Chemotherapeutic Agents. Lecture 1.

Paul W. Erhardt [1]
College of Pharmacy, The University of Toledo, 2801 West Bancroft Street, Toledo OH 43606-3390

PROLOGUE

The following text summarizes information conveyed[2] during an introductory lecture for a medicinal chemistry course that serves as a forum for in-depth discussions about representative chemotherapeutic agents wherein student and teacher work together to explore the key chemical features of selected compounds. In this opening lecture, ampicillin is used as an example to review some of the basic medicinal chemistry principles associated with carboxylic acids, amines, amides and 6-lactams, as well as to demonstrate the general approach that can be taken while considering all subsequent compounds.

COURSE INFORMATION

MBC 432 Chemotherapeutic Agents is the final offering within a series of five didactic Medicinal and Biological Chemistry courses required by all BS and PharmD students enrolled in the College's professional degree programs. This two-credit course serves as a forum for in-depth medicinal chemistry discussions about representative chemotherapeutic agents. It is offered during the fourth year of the curriculum in parallel with a four-credit complementary pharmacology course that provides a survey of the various agents within this field.

The lecture style is deliberately set up to rely heavily upon the use of the blackboard, rather than handout or transparency, such that student and teacher can be thought of as working together to explore the interesting chemical features of selected compounds. After brief, lead-in reviews, students are prompted to draw upon their previous exposures to organic chemistry and biochemistry while the properties of various compounds are examined in terms of their distinguishing chemical functionality and in terms of their distinct chemical interactions with the biological realm.

A copy of the most recent syllabus for MBC 432 is provided in Appendix A. The lectures pertaining to anticancer agents are delivered in tandem with pharmacy practice faculty who introduce this particular topic by providing a clinical perspective. No textbook is required. Lecture information has been culled from several different sources. All exam questions are taken directly from the class notes and occasional handouts. The instructor's lecture notes are made available in the Center for Drug Design and Development (CD3) office/lab area for individual or group study on a continual basis but not for photocopying. Likewise, additional reading materials for every topic are either available in the CD3 office area or can be readily tracked down from the library.

INTRODUCTION

Medicinal chemistry is considered at the interface between chemical structure and biochemical consequence, or what the students have already come to appreciate as structure-activity relationships (SAR). However, within the field of chemotherapy, the students are challenged to extend these same type of SAR principles by first thinking in terms of structure-toxicity relationships and, ultimately, in terms of structure-selective toxicity relationships. The process of extending the student's appreciation of SAR principles to include the notion of selective toxicity is begun by emphasizing the dynamic nature of chemical functionality relative to its presence in different molecular environments.

CHEMICAL STRUCTURES AS DYNAMIC ENTITIES

Students are immediately asked to consider a chemical question which has been placed on the back side of their syllabus (Figure 1). This question is similar to one in the 1995-1996 NABPLEX Candidates Review Guide and pertains to a chemical structure that is particularly relevant for this course.

Although the answer "A" should be relatively easy for these students, the actual intent of this exercise is to set the stage for a contrast which will be made between this type of placid structural representation versus a way of thinking about molecules that, instead, will examine the dynamic aspects of a structure's key chemical features within specific contexts that become meaningful to pharmacists. Thus, it is

[1]Professor of Medicinal and Biological Chemistry; Director, Center of Drug Design and Development.

[2]Since a student's learning experience is very much dependent upon how information is conveyed as well as upon what information is conveyed, an attempt has also been made to include many of the analogies which can be employed during the lecture to breathe life into the chemical information. Interested readers are welcome to request the initial version of this manuscript which, taken mostly verbatim from the lecture, further illustrates how these analogies are used as part of an ongoing dialogue between student and teacher.

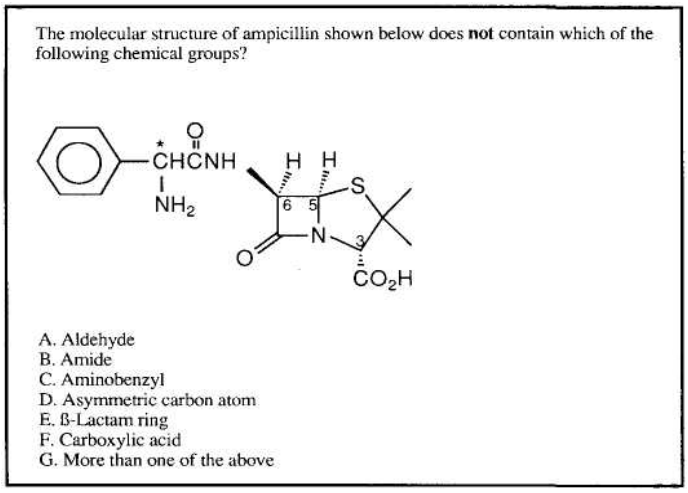

Fig. 1. Typical chemical structure-related state board question.

quickly pointed out that even to a seasoned medicinal chemist who can find delight in the mere drawing of a chemical structure, right or wrong the various answer choices to this question seem dull and uninspiring. Why? Because at this point the static chemical structure and its lifeless appendages lack a meaningful context. And most importantly, without such a context, purely memorized knowledge about organic chemistry, even though it may presently make this an easy question, is destined to be forgotten.

So although this question may be a satisfactory way to test an individual's general knowledge about medicinal chemistry during a multidisciplinary exam like the state board, it is emphasized that it is not the best way to actually learn something about chemical structures and it is certainly not the way that students should be thinking about them during MBC 432. Alternatively, it is pointed out that it is possible to breathe some life into such drawings by first examining the distinguishing chemical nature of each of their displayed functionalities. And that once these are in clear view, it then becomes much more meaningful to visualize how the chemical features result in the specific properties that ultimately translate drug molecules into unique therapeutic entities which interact, at times very aggressively, with the biological realm. To reinforce this concept, the students are then asked to consider again the same ampicillin question (Figure 1). But this time they are asked to start with the incorrect choice "F" and to slowly work backwards toward the correct answer "A."

CARBOXYLIC ACIDS OR CARBOXYLATE ANIONS (TO BE OR NOT TO BE)

Even the name of choice "F" should suggest some type of dynamic character for this particular group. It's an acid and it's very being or essence is that it's acidic. Thus, in an aqueous media this group can loose a proton according to an equilibrium (Figure 2, Line A) where the extent of the ionization process is related to its acid strength or pka as expressed by Line B. Student's are then challenged to recall that carboxylic acids want to do this because the resulting carboxylate anions can be resonance stabilized between the canonical forms depicted on each side of the double-headed arrow (Line C) so as to actually exist as a resonance hybrid structure which is of lower energy than either of its canonical forms alone.

Nevertheless, carboxylic acids with pKa's of about 4 to

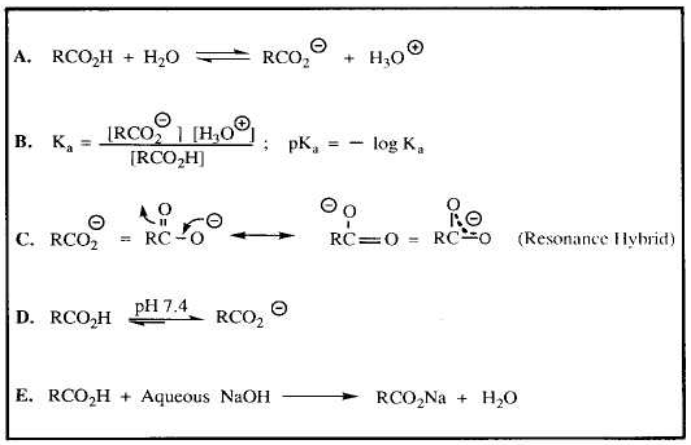

Fig. 2. Properties of carboxylic acids.

5, should be regarded as only weak acids and their ionization in water is not necessarily dramatic. But what about in the body? Students are now asked to recall that as a rule of thumb, the pka represents a number where if the pH is adjusted to this value, the acid will be about 50 percent ionized. Thus, if the pH is raised from 4 or 5, where this acid is already about 50 percent ionized, up to 7.4 (physiological), the more basic pull toward further removal of the proton is, by definition, logarithmic and the acid can now be thought of as being largely deprotonated or nearly fully ionized within the body (Line D). Actual reaction with a base (Line E) can be even more dramatic, essentially going to completion in a process which neutralizes both the acid and the base.

In the case of the penicillins, the chemical behavior of the carboxylic acid becomes extremely important. This is because the formation of sodium and potassium salts with this particular group provides stable, crystalline materials which are useful during the production, formulation and storage of these compounds. And without taking advantage of this specific salt forming reaction, the penicillins are, as a class, notoriously troublesome in all of these regards. But the role of the carboxylic acid group goes even further toward providing the pharmacist with a relevant context from which to consider and remember its display on the ampicillin molecule.

TROJAN HORSES AND HAPTOPHORES

The penicillins are able to disrupt the cell walls of bacteria. A detailed examination of this mechanism is undertaken in later lectures so only a quick, snap shot look at this process is provided at this point. Bacteria normally strengthen their cell walls by effecting a key cross-linking reaction between short peptide chains which would otherwise be loosely dangling from the cell wall structure (Figure 3). The bacterial enzyme responsible for this key reaction is called D-Alanyltranspeptidase.

Interestingly, the north and east edges of the penicillin structure resemble the terminal amino acid portion of these short peptide chains (*e.g.*, sequence B in Figure 3). And even more interesting, the resonance stabilized carboxylate anion hybrid (answer choice "F" at physiological pH) is thought to reside in a region of space which is similar to that of the D-Ala carboxylate anion terminus relative to its orientation at the end of chain B. Because of this very close resemblance, the transpeptidase enzyme becomes tricked into interacting

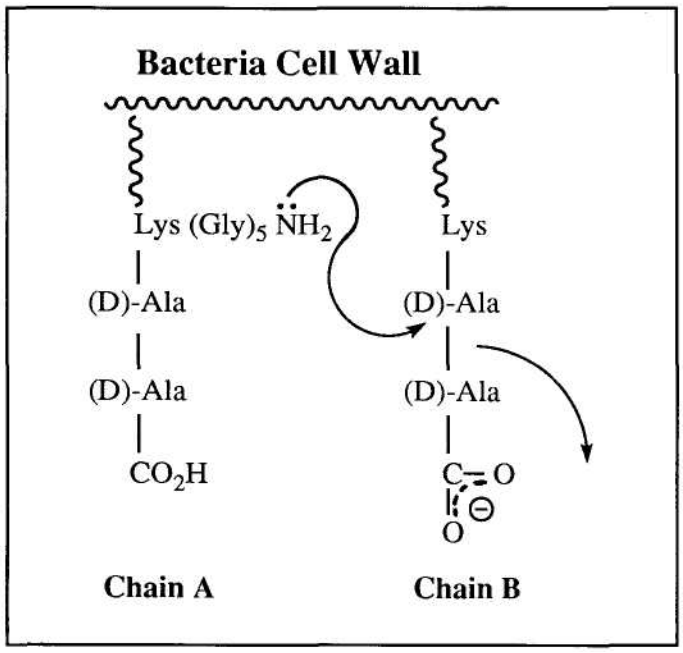

Fig. 3. Cross-linking reaction effected by D-Alanyltranspeptidase.

with a penicillin molecule rather than with its normal endogenous substrate. However, while initially receptive to this friendly looking "Trojan Horse," once inside the enzyme's active site the penicillin molecule becomes entwined with the transpeptidase in a manner that is inhibitory to the enzyme's function and the bacteria's key cross linking process is shut down. Ultimately, without a strong cell wall to prevent osmotic swelling and rupture, the bacteria's life is compromised and its invasion of a host becomes defeated.

In this way, one can see that the carboxylate anion is a key recognitional element that is critical for the penicillin's action within bacteria. During a subsequent lecture which provides a historical perspective to the field of chemotherapy, it is shown that the concept of having specific recognitional elements within a chemotherapeutic structure was first elaborated by Paul Ehrlich who called such elements "haptophores."

β-LACTAMS AND SCHIZOPHRENIC NITROGENS

If one takes β-amino propionic acid and allows for the formation of an intramolecular amide bond (Figure 4, Line A), one can obtain what is referred to as a β-lactam. So in some ways, choice "E" can be regarded as nothing more than a cyclic amide. And what might the students be expected to recall about the chemistry of amides, or even more generally, of nitrogens? Consider first an aliphatic amine (Figure 4, Line B) which by virtue of its lone pair of electrons, is basic and wants to accept a proton. Such systems are generally protonated at physiological pH and their behavior in vivo will often reflect their protonated ammonium character. Next consider an aromatic amine (Line C) whose electrons can now become involved with three canonical forms associated with aromatic ring resonance (the double-headed arrow leading to just one of these possibilities). Because of this, these electrons are less free to pursue a proton and such systems are typically much less basic than the aliphatic amines. The aromatic amines, therefore, can generally be regarded to be unprotonated in the body (note that in this case the direction of the equilibrium's major arrow at pH 7.4 is pointed to the left and away from the

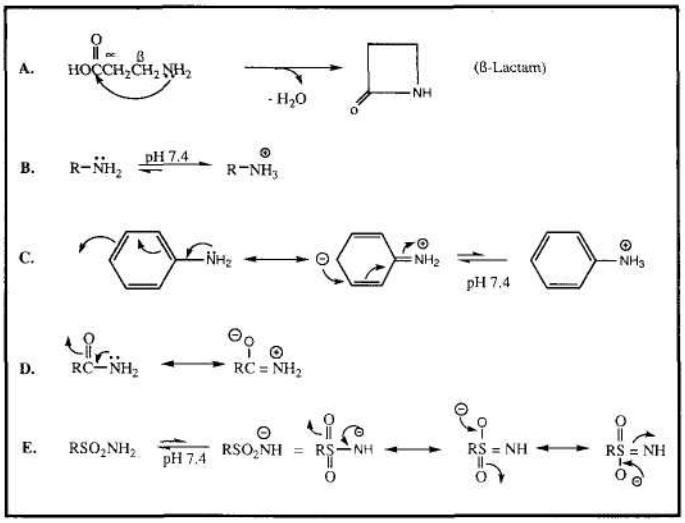

Fig. 4. Properties of some nitrogen containing systems.

protonated form). Which brings the students to an amide. Similar to an aromatic amine, the nitrogen's electrons are in resonance (Line D), this time with a carbonyl moiety. But also note that in this case the relevant canonical form happens to place a negative charge on an oxygen atom rather than, as in the case of benzene, on a ring carbon atom. And since an oxygen atom is a more electronegative element than carbon, this is a preferred arrangement (of lower energy) and this particular canonical form makes a more significant contribution to the overall resonance hybrid. Thus, the electron pair in the case of amides is even less available to act like a base. Although amides can become involved in key hydrogen bonding schemes and can undergo a variety of other significant chemical and biochemical reactions, for the present purpose the amides can essentially be considered to be inert as bases under physiologic conditions.

Interestingly, this change in a nitrogen's chemical personality can be even more dramatic. For example, during later discussions about sulfonamides, it is shown that this special type of nitrogen can become weakly acidic. This completely different chemical personality comes about because in this special case the anion that can result after removal of a proton can now be stabilized (Line E) between two desirable (low energy) canonical forms. Indeed, it will be shown that when the sulfonamide behaves like an acid, it becomes a key haptophore type element similar to that of the carboxylic acid group in ampicillin, although the sulfonamides interact with a completely different biochemical pathway within bacteria.

Nevertheless, despite this rather timid behavior exhibited by the nitrogen in terms of basicity, the β-lactam, which is present in all penicillins, is not at all inert or innocuous! Indeed, the β -lactam makes its own and very distinct contribution toward the behavior of the penicillins.

LOADED SPRINGS, WEAK LINKS AND TOXOPHILES

To appreciate this, the students are next asked to consider the β-lactam's cyclic nature. They are challenged to recall that a tetrahedral carbon atom prefers to have bond angles of about 109 degrees and that when these atoms are part of five and six membered rings, these bond angles are able to be maintained such that the systems can remain at reasonable energy levels (Figure 5 Line A). However, in order to

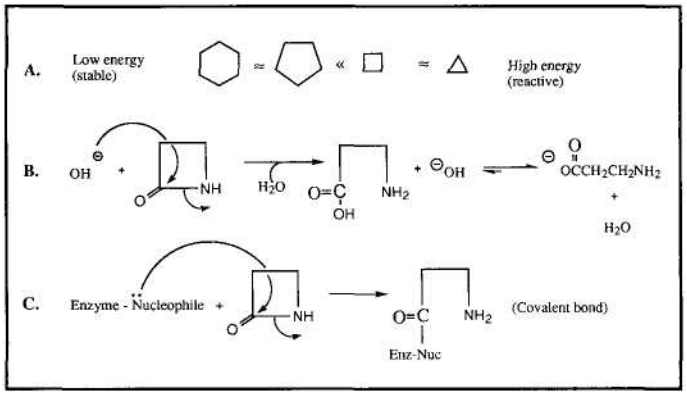

Fig. 5. Chemical reactivity of β-lactams.

form three and four membered rings, these bond angles must be constricted. This places a strain on such atoms and increases the relative energies of these smaller ring systems. In fact, the four-membered β-lactam, with its nearly 90° degree bond angles, is so strained that it can be thought of as sitting in an ampicillin molecule like a loaded spring waiting to be uncoiled. And within such a setting, the amide moiety can now be seen to take on a very special chemical role. For it is precisely the amide moiety that serves as the "weak link" which can be attacked chemically to allow the strained ring to be opened (Line B). Note that the reaction being depicted shows a slightly basic aqueous media serving as a nucleophile to attack the amide carbonyl group in a hydrolytic process that is essentially the reverse of the β-lactam forming reaction which was shown earlier (Figure 4). Indeed, it is this inherent strain which causes the decomposition of the penicillins in aqueous media to be such a problem. Because of this, solid materials must be carefully stored under dry conditions and aqueous formulations, when buffered in the optimal pH range 6 to 8, are only somewhat stable, generally for a matter of days even with refrigeration.

On the other hand, it is also this exact same chemical property which goes on to play a very distinct role in the mechanism that the penicillins ultimately invoke to disrupt bacterial cell walls. As previously indicated, a penicillin molecule is able to become entwined with bacterial transpeptidase enzyme. And while this is happening, the β-lactam, like a loaded spring, is ready to react and thereby uncoil with any unsuspecting nucleophile that may be present within the enzyme's pocket or active site (Line C). This reaction begins much like the simple hydrolysis reaction but instead leaves the penicillin now covalently bonded to the surface of the transpeptidase active site. And this results in a non-competitive type of enzyme inhibition which is difficult for the bacteria to overcome. Interestingly, this same type of behavior tends to dictate much of the chemistry of the penicillins which, simply stated, is thus driven by the β-lactam's perfectly understandable desire to relax and to enjoy a less stressful (strained) existence. In fact, in later lectures the students are shown how it is this same β-lactam chemistry which is responsible for both penicillin allergic responses and for the most prominent form of resistance that microorganisms can mount to thwart the presence of the penicillins. Finally, it can be noted that just like haptophore pertains to recognition, Ehrlich called reactive groups such as the β-lactam, "toxophiles" because they pertain to a specific interaction that becomes toxic to the microorganism.

THE BIOLOGICAL REALM IS ASYMMETRIC

Choice "D" prompts the students to recall that when a carbon atom is bonded to four non-identical groups, it is asymmetric. There are four of such carbon atoms within ampicillin. Three of these are within the central part of the molecule at positions 3,5 and 6, and the last is located on the side chain as denoted by an asterisk (Figure 1). In fact, it is shown later that the three centrally located asymmetric atoms, and always with the same depicted stereochemistry, are always present in all of the penicillins. This is a direct consequence of their common biosynthetic pathway.

Now since it is a combination of the north and east edges of ampicillin that, like the Trojan Horse, attempts to fool the transpeptidase into a friendly interaction, and since all living systems are composed of biomolecules which contain numerous asymmetric arrangements, specific three dimensional orientations of the key groups within drugs hoping to interact with these systems must also be appropriately matched in three dimensional space in order to be properly accepted. The specific stereochemistry portrayed at positions 3,5 and 6 within the penicillins meets this requirement because they precisely match the corresponding stereochemistry involved in the display of the analogous functionality present along the terminal portion of chain B (Figure 4). These particular asymmetric carbon atoms, then, are also part of the requisite components of the overall penicillin haptophore. Choice "C" is considered next.

HANGING TOGETHER AND NOT SO MUCH SCHIZOPHRENIC AS CHAMELEON-LIKE

Given the strain present within the β-lactam, it should not be surprising to appreciate that the penicillins are also subject to ring-opening reactions under acidic conditions, as well as when they are under basic conditions. And just like the propensity of basic hydrolysis to cause problems during formulation and storage, the propensity toward decomposition in acidic media causes major problems for the oral bioavailability of the penicillins. Simply stated, the early penicillins could not hang together while traversing the stomach and its pH of ~2 to 3.

Interestingly, the acid catalyzed decomposition is actually initiated by a nucleophilic attack involving the penicillin's own amide group (Figure 6, Line A). The entire scheme for this unique, intramolecular chemical process is elaborated later. For now, focus is placed just on this first step. Note that the flow of electrons produces a favorable canonical form which bears a negative charge on an oxygen atom and that this electron pair, just like an OH or a transpeptidase nucleophile, then attacks the weak link carbonyl moiety present within the strained β-lactam. Thus, if one wants to deter the acid decomposition pathway, one needs to place bulky, electron withdrawing groups such as an aminobenzyl group (choice "C") into position R. Both the size of this group and its impact upon the availability of the amide's electron pair can serve to attenuate the nucleophilic reaction. And exactly what type of electronic impact is desirable? As just stated, the availability of the amide's electron pair needs to be decreased because it is the initial movement of these electrons that becomes responsible for promoting the eventual nucleophilic reaction involving the carbonyl moiety. Therefore, placement of an electron withdrawing group at position R is desirable.

But then why does an electron rich aliphatic amine like the one present in choice "C work? After all, it was just

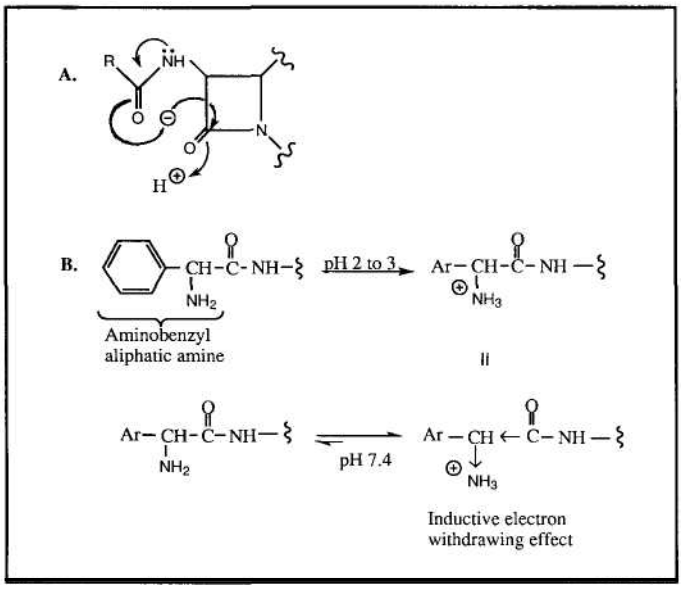

Fig. 6. Ampicillin's chemical behavior when exposed to acidic conditions.

shown that aliphatic amines are basic and, if anything, amines should be considered to be electron donating groups when attached to an aromatic ring (Figure 4, Line C). Now, while addressing this seeming paradox, the students can truly come to fully appreciate the chemically dynamic nature of an amine. Thus, the students are first led to recall what happens to an amine after it has played-out its role as a base and has already grabbed a proton. It's then a protonated ammonium species (Figure 6, Line B) that is no longer electron rich but instead bears a full-blown positive charge. And like a chameleon, in an acidic environment an amine changes to one of the most powerful electron withdrawing groups that is readily available. Indeed, exploiting this dynamic property can be especially clever because when the aminobenzyl traverses the stomach it exists almost exclusively as the protonated and powerfully electron withdrawing form which serves to effectively circumvent the acid promoted decomposition that otherwise precludes oral bioavailability. Then, once absorbed into the body and at pH 7.4, the aminobenzyl group reverts back to a more evenly distributed equilibrium that allows for a somewhat higher concentration of its free or unprotonated form to pass through membrane barriers on route to the penicillin's eventual site of action. And this does work! Remarkably, ampicillin and its closely related analogue amoxicillin are among the most stable of the oral penicillins. The model question is finally finished by moving on to the last, incorrect choice which is "B. Amide" (Figure 1).

PEPTIDE BACKBONES VERSUS MOLECULAR SCAFFOLDS

Besides the role that the amide group can be found to be playing during acidic decomposition, and in addition to the clever way that medicinal chemists upon fully appreciating the intramolecular nature of this mechanism, have manipulated its electronic character to afford good oral bioavailability, the amide group is important because, like the carboxylate anion and the specific stereochemical features mentioned earlier, it is also a key component of the haptophore within the penicillins. In the overall architecture of the penicillin's structure, the amide is part of a molecular scaffold system which mimics a corresponding key portion of the peptide backbone near the terminal end of sequence B (Figure 3).

CONCLUSION

Having thus accomplished a reasonable chemical review of several organic functional groups within the context of a molecule that is particularly relevant to this course, and having adequately demonstrated what is meant by the dynamic nature of chemical structures, the remainder of this first lecture is devoted to delineating a working definition for the phrase "chemotherapeutic agent" and toward describing, also by way of example, the key terms incorporated into this definition. A second lecture then completes the overall introduction to MBC 432 by providing past, present and future analyses about selective toxicity wherein magic bullets can be seen to be transcending into smart bombs as our continuing war against microbes actually appears to be heating-up with, among other things, the current trend in global warming.

Acknowledgement. This article is dedicated with sincere appreciation to the University of Toledo College of Pharmacy Fifth-Year Class of 1995 who responded to my enthusiasm for medicinal-related chemical structures by presenting me with their Outstanding Faculty Member award.

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APPENDIX A. RECENT COURSE SYLLABUS

MBC 432. Medicinal and Biological Chemistry V. Chemotherapeutics Agents

Winter Quarter 1997	Instructor:	Dr. Paul Erhardt
Time -10:00-11:50 AM	Office	UH 4670 (CD3)
Snyder Memorial 211	Hours:	R 10:00-Noon

Course Objectives: 1. To provide medicinal chemistry perspectives about anti-infective and antiviral agents selected to exemplify the early and continuing development of these fields; 2. To provide an introduction to the anticancer field and to examine the chemical aspects of some of its related chemotherapeutic agents.

Lecture	Date	Topic
1,2	Jan 7	Introduction, Selective Toxicity
3,4	Jan l4	Sulfonamides and Trimethoprim
5-8	Jan 21, 28	Penicillins & Cephalosporins; Protein Synthesis Inhibitors
____	Feb 4	Midterm Exam (Lectures 1-8)
9, 10	Feb 11	Agents affecting Membrane Permeability; Antitubercular, Antiprotozoal & Antifungal Agents
11-14	Feb 18, 25	Antiviral Agents
15, 16	Mar 4	Cancer Chemotherapy (Pharmacy Practice Faculty)
17, 18	Mar 11	Cancer Chemotherapy
____	Mar 20	Final Exam (Cumulative)

Reading: No formal textbook required. Supplementary reading materials are available on request