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Perspective

The Discovery of Ezetimibe: A View from Outside the Receptor

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Introduction

The term "rationale drug design" has been used for more than 30 years to describe a marriage of experiment and technology intended to promote a more directed approach toward drug discovery, often focusing on whatever the hottest technology of the time might be.[1] While the technology has changed over the years, the underlying goal has always been to provide a level of insight that would permit researchers to focus on compounds tailored to specific molecular targets with known function, in essence, to reduce the element of chance in the drug discovery process. The discovery of captopril is often cited as an early example of rational drug design, where a detailed knowledge of both the pharmacological rationale as well as the structure and function of the molecular target allowed a targeted synthesis of compounds specifically designed to elicit the desired effect.[2] The decades that followed this discovery have seen a procession of technological initiatives designed to provide an ever more detailed view of structure and function, of how potential drug molecules might interact with their molecular targets, and of how the effects they elicit might impact disease. The promise of these developments was eloquently captured in Strader's 1996 editorial in this journal,[3] "The View from Inside the Receptor". Increasingly, scientists would be able to view the interaction of small molecules from the perspective of the receptor, with a detailed understanding of what residues were key to providing affinity and function and how these residues interacted with a particular ligand. The pace of developments has not slowed in the ensuing years and in many ways has progressed even beyond that vision. Today, advances such as genomics and proteomics promise to tell us not only how drugs interact with their targets but also which targets are most relevant to human disease.

The discovery of the novel cholesterol-lowering agent ezetimibe represents a distinct outlier from these trends.[4] As the first new therapy for treatment of hypercholesterolemia since the discovery of the statins, ezetimibe represents a significant discovery by any measure, but the manner in which it was discovered was also clearly orthogonal to the technological currents of the time. Not only was it discovered without a clear understanding of the molecular target, even the existence of the target itself was unknown at the time the effort began. Even as the discovery process continued, the nature of the target could only be inferred from the existence of a consistent pattern of structure−activity relationships. There are several excellent review articles on the biology of ezetimibe and the results from clinical trials.[5] This perspective will focus on the unusual process that led to its discovery and what lesson can be gleaned from this process.

Discovery of 1,4-Bis(4-methoxyphenyl)-3-(3-phenylpropyl)azetidin-2-one 14 (SCH 48461): The Prototype Azetidinone Cholesterol Absorption Inhibitor

The discovery program that ultimately led to ezetimibe began as a program to discover novel ACAT (acyl-coenzyme A cholesterol acyltransferase) inhibitors.[6] Although ACAT was known to be involved in a variety of cholesterol trafficking events, including cholesterol absorption in rodents, the relevance of ACAT in non-

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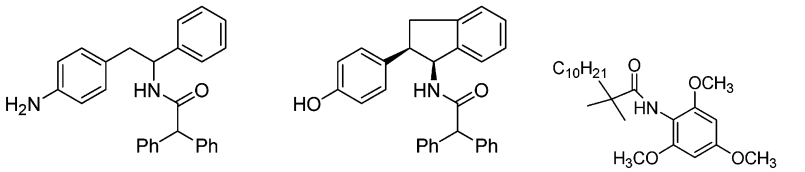
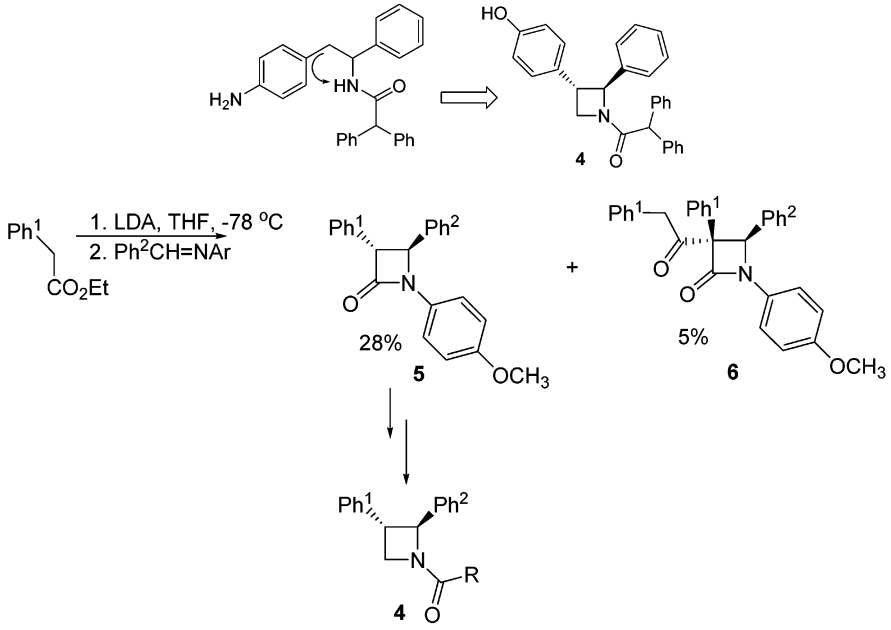


1	2	3 (CI-976)
ACAT: IC_{50} = 900 nM	ACAT: IC_{50} = 40 nM	ACAT: IC_{50} = 4200 nM
Hamster: @ 50 mg/kg PO	Hamster: @ 5 mg/kg PO	Hamster: @ 50 mg/kg PO
SC: NE	SC: -7%	SC: -10%
CE: -80%	CE: -88%	CE: -67%

Figure 1. Prototype ACAT inhibitors.

Scheme 1. Design and Synthesis of Early Azetidinones



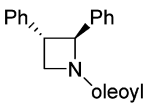
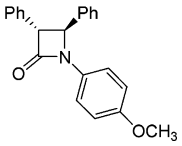
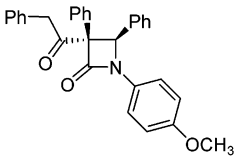
rodent species was still unclear at the time this program began. Nonetheless, a variety of structural classes were known to be potent ACAT inhibitors in vitro and to be active in rodent animal models that reflect a potential for lowering cholesterol levels. Among these models was the cholesterol-fed hamster.[7] A high-cholesterol diet dramatically increases liver cholesteryl ester (CE) levels in these animals, making them especially sensitive to ACAT inhibition. By contrast, serum cholesterol (SC) levels are not dramatically changed by cholesterol feeding in these animals, and most ACAT inhibitors have minimal effect on serum cholesterol levels. Figure 1 shows the in vitro and in vivo profiles of some typical early compounds from this effort, **1** and **2**,[8] as well as a reference ACAT inhibitor **3** (CI-976).[9] Consistent with a well-defined molecular target, ACAT inhibitors displayed clearly defined structure−activity relationships in these models that logically followed from structural changes. Thus, proper conformational constraint of **1** led to indane **2**, which showed both a significant increase in in vitro potency and a commensurate improvement in potency in the cholesterol-fed hamster.[8]

In addition to **2**, a number of alternative conformationally constrained analogues were prepared to probe their impact on in vitro and in vivo potency. Among these was azetidinone **4** proposed by Burnett et al.[10] These compounds were prepared by ester−enolate condensation to give azetidinone **5**, which was then deprotected by CAN oxidation, reduced, and acylated with a variety of acids (Scheme 1).

In practice, the ester−enolate condensation gave only a modest yield of the desired azetidinone **5** accompanied by a small amount of a byproduct **6**, apparently derived from deprotonation of **5** followed by Claisen condensation with the ethyl phenylacetate starting material. Both the desired product **4** and the intermediate **5** and byproduct **6** were evaluated for in vitro activity against ACAT as well as for in vivo activity in the cholesterol-fed hamster (Table 1).[10]

None of the compounds was a potent ACAT inhibitor, although compound **6** did show some modest ACAT activity with an IC_{50} of about 7 μM. Despite this relatively weak activity, **6** showed moderate in vivo activity in the cholesterol-fed hamster assay. This included effects on both CE and a modest but reproducible effect on serum cholesterol. Initial follow-up of this lead structure by Burnett and co-workers (Table 2) demonstrated that **6** was not an isolated finding, since

Table 1. In Vitro and In Vivo Activity of the First Azetidinones

		
4	**5**	**6**
ACAT(25 uM): 4 % Inh.	ACAT(25 uM): 64 % Inh.	ACAT(25 uM): 4 % Inh
		IC50: 7.5 uM
Hamster(100 mg/kg)	Hamster(100 mg/kg)	Hamster(100 mg/kg)
SC: NE	SC: NE	SC: -10 %
CE: NE	CE: NE	CE: **-26** %

several analogues of **6** displayed a similar profile of weak ACAT activity accompanied by a modest but reproducible effect on CE and SC in the hamster.[11] Even at this early stage, elements of a structure−activity relationship were apparent, such as the loss in activity with aliphatic derivative **9**. Improving the ACAT activity of these compounds, such as with **11** which adds the 2,4,6-trimethoxy moiety of **3**, had little or no impact on the in vivo activity of compounds. On the basis of this observation, Burnett et al. opted to disregard the ACAT activity of subsequent analogues and focus on optimizing the in vivo activity of compounds, guided solely by activity in the cholesterol-fed hamster. The absence of an in vitro assay clearly made this an extraordinarily challenging medicinal chemistry effort. Nonetheless, this effort culminated in the discovery of azetidinone **13** and its resolved form **14**, the prototype azetidinone cholesterol absorption inhibitors and the starting point for all subsequent work. It is noteworthy that the difference between **13** and less interesting analogues such as **12** is the addition of a single well-placed methoxy group at the C4 phenyl.

Figure 2 shows the in vitro and in vivo profile of **14**.[12] In addition to blocking accumulation of hepatic cholesteryl esters in the hamster, **14** reduces serum cholesterol levels in the cholesterol-fed rat, dog, and monkey. Of particular significance is the activity in the cholesterol-fed rhesus monkey. Figure 3 shows the effects of **14** on rhesus monkeys fed a high-cholesterol diet for 4 weeks.[12] Control animals show a profound hypercholesterolemia after 3 weeks that is completely blocked by 1 mg/kg dose of **14** admininistered in the diet. When the control animals are then treated with 1 mg/kg of **14**, their cholesterol levels return to nearly baseline within 1 week, while withdrawal of drug causes a gradual rise in cholesterol levels over the same time period.

While ACAT inhibitors are known to inhibit cholesterol absorption in rodents, such potent antihypercholesterolemic activity in nonrodents was unprecedented with ACAT inhibitors. Furthermore, Salisbury et al. demonstrated that ACAT inhibitors and **14** have different effects on intracellular cholesteryl levels.[12] In these experiments, the ACAT inhibitor **3** blocked the accumulation of ^{14}C-cholesteryl esters but had no effect on free cholesterol in the intestinal wall of cholesterol-fed hamsters. By contrast, **14** inhibited the accumulation of both esterified and unesterified cholesterol. The reduction in cholesteryl ester levels was due entirely to reduction in free cholesterol substrate, while ACAT activity remained essentially unchanged. Thus, both the in vitro data and the in vivo pharmacology suggested that the azetidinone cholesterol absorption inhibitors act via a unique mechanism that is upstream from ACAT.

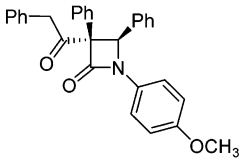
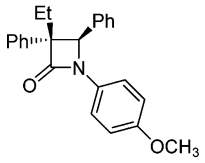
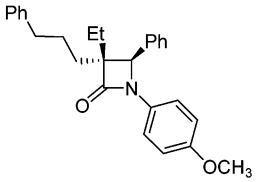
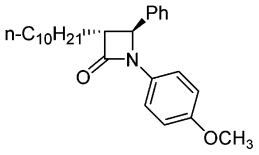
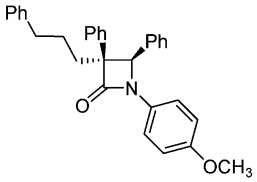
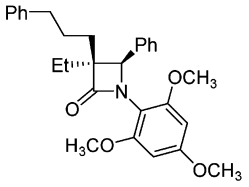
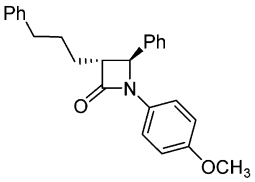
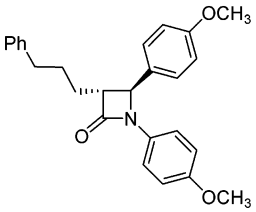
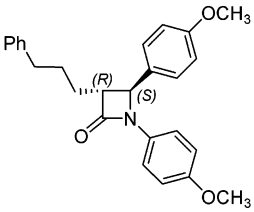
From Serendipity to Design: The Discovery of Ezetimibe

A substantial part of the chemistry program that followed the discovery of **14** was devoted to understanding the nature of the molecular target. A complete discussion of the ensuing SAR is the subject of other reviews and is beyond the scope of this perspective.[13] Although the evidence is clearly circumstantial, these studies showed that the effects of **14** and related compounds were consistent with interaction with a well-defined molecular target. Among the most compelling evidence was once again the effects of conformationally constrained analogues such as **15**−**18** (Table 3).[14,15]

While these data provided encouragement, the absence of an in vitro assay made it difficult to separate potential effects on intrinsic potency from effects on pharmacokinetics. The latter was confounded by the fact that **14** is extensively metabolized in vivo, making it unclear exactly what the active species is.[16] To address this latter issue, Van Heek and colleagues devised a unique experimental protocol designed to determine if there were active metabolites and how they contributed to the overall in vivo profile of **14**.[17] The experiment was divided into two parts, both of which used an intestinally cannulated, bile-duct-diverted rat model (Figure 4).

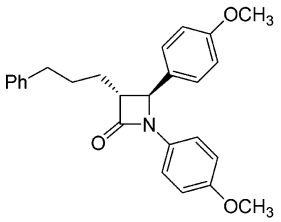
In the first part of the experiment, animals were dosed via intraduodenal cannula with ^{3}H-**14**. Bile from these animals was collected via the bile-duct cannula to give the so-called "metabolite" bile, which on the basis of previous experiments was known to contain the majority of the metabolites of **14**. Concurrently, control animals were dosed with vehicle, bile was collected, and then ^{3}H-**14** was added to this so-called "parent" bile to match the specific activity of the metabolite containing bile. In the second part of the experiment, both the metabolite bile and the parent bile were again dosed intraduodenally into a second group of diverted animals

Table 2. Early Azetidinone Structure−Activity Relationships

		
6	**7**	**8**
ACAT IC_{50}= 7500 nM	ACAT IC_{50} >10,000 nM	ACAT IC_{50} >10,000 nM
Hamster: @100 mg/kg	Hamster: @50 mg/kg	Hamster: @50 mg/kg
SC: -10%	SC: -15%	SC: -12%
CE -26%	CE -39%	CE -29%
		
9	**10**	**11**
ACAT IC_{50}> 10,000 nM	ACAT IC_{50}= 6000 nM	ACAT IC_{50}= 1700 nM
Hamster: @50 mg/kg	Hamster: @50 mg/kg	Hamster: @50 mg/kg
SC: NE	SC: -11%	SC: NE
CE NE	CE -16%	CE -31%
		
12	**13**	**14**
ACAT IC_{50} > 10,000 nM	ACAT IC_{50} = 18,000 nM	ACAT IC_{50} = 26,000 nM
Hamster: @50 mg/kg	Hamster: @10 mg/kg	Hamster: @10 mg/kg
SC: NE	SC: -21%	SC: -43%
CE -15%	CE -60%	CE -93%

along with ^{14}C-cholesterol, and the counts of both ^{14}C and ^{3}H in various tissues were measured. In this way, both the pharmacological effects as well as the disposition of drug could be measured in a single experiment by following counts of ^{14}C and ^{3}H, respectively. Furthermore, because of the bile duct diversion, the pharmacological effects reflected primarily the activity of the species being dosed and not the effects of any subsequently produced metabolites. In a third control arm of part 2, parent bile was dosed into intact, undiverted animals, where formation of active metabolites could still contribute to activity. Figure 5 shows the results of these experiments.

While the parent bile reduced the appearance of ^{14}C-cholesterol in both the plasma and liver, the metabolite bile was clearly more effective and reduced ^{14}C levels comparably to the parent compound **14** in intact animals. These data strongly suggested that formation of one or more active metabolites plays a significant role in the in vivo activity of **14**. Consistent with this, when ^{3}H-**14** bile was dosed, the majority of the tritium counts were recovered in the bile. However, when ^{3}H metabo-



14

(Burnett et al.)

ACAT: IC_{50}= 26 uM

Cholesterol Fed Animal Models:

ED_{50}:	Hamster:	2.2 mg/kg[1]
	Rat:	2.0 mg/kg
	Monkey:	0.2 mg/kg
	Dog:	0.1 mg/kg

[1] Endpoint in hamster is hepatic cholesteryl esters, others are total plasma cholesterol.

Figure 2. Profile of **14** in cholesterol-fed animal models.

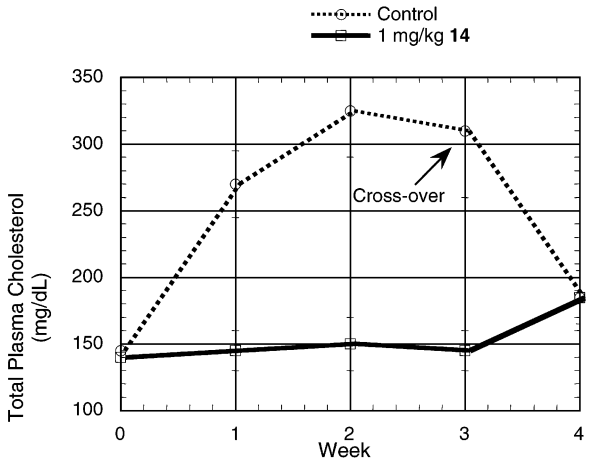
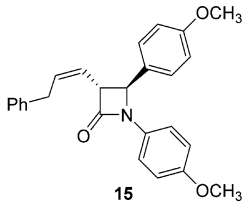


Figure 3. Effect of **14** in the cholesterol-fed rhesus monkey.

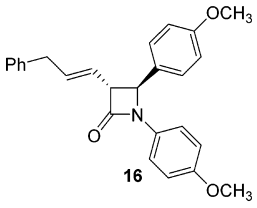
Table 3. Conformationally Constrained Analogues of **14**



15

CE: -73%

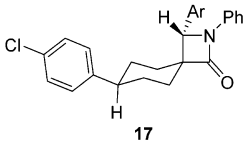
SC: -20%



16

CE: NE

SC: NE

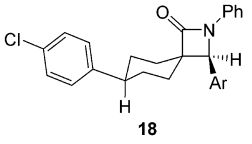


17

CE: -97%

(ED_{50}= 0.66 mg/kg)

SC: -46%



18

CE: NE

SC: NE

lite bile was dosed, the majority of the counts remained in the intestinal wall and lumen. These data suggest not only that are there active metabolites but that these metabolites localize at the putative site of action more efficiently than **14** itself.

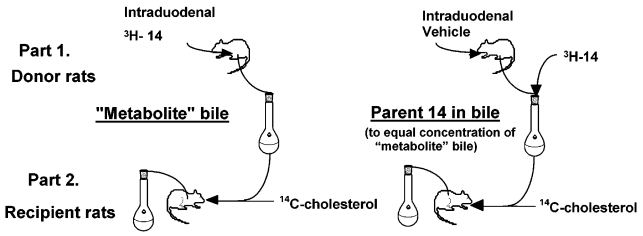


Figure 4. Intestinally cannulated bile-duct-diverted rat protocol.

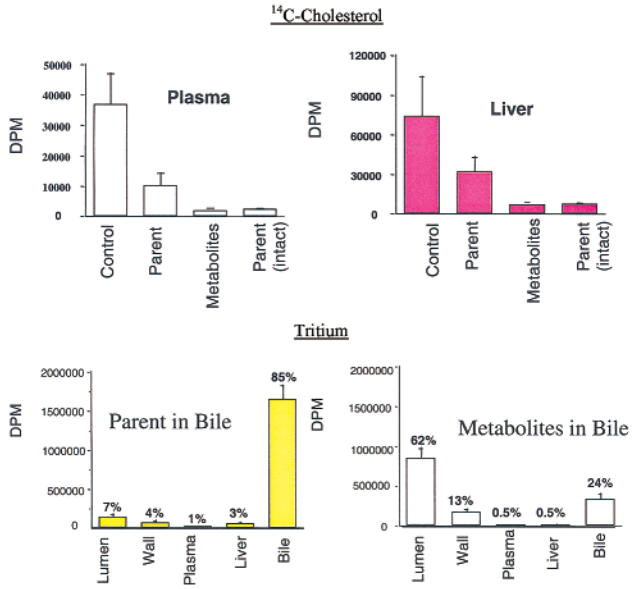


Figure 5. Distribution of ^{14}C-cholesterol and ^{3}H-14 metabolites in bile-duct-diverted rats.[17]

These data begged the question of the identity of the active metabolites. To address this, metabolite bile was fractionated by HPLC and each fraction was evaluated according to the paradigm described in part 2 above. The results of this experiment are shown in Figure 6. On the basis of the appearance of ^{14}C-cholesterol in plasma, these data showed that the bulk of the activity resided in fraction 6, and subsequent analysis showed that this fraction was composed primarily of the glucuronide of compound **19**, a phenolic metabolite of **14**.

To complete the experiment, the activities of the metabolite bile, crude fraction 6, and authentic **19** were compared in bile-duct-diverted rats (Figure 7). These data show that the activity of **19** is identical to that of fraction 6 and both are substantially more active than the crude mixture of metabolites.

In total, these data strongly suggested that much of the in vivo activity of **14** was due to the formation of **19**. Furthermore, they suggested that metabolism of **14** to **19** helped to localize the compound in the intestines at the putative site of action of the compound.

The experiments by Van Heek et al. provided compelling evidence for the presence of at least one active metabolite of **14**. Nonetheless, there were also other less prominent metabolites whose formation could either contribute to the activity of **14** or diminish it. To understand this, Rosenblum et al. prepared authentic samples of a number of known or putative metabolites that were evaluated for activity in the cholesterol-fed hamster (Table 4).

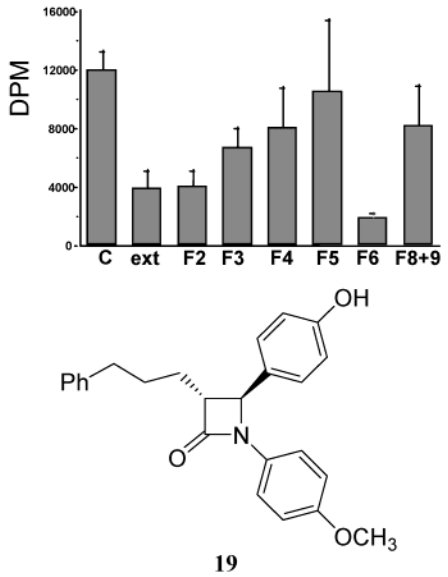


Figure 6. Efficacy of total bile extract (ext) and fractionated bile.

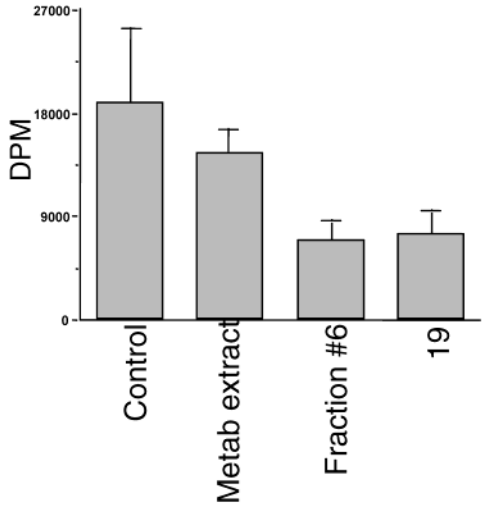


Figure 7. Efficacy of fraction 6 versus **19** in bile-duct-diverted rats.

Among the various putative metabolites of **14** were a variety of phenols produced by dealkylation of either or both of the methoxy groups or via aromatic hydroxylation.[18] Both phenol **20** and bisphenol **21** showed substantial activity in the cholesterol-fed hamster, although neither was more active than **14** or **19**, suggesting that metabolism on the *N*-aryl moiety was not required for activity. On the other hand, phenol **22** was less active than **14**, suggesting that this route of metabolism might be detrimental to activity. In addition to the phenols, another route of metabolism involved hydroxylation of the 3-phenylpropyl side chain to produce alcohols and ketones. (*S*)-Alcohol **23** was substantially more active than **14**, the (*R*)-isomer **24** was less active, and the corresponding ketone **25** had intermediate activity. This suggested that metabolism to the (*S*)-alcohol, if not required for activity, might improve the activity of the compound. Other combinations of these routes of metabolism were also investigated with similar results.

On the basis of the combined observations of experiments in bile-duct-diverted rats and the activity of various putative metabolites, a strategy emerged for the design of a second-generation compound, namely, (1) premetabolize profitable sites of metabolism on the C4 aryl and the phenylpropyl side chain to improve activity, minimize plasma levels, and localize the compound in the intestines and (2) block unprofitable sites of metabolism to maximize activity and limit further oxidative metabolism. This strategy was in fact applied to a number of chemical series related to **14**[19] but most successfully by Rosenblum et al. to give azetidinone **26**, now known as ezetimibe.[20]

Figure 8 compares the activity of ezetimibe to **14** in a number of cholesterol-fed animal models.[21] In every case, but most dramatically in the monkey, ezetimibe is substantially more active than **14** and shows substantially lower plasma levels.

Synthesis

A number of syntheses of ezetimibe and related compounds have been reported, several of which were utilized in the course of these investigations.[22] Many of these are based on an Evans-type oxazolidinone condensation to establish the correct stereochemistry on the azetidinone ring. Scheme 2 shows a representative synthesis of ezetimibe. In addition to the use of the oxazolidinone, this synthesis also features a Corey oxazaborolidine reduction to set the (*S*)-stereochemistry of the side chain hydroxyl group.

Ezetimibe and Statins

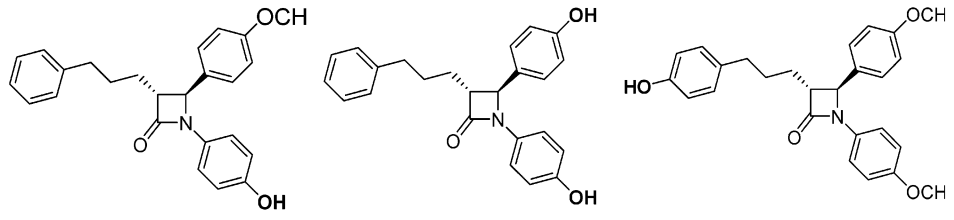
While the activity of ezetimibe in cholesterol-fed animals was clearly impressive, it is noteworthy that these models involve diets that are substantially higher in fat and cholesterol than the animals' normal chow diet. Despite the substantial activity of ezetimibe and other azetidinone cholesterol absorption inhibitors in these models, none of the compounds tested significantly reduced plasma cholesterol levels in animals fed a normal chow diet. Rather than being a liability, this observation in fact led to one of the most important aspects of the profile of ezetimibe. In considering the possible reasons for the lack of substantial effect on serum cholesterol in the absence of a high cholesterol diet, Davis reasoned that a cholesterol absorption inhibitor might stimulate hepatic HMG-CoA reductase activity. This could compensate for a reduced cholesterol load due to inhibition of intestinal cholesterol absorption. If this were the case, then coadministration of a cholesterol absorption inhibitor and an HMG-CoA reductase inhibitor should produce an enhanced reduction in serum cholesterol at doses that were less effective or ineffective as monotherapy. To test this hypothesis, Davis et al. administered ezetimibe (0.007 mg/kg) or lovastatin (5 mg/kg) to chow fed dogs over 14 days.[23] While neither compound had a substantial effect on serum cholesterol alone, the combination produced a profound reduction in serum cholesterol (Figure 9).

Similar experiments demonstrated a comparable effect in other species and with other statins. These data suggested that ezetimibe would be effective at reducing cholesterol levels in humans and would be particularly effective in combination with HMG-CoA reductase inhibitors.

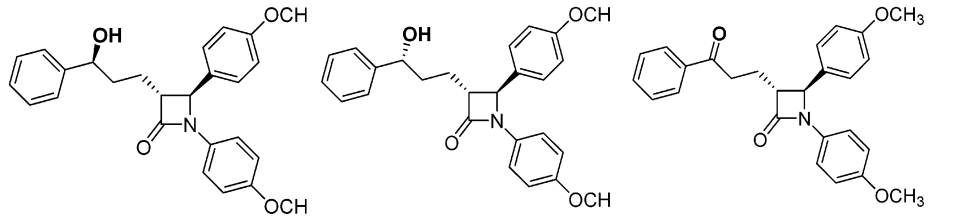
Clinical Results

Human clinical trials with ezetimibe supported the expectations of animal studies with ezetimibe both as

Table 4. Activity of Possible Metabolites of **14** in Cholesterol-Fed Hamster



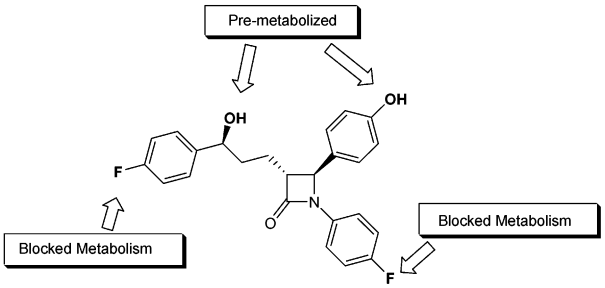
20	**21**	**22**
CE: - 68% @ 50 mg/kg	CE: - 78% @ 50 mg/kg	CE: - 78% @ 50 mg/kg



23	**24**	**25**
CE: ED50 = 0.9 mg/kg	CE: ED50 = 5.0 mg/kg	CE: ED50 = 1.9 mg/kg

monotherapy and in combination with statins.[24] Table 5 shows the results of phase III human trials with ezetimibe as monotherapy. Ezetimibe produced a significant reduction in total cholesterol, LDL cholesterol, and triglycerides as well as a small but significant increase in HDL cholesterol.

Figure 10 compares the effect of simvastatin or atorvastatin either alone or when coadministered with ezetimibe on LDL cholesterol. In each case, ezetimibe produced an additional 15−18% reduction in LDL cholesterol above that achieved by the statin alone.



26
(Ezetimibe)

ED50:	**14**	**Ezetimibe**
Hamster :	2.2 mg/kg	0.04 mg/kg
Rat:	2.0 mg/kg	0.03 mg/kg
Monkey:	0.2 mg/kg	0.0005 mg/kg
Dog	0.1 mg/kg	.007 mg/kg
Blood Levels:		<< **14**

Figure 8. Comparison of structure and in vivo profile of **14** and ezetimibe.

Finally, Figure 11 compares the effect of a high dose of statin alone with a low dose of statin coadministered with ezetimibe. In each case, coadministration of ezetimibe and low-dose statin produced an equivalent reduction in LDL cholesterol as the high dose of statin alone. Combined, these data demonstrate that ezetimibe alone or coadministration with statins provides favorable effects on the major lipid parameters in patients with hypercholesterolemia.

Conclusions

Case histories of drug discoveries that have made extensive use of a novel technology are often used as evidence for the validity of that technology as a drug discovery tool. Remarkably, essentially none of the technologies often associated with "modern" drug discovery played a significant role in the discovery of

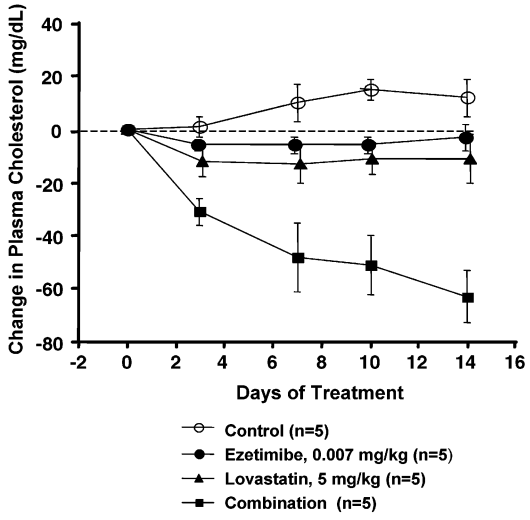


Figure 9. Effect of ezetimibe and lovastatin in chow-fed dogs.

Scheme 2. Representative Synthesis of Ezetimibe

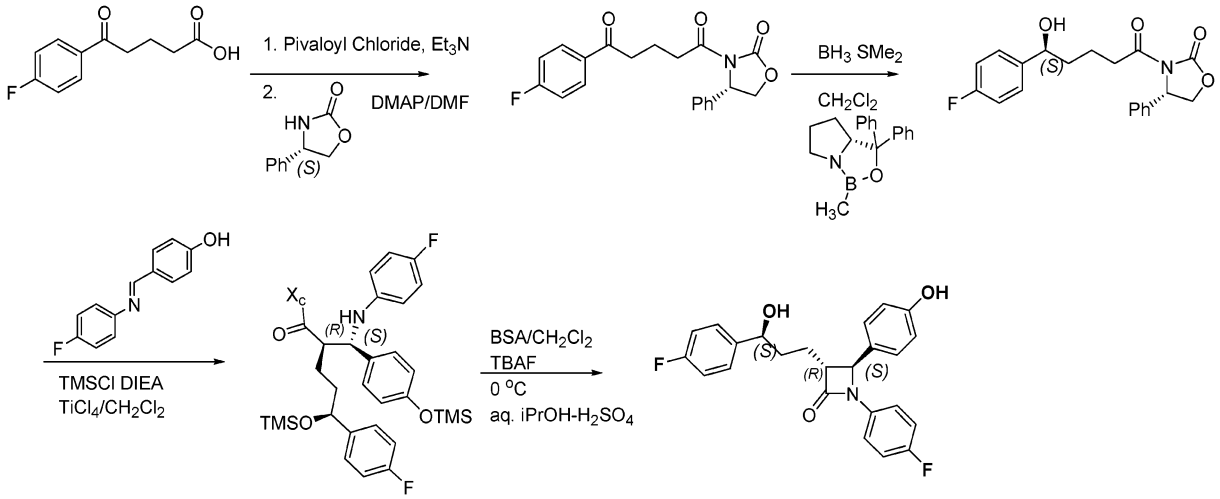


Table 5. Ezetimibe Phase III Monotherapy Efficacy Results

	mean % change from baseline at endpoint			
treatment	LDL cholesterol	total cholesterol	HDL cholesterol	triglycerides
placebo (n = 226)	+0.4	+0.8	−1.6	+5.7
ezetimibe, 10 mg (n = 666)	−16.9[a]	−12.5[a]	+1.3[a]	−5.7

[a] Significantly different from placebo results (p < 0.01).

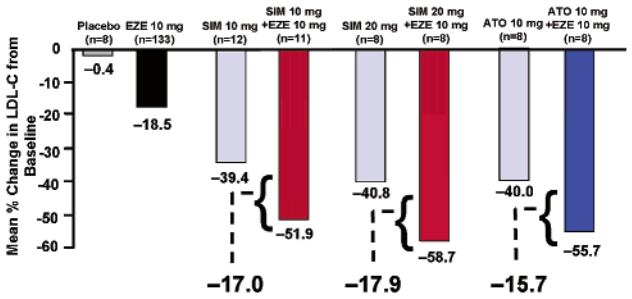


Figure 10. Ezetimibe coadministered with simvastatin or atorvastatin.

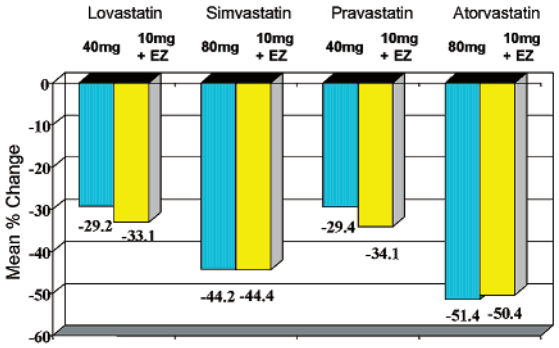


Figure 11. Effect of high- and low-dose statin coadministered with ezetimibe.

ezetimibe, although they may be important for our understanding of the molecular target and the implications of this finding on cholesterol management and cholesterol homeostasis. The absence of these tools clearly created a number of challenges that required creative solutions, but in the end the entire process from initiation of the discovery program to discovery of the first clinical candidate **14** and finally to the discovery of ezetimibe was still completed in about 6 years, a time frame not out of line with more biochemically driven programs of that time. The most important lesson from this discovery may be that while the technological advances of the past decade have unquestionably had a profound and positive impact on the discovery process, they have not reduced the importance of the discovery scientist in shaping and guiding this process. Even as it becomes more focused and technologically driven, drug discovery is still an experimental science. It remains a risky but distinctly human endeavor where serendipity and the opportunity to capitalize on it still play an important role.

Acknowledgment. Ezetimibe was discovered by a team of Schering-Plough scientists from many scientific backgrounds who shared a common dedication toward scientific excellence and close collaboration. Without all of them and the unfailing support of Schering-Plough management, this project could not have succeeded. The author is privileged to present this case history on their behalf. Special thanks are given to Drs. M. Van Heek and H. Davis for many helpful comments and suggestions.

Biography

John W. Clader received his Ph.D. in organic chemistry in 1980 from Indiana University. After 2 years at the University of Notre Dame, he began his career in medicinal chemistry at Hoffmann-La Roche in Nutley, New Jersey. He moved to the Schering-Plough Research Institute in 1985, where he is currently Distinguished Research Fellow in Medicinal Chemistry. In addition to ezetimibe, his research interests at Schering-Plough have included potential treatments for Alzheimer's disease and HIV infection as well as the application of chemoinformatics and other computer methods to facilitate drug discovery.

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