allylic ketone). It also inhibits aromatase [Andrews et al., 2005], the enzyme that converts androgens to estrogens and thereby stimulates the conversion of Cer to glucosylCer (an antiapoptotic sphingolipid) [Shukla et al., 1992].

Cer induces production of the death-inducing mitochondrial protein, BNIP3 [Daido, 2004], influences angiogenesis, Ca^{2+} transport, tubulin polymerization, and many physiological functions. Cer, like temperature elevation, can induce heat shock protein αβ-crystallin [Chang et al., 1995]. Metabolites of Cer, such as sphingosine and ganglioside GD3, also control protein phosphorylation. Various Cer metabolites have roles in cell physiology, e.g., control of multi-drug resistance (via MDR1) [Derosa et al., 2007; Gouaze-Andersson et al., 2007], providing binding sites for microbes and toxins [Radin, 2006], control of water homeostasis [Stoll et al., 1980], blood vessel diameter, apoptosis, and ischemic recovery. Cathepsin D is stimulated by Cer [Heinrich et al., 2000], while ceramides stabilize raft domains in cell membranes, displacing cholesterol and binding various growth-promoting factors.

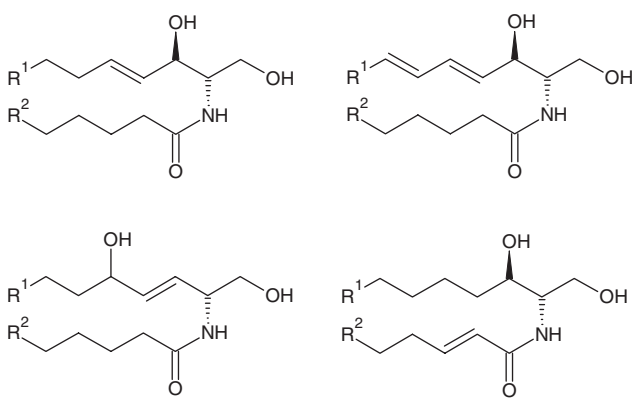

Fig. 2. Ceramide (top left) and some analogues that also show good apoptotic activity.

The importance of protein phosphate groups is well established, but the number of phosphoproteins controlled by Cer and other sphingolipids remains unknown. Cer affects the phosphorylation state of proteins such as Akt, Jun, JAK3, Bax, PKCα, PKCζ, RAF-1, KSR, Bcl2, p38 MAPK, Tau, cd25, STAT1/3, and Rb. These pleiotropic activities of exogenous and endogenously produced Cer are not seen with exogenous dihydroCer, which lacks the key allylic double bond supporting the importance of the allylic structure. The apoptotic activity of some Cer analogues (Fig. 2) is independent of the *location* of the allylic moiety in Cer. Interchanging the OH and double bond, so the latter is at Δ^3 and the OH is at C–5, yielded higher activity [Crawford et al., 2003]. Adding an extra, conjugated double bond to Cer at Δ^6 enhanced apoptotic activity [Struckhoff et al., 2004]. Moving the Δ^4 double bond of Cer into a methylene side chain, which still maintained the allylic structure, increased the antiproliferative activity [Lu et al., 2005]. Moreover, the absolute orientation of an allylic OH seems relatively unimportant, as shown with the C–15 OH in prostaglandin A_2 [Honn and Marnett, 1985]. The allylic fragment in Cer need not even be in the sphingosine chain (see Fig. 2, right bottom) [Azuma et al., 2003]. This lack of specificity requirement helps explain why so many different allylic drugs have similar activity.

Some metabolites of Cer have opposite effects, stimulating cell growth and countering the influence of Cer. It is evident that a dynamic balancing process operates in animals, normally maintaining a healthy state. The balance between the apoptotic and anti-apoptotic sphingolipids is controlled by many factors, such as tumor necrosis factor-α (TNF-α), GSH, fatty acids, phospholipids, chlorpromazine, and microbial toxins. Many drugs elevate the level of Cer so it is not always clear whether the biological effects are due only to the Cer or to both the drug and Cer. It is also apparent that some allylic drugs mimic or inhibit the action of Cer on some proteins.

BRINGING ALLYLIC MOIETIES OUT INTO CLEAR VIEW

Naturally occurring allylic compounds are widely distributed, but it is often difficult to recognize the presence of the allylic moieties. Perhaps the best way to view one is to look for an isolated double bond or a series of conjugated double bonds. Then look at the adjacent C atom at each end of the bond(s) and see if it

is attached to an O or N atom. Conversely, one can look for an O or N atom, and then count off the closest chain of three C atoms connected to it. The second and third atoms should be connected by a double or triple bond. Figure 1 lists a large number of allylic clusters. In some drugs, the S atom in a thio ether with an adjacent propene chain appears to show allylic properties. Another technique is to look at rings, aliphatic or heterocyclic. If the ring contains only one or two double bonds, there may be an allylic O or N on an adjacent carbon or part of the ring. A hydroxymethyl group is sometimes attached to the double bond. An isolated methylene carbon protruding from a ring or chain is often a clue to an adjacent allylic O or N. In the case of Δ^2-carboxylic acid esters (–C=C–C(=O)OR), either O can be considered allylic but the carbonyl O appears to be more important. A similar question arises for Δ^2-amides. In N-ethylmaleimide (Fig. 1), the popular reagent for Michael condensation with thiols, the carbonyl O appears more important.

Searching for the hidden allylic moiety in many synthetic drugs can be confusing because one or both of the C atoms in an allylic double bond may be attached to an O or N atom. These "extra" polar atoms offer extra H-bonding sites that probably improve the protein fit and also affect the reactivity of the allylic O or N. They certainly improve water-solubility. Consider, for example, the ease with which the OH of indole-3-carbinol (an allylic alcohol) dissociates to form a carbocation. In this case, one of the double bond carbons is attached to the N atom. A nearby trifluoromethyl group also appears to increase activity [Motohashi et al., 2001]. A tertiary amine near an allylic C–O can enhance the ability of a drug to condense with endogenous thiols [Pati et al., 2007a].

Quinones are double allylic ketones. The allylic O can also be present in an OH, or ether, or ketal, or ester linkage, or a simple carbonyl group. Other compounds contain an allylic N (amine or imine linkage) instead of an O. Identifying the allylic N in drugs/compounds bearing multi-nitrogen, basic moieties (e.g., imidazoles, pyrazoles) calls for clear eyes.

Not infrequently, two different allylic oxygens share the same double bond, or two double bonds share the same O. Some structures that are nonplanar are especially difficult to analyze in a 2-dimensional representation.

Allylic compounds occur in a very wide range of structures; often (in drugs) there is more than one allylic moiety in each molecule. See the variant campothecin structure (Fig. 1), with three allylic Os and an allylic N. Multi-allylic drugs can probably react with more than one kind of protein, adding to their versatility. Allylic moieties occur in sphingolipids, prostanoids, polyunsaturated fatty acids, vitamins, coenzymes, steroids, porphyrins, and various metabolites. In plants, molds, and bacteria, allylic compounds appear to operate as biological defense or offense weapons.

HOW ALLYLIC COMPOUNDS PRODUCE THEIR SPECIAL EFFECTS

Control of Protein Size and Phosphorylation State

Many of the biological effects of allylic alcohols can be attributed to the reactivity of the C–O bond, which is relatively easily broken to yield a transient free radical or a carbocation. This can happen even with allylic esters, ethers, and (probably) ketals. The cation can condense with a variety of anions, such as a phosphate derivative. Moreover, the original anion can return in the original or isomeric form. The double bond in the carbocation readily migrates (the "allylic rearrangement"), so the condensation with an anion might take place on an adjacent C atom. An example of double bond movement is seen in an allylic ester, dimethylallylpyrophosphate, formed during the course of cholesterol biosynthesis.

The same carbocation intermediate formed from Cer and related sphingolipids has been proposed to explain why they activate or stimulate protein kinases, protein phosphatases, and proteases [Radin, 2004]. In the case of kinases, this hypothesis suggests that the terminal phosphate residue of ATP displaces the allylic OH from enzyme-bound Cer and is then hydrolyzed at the other ester link to yield ADP and (transiently) Cer-3-phosphate. The latter high-energy allylic phosphate group is then transferred to a hydroxy amino acid moiety in a protein, forming a phosphoprotein and Cer. The latter is then ready for a new cycle. This cycle can also operate in reverse fashion, with a phosphoprotein forming a phosphate diester with a molecule of Cer that functions in the active site of a phosphatase. The site of hydrolysis is then between the phosphate and protein, yielding the dephosphorylated protein and transient Cer-3-phosphate. The latter is then hydrolyzed to P_i and Cer, ready for the next cycle. Similar transfers of anion peptides with proteases, such as cathepsin D may be explained the same way. Thus, these important enzymes appear to use a sphingolipid as a bound coenzyme. Since the enzymes function to some extent even without addition of Cer, they apparently exist in part as apoenzymes, allowing local levels of Cer to control their activities. Many drugs containing allylic alcohol residues elicit changes in kinase activity or phosphoprotein levels. Some may act as Cer antagonists in these enzymes (e.g., okadaic acid,

an allylic alcohol, inhibits protein phosphatase, blocking activation by Cer and producing cancer).

Enzymes that control phosphate linkages in DNA and RNA also respond to Cer and allylic drugs. Telomerase, which protects DNA, is inhibited by Cer and allylic drugs (e.g., paclitaxel, etoposide) [Ogretmen et al., 2001]. RNA polymerase is also inhibited by allylic drugs with widely differing structures, e.g., rifampin, streptolydigin, sorangicin A, thiolutin, holomycin, and corallopyronin A [O'Neill et al., 2000]. Some of these compounds contain five allylic groups.

Interference With Mitochondrial Energy and Integrity

Several allylic ketones interfere with the operation of ubiquinone, the mitochondrial double allylic ketone essential for respiration. Ubiquinone constantly cycles through reduction to a free radical, ubisemiquinone, is reduced further to the non-allylic hydroquinone form, then back again. Cer, which is not an allylic ketone, also interferes with ubiquinone operation, probably via enzymatic oxidation of the allylic OH to an allylic ketone via a free radical intermediate. Also in the respiratory chain is the electron carrier NADPH, in which the C=O of the nicotinamide moiety is allylic with respect to the adjacent double bond in the ring. NADPH oxidase generates ROS, so interference with a suitable allylic compound can generate or reduce ROS.

The cholesterol-reducing statins slow the ubiquinone synthesis from hydroxymethylglutaryl CoA, thus augmenting the potency of anti-ubiquinone drugs. Some statins are also allylic (cerivastatin, atorvastatin, pravastatin, fluvastatin) and may act to produce apoptosis [Kaneta et al., 2003]. Statins also influence Cer levels by reducing the concentration of cholesterol, which exists in part as a stabilizing complex with sphingomyelin. Reduction in cholesterol makes sphingomyelin more available to hydrolase activity, thus elevating Cer.

Cer is attacked by isolated or in situ mitochondria, producing ROS and mitochondrial degeneration, with production of cytochrome c and other enzymes. It is likely that mitochondria oxidize the allylic OH in Cer to form 3-ketoCer, an allylic ketone, which is the actual inhibitor [Radin, 2001a]. Supporting this hypothesis is the finding that ketoCer is more apoptotic than Cer [Azuma et al., 2007]. It is also possible that the free radical intermediate in Cer oxidation is the primary inhibitor. KetoCer may also be formed to some extent by direct oxidation by allylic ketone drugs, via a quinone reductase exchange reaction. Drugs that produce ROS also lower GSH levels, thus liberating sphingomyelinase from its inhibited state. Since Cer itself causes an increase in mitochondrial ROS production, even more Cer is formed from sphingomyelin, ultimately leading to cytotoxic effects by the Cer.

Reaction of Allylic Ketones With Cellular Thiol Groups

Allylic ketones undergo Michael reactions, the condensation on the double bond with biological thiols (e.g., glutathione, cysteine) and reactive amines. This reaction explains the frequent observation that allylic ketone drugs lower the cellular level of thiols and thus control the activity of many enzymes. It is important that GSH inhibits several enzymes that control the tissue level of Cer and its metabolites. A major example mentioned above is neutral Mg^{2+}-stimulated sphingomyelinase, which hydrolyzes sphingomyelin to Cer + phosphocholine but is held in check by GSH. This effect is especially important in cancer cells, which protect themselves with elevated GSH concentrations.

Adducts of allylic ketone drugs with GSH and protein-linked CySH have been isolated and identified, but have not been extensively characterized. Drugs containing a catechol-like structure are typically metabolized to *o*-quinones that crosslink protein chains via nearby CySH residues. Unfortunately, such thiol condensation products are probably antigenic, provoking the allergic reactions that are so common in drug therapy. This may call for the use of immunosuppressants like FTY720, a sphingosine-like drug. KetoCer can be expected to react like other allylic ketones, probably binding to protein CySH moieties.

This condensation may explain some unexpected effects, such as the report that fenretinide (4-HPR) treatment of DU145 cells produced a large increase in dihydroCer but not in Cer [Zheng et al., 2006]. Most analyses of Cer content have relied on methods that do not distinguish between Cer and dihydroCer, but a mass spectrometer was used in this instance. Thus, the possibility was raised that dihydroCer can show biological activity in some situations. However, it is likely that fenretinide acts by stimulating the production of ROS [Morales et al., 2005], which means sphingomyelin hydrolysis will yield Cer plus some dihydroCer (dihydrosphingosine is a minor component of all the sphingolipid families). According to the allylic hypotheses, Cer will undergo oxidation to ketoCer, and a large portion of the allylic ketone form will condense with protein and thus be lost to the analytical procedure. The dihydroCer can accumulate temporarily but will ultimately undergo desaturation or hydrolysis. It is also possible that the cells used in the study by Zheng et al. preferentially hydrolyze Cer faster than dihydroCer.

Using mass spectrometry to better understand sphingolipid effects, shows that the fatty acid chain length in Cer plays an important part [Karahatay et al., 2007]. The rate of sphingosine acylation varies somewhat with the different fatty acids [Morell and Radin, 1970]. Incidentally, this article shows that it is possible to separate Cer from dihydroCer by thin-layer chromatography with borate-impregnated silica gel. An immunoassay specific for Cer offers another method for specific analysis [Kawase et al., 2002]. Other sources of information are Obeid et al. [1993], Hannun [1996], Brodesser et al. [2003], Woodcock [2006], Modrak et al. [2006], Maccioni [2007], Hoetzl et al. [2007], Bieberich [2004] and papers by Hannun and colleagues. An important review of the alkylation reaction describes unexpected features that need consideration in designing allylic ketones [Pati et al., 2007a].

Pore Formation in Mitochondria

Cer also has an unexpected function: the formation of mitochondrial pores [Siskind et al., 2006]. If sufficient Cer is added to mitochondria, the lipid assembles into hollow cylinders in the outer membrane, allowing small molecules (cytochrome *c*, procaspases, heat shock proteins) to leak out of the mitochondria and initiate mitochondrial breakdown and apoptosis. DihydroCer does not do this, and the fatty acid chain length is unimportant, suggesting that the allylic properties of Cer are involved in the assembly (and function?) of the channels. Apparently the polar ends of the Cer molecules face the inside of the channels, forming a cylinder of allylic alcohol moieties. This leads me to wonder if these allylic hydroxyls actively transfer phosphate moieties through the mitochondrial membranes (see discussion above of Cer as a coenzyme). This might enable the transfer of ATP (and linked Na^+ or K^+?) by a sort of "bucket brigade" process with stepwise movement from one Cer OH to the adjacent one.

BIOACTIVATION OF "ALMOST ALLYLIC" DRUGS

Exceptions to the "allylic hypothesis" certainly exist, so other modes of drug action are operational. However, some non-allylic compounds are pro-allylics, converted in the body to allylics, which are probably the active forms. There are oxygenases (P-450 enzymes) that hydroxylate many compounds on a C atom next to a C=C moiety (often near or in a branched methyl group), thus creating an allylic fragment. This is seen with fenretinide, which is hydroxylated on its cyclohexene ring (Fig. 1, bottom right) [Villani et al., 2006]. The resultant allylic alcohol is subsequently oxidized to an allylic ketone that is more effective than the original drug. Vitamin D_3 is similarly activated by hydroxylation on the hydroxycyclohexane group. The activities of these oxygenases vary considerably between individuals, as does the rapidity with which they are induced. This may explains why there is so much variability in patient response to drugs and why many promising non-allylic drugs fall by the wayside late in testing. If such drugs were offered in the allylic version instead, dosages could be lowered, which might reduce the problem of toxic side effects.

Conversely, desaturases can produce an allylic double bond next to a –C–O– moiety. This is an essential reaction in the case of dihydroceramide, which is made from the basic sphingolipid (3-keto-2-amino-1-octadecanol) by reduction and fatty acylation. The pyrrolidine ring in indoline is desaturated by cytochrome P450 to form indole, and 3-substituted indoles (e.g., 3-methylindole) are dehydrogenated to form a reactive electrophilic intermediate that reacts with GSH and thiols [Sun and Yost, 2008].

A common method for producing allylic structures is the oxidation of two phenolic groups on the same ring to form a *p*- or *o*-quinone. In many polyphenols, there is at least one methyl ether that can be oxidatively demethylated and then oxidized. This is often seen in antioxidants, which become quinones when they act to protect cells. A similar oxidation appears to occur with *o*-diphenols (catechols) that are bound in ether linkage to the same methylene C. One example is paroxetine, a selective serotonin reuptake inhibitor, in which a complex series of reactions take place at the ether site [Zhao et al., 2007]. Each of the two allylic ketone groups forms the expected Michael sulfide link with GSH, followed by an unusual conversion of the glycylcysteine moiety of GSH to an allylic imino N ring. Thus, even the final product is allylic. Some paroxetine binds to tissues, presumably by a Michael reaction with protein thiols, including those on p450, which is itself inactivated. It is not surprising that paroxetine can also produce apoptosis in cancer cells, apparently due to the *o*-quinone moiety, independently of its action on 5HT [Schuster et al., 2007]. A typical study of allylic ketones, especially adjacent allylic ketones, showed tight binding of an allylic toxin, aflatoxin M1, to milk proteins [Barbiroli et al., 2007]. γ-Tocopherol is also metabolized to a quinone that can produce apoptosis and condense with GSH and protein thiols [Cornwell et al., 2003].

Etoposide, itself not an allylic drug, is metabolized this way. The oxidation appears to go through a semiquinone radical intermediate, as in normal ubiquinone functioning. Similar quinone formation occurs with hydroquinone rings, which form *p*-quinones.

Doxorubicin, which has a *p*-quinone ring adjacent to a hydroquinone ring, may become oxidized to two adjacent quinone rings, probably a very toxic product that may limit the usefulness of this anticancer drug. With suitable connections between two phenyl rings, each containing a single OH, several conjugated double bonds will form together with two carbonyl oxygens, which probably improves reactivity with thiols, since more sites are available for the Michael condensation.

Polyunsaturated fatty acids, characterized by methylene-interrupted double bonds, are oxidized by oxygen to allylic peroxides, which can be reduced enzymatically to allylic alcohols. These are split to some extent by ROS to form the reactive allylic aldehyde, 4-hydroxy-2-nonenal. Such steps are part of arachidonate conversion to the HETEs and prostanoids.

Some phenyl-containing drugs, e.g., tamoxifen, are oxidized to phenols, which are in turn oxidized further to quinones as discussed above. A detailed description of such bioactivations appears in Dowers et al. [2006], which describes three different allylic tamoxifen metabolites and a carbocation. Other phenylated drugs can be metabolized to an epoxy derivative, which is then opened by epoxide hydrolase to form a catechol ring, which then is oxidized to an *o*-quinone. In the case of flavone-8-acetic acid, the resultant catechol derivative [Pham et al., 2007] can be expected to form an *o*-quinone in which the double bonds are conjugated with the adjacent flavone pyrone group. This additional allylic ketone group should enhance the activity.

As mentioned above, a double bond can move over one C atom closer to an OH, to yield an allylic alcohol. This kind of shift is seen in an early step in the biosynthesis of squalene. Here, the double bond in isopentenyl pyrophosphate is shifted (reversibly) one C closer to the OH, to form dimethylallyl pyrophosphate, an allylic pyrophosphate ester. Prenyl transferase then shifts the same double bond of a second molecule of isopentenyl pyrophosphate over one C atom to accommodate condensation with the dimethylallyl carbocation formed by dissociation of the pyrophosphate moiety. This forms geranyl pyrophosphate, then repeats the condensation to add another unit, forming farnesyl pyrophosphate. Allylic alcohols from both esters can produce apoptosis in cancer cells. The same shift occurs in pregnenolone, an "almost allylic" sterol that forms progesterone, an allylic ketone. In the conversion of prostaglandin J_2 to 15-deoxy-D12-PGJ_2, dehydration of the allylic OH forms an extra double bond and moves the adjacent one over by one position. Several allylic drugs also contain an "almost allylic" OH.

The structures of most drug metabolites are unknown or difficult to track down, but one can quickly obtain a clue by testing the effectiveness of a drug after adding a P-450 inhibitor or a thiol, e.g., N-acetyl-CySH. Searching for a protein-bound metabolite of an allylic ketone drug can be done with a radioactive drug or mass spectrometry.

PARTIAL LIST OF ALLYLIC AND PRO-ALLYLIC DRUGS

The claim "many drugs are allylic" can be tested by simply looking at various drug and metabolite structures. Space limitations prevent showing them here, but my previous papers on the subject show some [Radin, 2003, 2007a,b]. Only names are shown in Table 1. You may be surprised to recognize some of the names.

It is significant that the use of combinatorial chemistry to create a library of paclitaxel analogues has produced a new compound that enhances the effectiveness of paclitaxel on multi-drug resistant cells [Edwards, 2007]. Containing three allylic oxygens, its IC_{50} value was 31 nM. A scan of 10,000 compounds for anticancer activity yielded an allylic ketone (DBPT = 5-(2,4-dihydroxybenzylidene)-2-(phenylimino)-1,3-thiazolidin) [Teraishi et al., 2005]. The use of structural bioinformatics to design protein kinase inhibitors led to an irreversibly binding allylic ketone [Cohen et al., 2005]. A computerized search for inhibitors of Stat3, based on the structure of the protein, examined ~429,000 potential small molecules and yielded a 4-ring *p*-quinone, STA-21 [Song et al., 2005].

DISCUSSION

While there is considerable evidence to support the allylic hypothesis, additional experiments are needed to make it more practical. Comparing an allylic drug with the same compound in which the double bond has been hydrogenated might strengthen the hypothesis. How does the loss of the double bond affect the activity, metabolism, and side reactions? The non-allylic version might be rapidly converted biologically to the allylic version, or it might interfere with the action of a natural allylic metabolite or drug, by competing for binding to the same enzyme active site. However, binding of the drug to the enzyme might require simultaneous formation of the allylic carbocation or condensation with a specific enzyme thiol, so the non-allylic imposter drug might have little affinity for the active site. For example, binding might require bringing an allylic OH close to the strongly basic guanidine group of an Arg in the active site.

TABLE 1. Allylic and Pro-allylic Drugs

Acetaminophen, acolbifene, actinomycin D, aflatoxins, AGN193198. aldosterone, 17-allylamino-17-demethoxygeldanomycin, amiodarone, amphidinolides, amphotericin B, anandamide, annonacin, antipyrine, apigenin, arzoxifene, ascididemin, atorvastatin, atovaquone, atractyloside, aureothin, avermectin

Bengamide A, bimatoprost, bleomycin, bongkrekic acid, briaranes, brinzolamide, bruceantin, brucine, bufadienolide, bullatacin

Calcipotriol, calicheamicin, calotropin and other cardenolides, calyculin A, camptothecin, capsaicin metabolite M8, carbenoxolone, CDDO-Im (2-cyano-3,12-dioxooleana-1,9-dien-28-imidazolide), cefdinir, cerivastatin, chalcones, chlordiazepoxide, cimetidine (MI 2300), cinnamedrine MI 2320), cinnamaldehyde, ciprofloxacin, clobetasol, clozapine, colchicine, concanamycin, corallopyronin A, cortexolone, crocin, crotonyloxymethyl-trihydroxy-cyclohexenone, cryptophycins, curcumin, 2-cyclohexen-1-ones and 1-ols, 2-cyclopentene-1-ones and 1-ols, cyclovalone, cytarabine, cytocholasin D, cytotorienin A

Daidzein, dajisentan, dantrolene, dasitinib, daunorubicin, 2-deacetoxyaustrotropicatine, 2-deacetoxytaxinine J, dehydrozingerone, 7-deoxynarciclasine, desonide, dexamethasone, digoxin, 5-(2,4-dihydroxybenzylidene)-2-(phenylimino)-1,3-thiazolidin, docetaxel, doxorubicin, doxycycline, 15-deoxy-$\Delta^{12,14}$-prostaglandin (PGJ_2)

Efavirenz, emodin, epithilone, eplerenone, esculetin, etoposide

F11334, farnesol, fenretinide, ferulic acid, flavopiridol, floxuridine, fluvastatin, fodosine, folinic acid (leucovorin), forskolin, fostriecin, FR901464, fenpyroximate

Gambogic acid, gangliosides, ganoderol, gefarnate, gefitinib, gemcitabine, genestein, geraniol, gimatecan, gossypol, guanacatepene A, GW5810

Herbimycin A, holomycin, 14β-hydroxybaccatin, 7-hydroxycholesterol, hydroxyjasmonic acid, 4-hydroxynonenal, hymenialdisine

Idabenone, illudins, imidazo[2,1-b]thiazoles and 2,3-dihydroimidazo[2,1-b]thiazoles connected by means of a methylene bridge to CoQ, indole-3-carbinol, irinotecan

Kaempferol, khafrefungin

Landomycin, lapachol, lapatinib, lavendamycin, LAQ824, linalool, luteolin, LY 294002

Manumycin A, manzamine A, marinomycin A, masoprocol, meayamycin, menadione, meridine, 5-methyltetrahydrofolic acid, methymycin, mitomycin D, mitoxantrone, monocrotaline, morin, morphine, MPTP (methylphenyldihydropyridine), mupirocin, myxothiazol

Navelbine, nelfinavir metabolites M1 and M3, nifedipine, nobiletin, nocodazole, NSC 668602, NSC606985, 618296, 314622

Oltipraz (a thio analog), ortataxel, OSU-A9 (a derivative of 3-OH-methyl indole), ouabain, 2″-oxovoruscharin

Pachyclavulide, paclitaxel, 6-paradol, paroxetine, parthenolide, PD98059, pectenotoxin-1, pentostatin, D-perillyl alcohol, phenoxodiol, pheophorbide a, phorbol diesters, phytol, piceatannol, picromycin, piericidin, pimecrolimus, plakorstatin, plaunatol, plitidepsin, porphyrins, pravastatin, prostacyclin, pyrantel, pyridaben

Quassinoids, quercetin

Radicocol, raloxifene, rapamycin, resiniferatoxin, resveratrol, retinoic acid, rhizoxin, rifampin, rofecoxib (Vioxx), rotenone, rottlerin, ryanodine

Salutaridine, salvicine, scyphostatin, seco-temsirolimus, seocalcitol, sorangicin A, SPD-304, squamocin, STA 21 (NSC #628869), stigmatellin, streptolydigin, strychnine, SU11652, subcoriacin, sungucine, sunitinib, swinholide A

Tacrolimus, TAK-242, tamoxifen, tautomycin, tedanolide C, temzolimide, tetracycline, Δ^9-tetrahydrocannabinol, thapsigargin, thenoyltrifluoroacetone, thiolutin, tibolone and its Δ^4 isomer, tilosin, topotecan, triamcinolone, trichostatin A, trichothecenes, troxacitabine, tryptolide

Vinblastine, vincristine, vinclozolin, vitamin A; vitamin B_{12}, vitamins K, warfarin, wogonin, wortmannin, zafirlukast, xanthohumol, zaragozic acid A

More information is needed about the properties of allylic amines and imines; are they really much like the corresponding oxygen compounds? Perhaps they must first undergo N-dealkylation and oxidation to allylic aldehydes. In drugs containing an allylic double bond shared by a C=O and an allylic tertiary N, the amine group is readily displaced by an OH [Pati et al., 2007b]. High anticancer potency was observed with a simple related allylic structure in which an amino N and a carbonyl O were activated by two different double bonds (3,5-distyryl-4-piperidone) [Pati et al., 2007a].

Are the oxygen and nitrogen atoms often seen on the double bonds of commercial drugs really useful for binding or functioning, or is their main value an increase in water-solubility? The author's experience with the solubility problem is that crude, uncontrolled suspensions, made by injecting an alcoholic solution of drug into growth or assay medium, yields misleading data [Radin, 2007b]. It seems wise to compare only well-dispersed drugs, especially in animal testing. The alternative dosing technique, gavage, must introduce a strong stress response. In human use, dispersed drugs circulate safely in the blood for longer periods and also react preferentially with the proteins that most strongly bind them. This enhances their specificity and reduces toxic side effects.

Is Cer (or another allylic sphingolipid) an activator of all protein kinases or just primary ones, which then act on many other kinases? If it acts in all of them, a column containing Cer bound to the support particles [Heinrich et al., 2000] might be useful for kinase purification. How many proteases contain Cer? Mass spectrometric analysis of Cer-treated proteins might answer this question. Since there are hundreds of protein kinases, it is possible that different (allylic

alcohol) drugs differ in their specificity as Cer agonists/antagonists.

If the allylic structure is indeed desirable in potential drug designs, limiting new designs to allylic compounds might save a good deal of work. From a preparative point of view, it might be worth using available non-allylic versions and convert them to allylic analogues with a mixture of microbial P-450 s.

Allylic drugs not only typically contain regions that seem to be superfluous, but also regions that are relatively reactive (oxirane rings, aldehydes, peroxides). These may provoke unnecessary side effects. They may react covalently with proteins that side-track the drugs before they reach their target, forming allergenic products. In the author's view, it would be wise to avoid unusual groups and instead incorporate multiple allylic fragments, some of which involve nitrogen. Free OH groups tend to waste drugs, via sulfation or glucuronidation, so pro-drug versions, i.e., methyl ethers [Walle, 2007] or esters, may be more effective. In general, researchers who test simple allylic compounds in animals have been struck by their low toxicity and lack of interference by multi-drug resistance.

Since Cer elevation by drugs seems to play a major role in their utility, it is worth examining the mechanisms by which Cer is elevated. This is done by using the different inhibitors available for controlling the multiple routes of Cer biosynthesis. Some drugs stimulate de novo synthesis from palmitic acid and serine, some stimulate hydrolysis of sphingomyelin by acid or neutral sphingomyelinase, and some inhibit the loss of Cer by preventing its conversion to glycosphingolipids, or hydrolysis by ceramidases [Liu et al., 2008]. Thus, the ideal therapeutic approach would be the use of a mixture of drugs, one for each of the reactions [Radin, 2001b]. This approach also reduces the compensating induction of enzymes that normally occurs when a single drug inhibits only one enzyme. As Modrak et al. [2004] have pointed out, sphingomyelin is a simple nontoxic source of Cer that belongs in any drug cocktail. A Cer-like analogue that is more efficient than Cer would also improve the effectiveness of a cocktail. Even when administered alone, in the diet, sphingomyelin and Cer show anticancer activity.

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