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Evaluation of a Simple Carrier Molecule to Enhance Drug Penetration of Dermal Layers by Utilizing Multivariate Methods, Structure Property Correlations, and Continuous System Modeling

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Abstract: Nicotinic acid is shown to be comparable to dihydropyridine in its capacity to facilitate penetration of an attached antibacterial drug through dermal layers. Antibacterial drugs examined with nicotinic acid or dihydropyridine carriers were β-lactam antibiotics: methicillin, oxacillin, benzylpenicillin, penicillin F, penicillin dihydro F, propicillin, carbenicillin, penicillin K, penicillin X, and ampicillin. An oxymethyl (-O-CH2-) group is inserted as the linker between the antibiotic and the carrier group. Structure Property Correlations and multivariate methods such as regression analysis, cluster analysis, principal component analysis, discriminate analysis, self-organizing tree algorithm, and factor analysis clearly showed that nicotinic acid performs as an effective carrier drug and is comparable to dihydropyridine. The skin penetration constant Kp was calculated for all 10 antibiotics having either dihydropyridine or nicotinic acid as carrier, and was found to have a mean of 5.13E-05 cm/hour and 1.83E-05 cm/hour, respectively. The standard deviation for each group showed the numerical values overlap as did the 90% confidence interval for each group. A hierarchical tree organization of skin shows the overlapped dermal layers as they exist in normal skin and for the model utilized in this work. A Deming-Regression analysis also shows the nicotinic acid and dihydropyridine structures to have similar and correlated water solubility. Plotting Kp of the dihydropyridine structures as independent variable versus Kp of the nicotinic acid structures show good correlation (Pearson correlation r = 0.6606) and no significant departure from linearity. Connected box plots showed the majority of Kp values for each group of modified antibiotics to exist in a tight cluster. Polar graph of the Log Kow values showed the two groups of modified antibiotics to be correlated and numerically adjacent in trend. ChemSketch property calculations and modeling demonstrates the affects of structural oxygens, nitrogens, carbonyl groups, amide groups, and aromatic rings that are important in understanding the pervasiveness through dermal layers. Continuous model analysis by acslXtreme is utilized and demonstrates three models of the dispersion of drugs through dermal layers based on diffusivity constant (D), Log Kp from Log Kow and formula weight, and Kp as a function of time.

PENICILLIN ANTIBIOTICS are bactericidal and interfere with the formation of the bacterial cell wall. Penicillins diffuse well into the body tissues and fluids. Penicillins are also referred to as the β-lactam antibiotics, a term which indicates the active amide ring that nullifies the membrane closing activity of transpeptidase enzyme. Ampicillin, one of the most widely used of the β-lactam antibiotics, is utilized as a topical drug but limited by resistant *Staphylococcus aureus*. Important classes of the β-lactam group are the penicillins, cephalosporins, monobactams, and carbapenems.

Bacteria which are important for skin and soft tissue considerations are the *Staphylococcus (S.) aureus, S. pyogenes, S. epidermidis,* and *Pasteurella*. Strains of the genus Pasteurella (gram-negative, coccobacilli) are susceptible to penicillin as well as tetracycline. Staphylococci are gram positive spherical bacteria that are found primarily on the skin or the mucous membranes for which methicillin or vancomycin are effective treatments. Isolates of *Staphylococcus aureus* can still be inhibited by penicillin, however resistance to this antibiotic is a significant problem in health facilities. Staphylococcus are opportunistic pathogens, not significantly invasive unless given the opportunity by skin trauma, lacerations, macerations, etc. *S. aureus* causes localized skin infections. *S. epidermidis* resides primarily in the skin and can cause blood infections if an invasive trauma provides the opportunity. The Staphylococci can adhere to epithelial cells causing problems of multiplication and spread.

To treat the infection threats described above steps have been taken to improve the delivery of antibiotics into skin tissue. Particularly the application of dihydropyridine attached β-lactam antibiotics to improve delivery through biological membranes (1, 2, 3). Induction of skin blisters have been utilized to understand the process of skin absorption of antibiotics (4). The penetration of skin by antibiotics has been shown to be inhibited by other medicinal agents such as hydrocortisone (5). Utilizing tissue cages has shown useful in determining the extent of antibiotic skin penetration (6).

The β-lactam antibiotics are highly effective against most bacteria that cause skin and soft tissue infections (7) and are considered to have good tissue penetration and long duration of activity (7) (with the low molecular weight members being optimal). Previous studies have shown that electrophoresis and phonophoresis can improve tissue penetration of penicillin and streptomycin (8, 9). Use of 6-ketocholestanol has been shown to enhance transdermal delivery of antibiotic (10).

This work shows that nicotinic acid is an effective carrier vehicle which is comparable to dihydropyridine for enhancing skin penetration by β-lactam antibiotics. Ten antibiotics that are currently used in clinical applications were selected for evaluating this mode of enhanced penetration. Each having an attached dihydropyridine or nicotinic acid molecule at the carboxyl group of the antibiotic (total of 20). Use of nicotinic acid as a carrier vehicle will benefit the clinical treatment of skin infections.

Materials and Methods

Numerical Analysis Methods

Multivariate methods such as regression analysis, cluster analysis, principal component analysis, discriminate analysis, self-organizing tree algorithm, and factor analysis were applied to molecular properties of the antibiotic structures having nicotinic acid or dihy-

dropyridine substituents. Dendrograms were utilized to portray distances obtained from cluster analysis. Descriptive statistics were applied to demonstrate numerical relationships of molecular properties for all compounds.

Software and Algorithms

Molecular properties were calculated by utilizing ChemSketch (Advanced Chemistry Development, Toronto, Canada M5H 3V9), Molinspiration Cheminformatics (SK-84104 Bratislava, Slovak Republic), and Syracuse Research Corp. (North Syracuse, New York 13212). Values of Log Kow, rate of ester hydrolysis, water solubility, and dermal penetration rate were evaluated by EPISUITE (US Environmental Protection Agency, Washington, DC USA). Self organizing tree algorithm (SOTA) was accomplished by GEPAS Bioinformatics (http://gepas.bioinfo.cnio.es/cgi-bin/sotarray). Hierarchical tree organization of skin structure was accomplished by Treepad (Freebyte, Interland Copyright © 1995 – 2003) and FLOW (Version 4.00.074 IMSI and COREL Corp. Copyright © 1996 – 1998).

Continuous Model System

The acslXtreme software was utilized to evaluate dermal penetration by a continuous model system and determine skin absorption parameters (Aegis Technologies Group, 631 Discovery Drive, Huntsville, AL 35806).

Descriptors of Dermal Absorption

Skin Permeability Coefficient, Kp (cm/hour):
Log Kp = −2.72 + 0.71LogKow − 0.0061(Formula Weight)

Skin Diffusivity Constant, D (cm^2/hour):
Log D = −2.72 − 0.0061(Formula Weight) + Log(L),
where, L = length of stratum corneum (0.004 cm)
Lag Time, τ (hour):
τ = (L)^2/6(D), see L and D above.

Drug penetration (cm) as function of Time (hours) and Kp (see above):
Penetration depth = Kp(Time)

Permeation Coefficient of Protein Layer, Kpol
Kpol = 0.0002976/(Formula Weight)$^{0.7}$

Permeation Coefficient of Aqueous Layer, Kaq
Kaq = 4.209/(Formula Weight)$^{0.7}$

Results and Discussion

The skin is a highly organized, multilayered, and heterogeneous organ. The skin produces factors that regulate growth, differentiation, and mediators of inflammation and immune response. The skin is made up of three distinct layers: 1) Epidermis (keratinized surface

layers), 2) Dermis (fibro-elastic connective tissue), and 3) Hypodermis (loose connective tissue). The dermis contains blood vessels which serve as an entrance portal to the body for drugs administered as topical agents. General functions of the skin can be described as: 1) Protective, 2) Inhibitory of microbial activity, 3) Regulation of temperature, and 4) Repressing of U.V. light damage. For considerations of skin modeling and skin permeation, it is taken to consist of three compartments: 1) Protein layer of the stratum corneum, 2) Lipid layer of the stratum corneum, and 3) Aqueous layer below the stratum corneum (drugs applied topically must permeate the aqueous layer to enter the bloodstream). These factors of skin constitution and activity are considered here in the comparison of antibiotics having either a dihydropyridine or nicotinic acid substituent. The stratum corneum is the rate-limiting diffusion barrier for most compounds and will be considered in this study.

Dihydropyridine has been used as a substituent of different classes of drugs to facilitate the penetration of membrane tissue such as the blood-brain-barrier. It has been utilized previously to enhance the membrane penetration of β-lactam antibiotics for targeting the central nervous system (11, 12, 3). The synthesis of the dihydropyridine variants of these antibiotics is straight forward and would be accomplished in the same manner for all antibiotic nicotinic acid variants. Briefly, all of the β-lactam antibiotics studied have a carboxylic acid group which is converted to a potassium salt by mild base. Then chloromethanechlorosulfate is introduced which produces a chloromethyl ester group (ie., $-C(O)OCH_2Cl$). Upon introduction of nicotinic acid or dihydropyridine, the formation of the final daughter compound having the desired substituent is energetically favored and is readily obtained (see Figure 1 for example final structures). It has been shown in previous studies that nicotinic acid functioning as a carrier drug will enhance the penetration of antineoplastic alkylating nitrogen mustard groups through the blood-brain-barrier (13).

Figure 1 shows a methicillin nicotinic acid and methicillin dihydropyridine products of the reaction of chloromethanechlorosulfate with a β-lactam antibiotic, followed by the desired substituent. Notice the β-lactam ring is intact and a methoxy (-O-CH2-) linker is in place between the antibiotic proper and the subtituent (see dotted rectangle). The SMILES notation (www.daylight.com) for each compound is given. The nicotinic acid and dihydropyridine constitute the "carrier" portion of these antibiotic variants which enhances their penetration through body membranes.

Organization of dermal layers comprising the skin is presented in Figure 2 as a hierarchical tree organization. The layers which are significant in their influence on drugs applied topically are shown. On the left is the tree organization with the important layers designated as nodes of that tree. To the top of the left-hand tree is the initial administered drug, followed by the epidermis which is in turn comprised by the protein layer, lipid layer, and aqueous layer in sequence as the drug penetrates the skin in depth. The drug must pass the aqueous layer prior to reaching the dermis, which has the blood capillaries which provide passage of the drug to other parts of the body. The hypodermis being a major layer also. The right-hand tree shows the important layers of the epidermis more clearly distinct from the underlying dermis and hypodermis.

Molecular properties for both the nicotinic acid antibiotic variants and the dihydropyrilidine antibiotic variants are presented in Table I. Note that the antibiotic forms and their properties are designated by columns and placed in the same vertical columns to facilitate comparisons of values. The molecular properties considered are as follows from left to
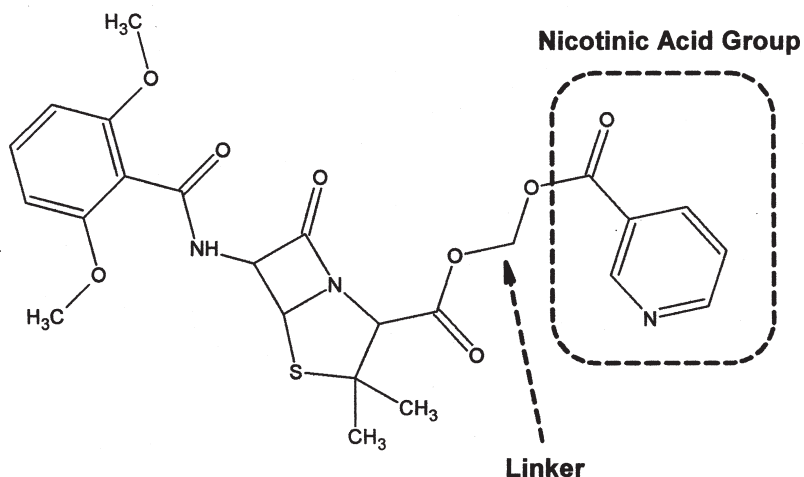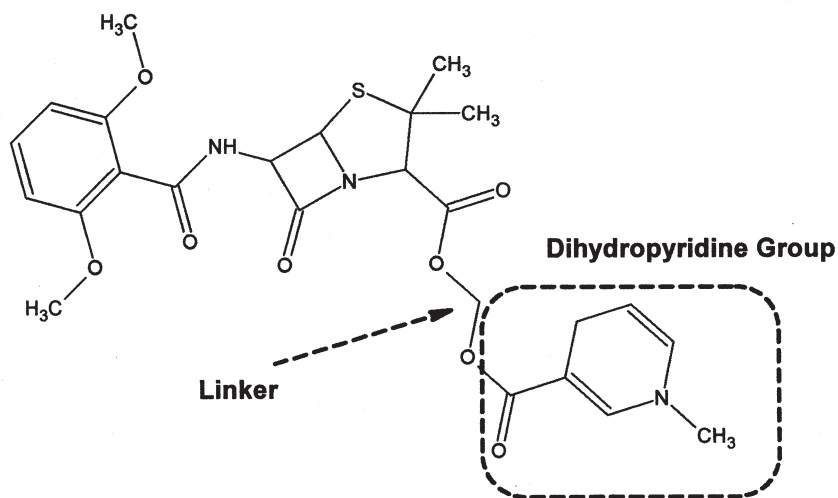
Methicillin-Nicotinic Acid

O=C(OCOC(C3C(SC2C(NC(c1c(cccc1OC)OC)=O)C(N23)=O)(C)C)=O)c4cccnc4

Methicillin-Dihydropyridine

CN/4/C=C(/C(=O)OCOC(C3C(SC2C(NC(c1c(cccc1OC)OC)=O)C(N23)=O)(C)C)=O)C\C=C\4

FIGURE 1. Examples of the molecular structures for each group of antibiotic derivatives of nicotinic acid or dihydropyridine utilizing methicillin as the parent compound. SMILES notation is given for each structure. Note the (-O-CH2-) linker connecting the antibiotic to the carrier group.

- Drug on Skin
  - Epidermis
    - Protein Layer
    - Lipid Layer
    - Aqueous Layer
  - Dermis (blood capillaries)
  - Hypodermis

EPIDERMIS

PROTEIN

LIPID

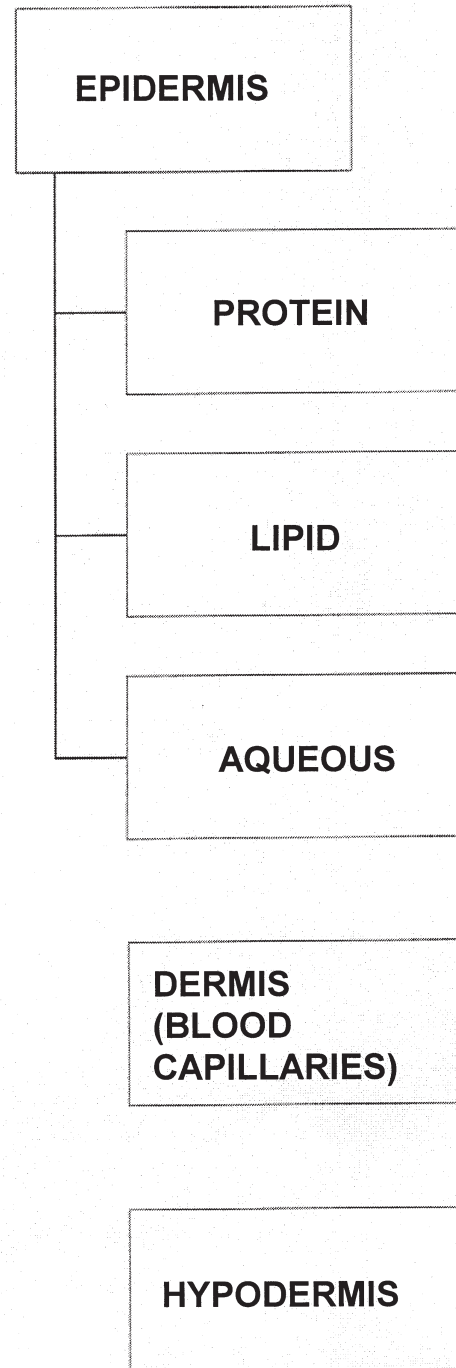AQUEOUS

DERMIS (BLOOD CAPILLARIES)

HYPODERMIS

FIGURE 2. Showing the hierarchical tree organization of normal skin, any topically applied drug will pass through the protein, lipid, and aqueous layers of the epidermis prior to reaching the blood capillaries of the dermis layer.

TABLE I

	FW	Molar Refractivity	Molar Volume	Parachor	Index of Refraction	Polariza-bility	nOnN	nNHOH	miLogP	TPSA
				Nicotinic Acid Structures of Antibiotics						
Methicillin	515.54	128.28	357.7	1030.5	1.636	50.85	11	1	2.563	133.28
oxacillin	538.57	138.11	358.7	999.6	1.696	54.75	11	1	3.232	136.51
benzylpenicillin	469.51	120.18	330.6	953.4	1.647	47.64	9	1	2.801	114.91
Penicillin F	447.51	113.9	329.9	927.4	1.606	45.15	9	1	2.88	114.91
Dihydro F	449.52	113.99	336.6	939.8	1.592	45.19	9	1	3.12	114.91
Propicillin	513.56	131.16	389.4	1051.8	1.628	51.89	10	1	3.629	124.143
Carbenicillin	513.52	126.44	341.3	1013.6	1.662	50.12	11	2	1.734	152.21
Penicillin K	477.58	123.25	368.8	1019.9	1.582	48.86	9	1	3.988	114.91
Penicillin X	485.51	121.71	327.4	968.8	1.665	48.25	10	2	2.417	135.137
Ampicillin	484.53	123.78	334.7	981.1	1.661	49.07	10	3	1.12	140.93
				Dihydropyridine Structures of Antibiotics						
Methicillin	531.58	134.98	377	1069.9	1.634	53.51	11	1	2.339	123.723
oxacillin	554.62	143.43	379.9	1045.1	1.679	56.86	11	1	3.008	126.853
benzylpenicillin	485.55	126.88	349.9	992.7	1.645	50.3	9	1	2.577	105.26
Penicillin F	463.55	120.6	349.2	966.8	1.607	47.81	9	1	2.436	105.26
Dihydro F	465.56	120.69	355.9	979.2	1.593	47.84	9	1	2.676	105.28
Propicillin	529.61	137.86	388.8	1091.2	1.627	54.65	10	1	3.405	114.49
Carbenicillin	529.56	133.14	360.6	1052.9	1.66	52.78	11	2	1.51	142.55
Penicillin K	493.62	129.96	388.1	1059.3	1.584	51.52	9	1	3.544	105.28
Penicillin X	501.55	128.41	346.7	1007.9	1.662	50.9	10	2	2.194	125.48
Ampicillin	500.57	130.49	354	1020.4	1.658	51.73	9	3	0.896	131.28

right in Table I: formula weight, molar refractivity, molar volume, parachor, index of refraction, polarizability, nOnN (number of oxygens and number of nitrogens), nNHnOH (number of -NH and number of -OH), Log P by Molinspiration (miLog P), and polar surface area by Molinpiration (TPSA). Polar surface area has been shown to be an accurate indicator of absorption of a drug through the intestinal tract (14). All of the antibiotic variants shown in Table I (all of dihydropyridine and nicotinic acid forms) have TPSA values ranging from about 105 A^2 to about 142 A^2, which indicate levels of intestinal absorption of 10% to 30%, respectively (14).

The skin permeability coefficient Kp is an important property and can be utilized to approximate the distance into the skin a drug will travel as a function of time. The values of Kp for all antibiotics with nicotinic acid or dihydropyridine as carrier is given in Table II as calculated by EPISUITE. The average value of Kp for each group are comparable, being 5.13E-05 cm/hr and 1.83E-05 cm/hr for dihydropyridine and nicotinic acid structures, respectively. Frequency analysis of the Kp values for the dihydropyridine structures reveal 90% confidence interval of 1.47E-05 cm/hr to 8.79E-05 cm/hr (outliers will be >0.0000795 from the median of 2.46E-05 cm/hr), skewness of 1.794, and kurtoses of 2.388. Frequency analysis of the Kp values for the nicotinic acid structures reveal 90% confidence interval of 5.70E-06 cm/hr to 3.09E-05 cm/hr (an overlap of the same range for the dihydropyridine structures), having skewness of 2.430, and kurtoses of 6.454 (outliers will be >0.0000301 from the median of 1.09E-05 cm/hr). Water solubilities for all antibiotics of nicotinic acid and dihydropyridine structure is shown in Table II in mg/mL. Although values vary widely within each group of compounds the numerical values themselves are comparable as a group. Note that each group has only a single outlier as a function of water solubility, that compound being benzylpenicillin for each group. The Grubb's Test for detecting outliers is also called the ESD method or extreme studentized deviate, and indicates that a value is unlikely to come from the same Gaussian population. Z values are calculated and a high Z value indicates that data is far from the other members of the group. Consequently, in terms of the two important properties Kp and water solubility the nicotinic acid group of structures are clearly similar to the dihydropyridine structures of antibiotics.

Analysis of molecular properties for these two groups of compounds by self organizing tree algorithm (SOTA) clearly showed mutual similarities and interrelationships. The results of SOTA analysis is in the form of cluster output which can also be defined in terms of proximity and distance associations (ie., single linkage and euclidean distance). SOTA (15) analysis was applied to seven molecular properties of these two groups in the following order: formula weight, molar refractivity, molar volume, parachor, index of refraction, polarizability, and number of oxygens and nitrogens. For ten compounds in each group that gives a total of 140 data points analyzed by SOTA. Parameters of the SOTA analysis were single linkage and standard euclidean distances. The results consisted of two clusters having members of both groups interwoven as follows: CLUSTER 1: Nicotinic acid structures of methicillin, propicillin, penicilliln K with dihydropyridine structures of methicillin, oxacillin, propicillin, carbenicillin, penicillin K, and ampicillin. CLUSTER 2: Nicotinic acid structures of oxacillin, benzylpenicilliln, penicillin F, dihydropenicillin F, carbenicillin, penicillin X, ampicillin with dihydropyridine structures of benzylpenicilliln, penicillin F, dihydropenicillin F, and penicillin X. Interrelationships and similarities are clearly shown when members of either the nicotinic acid structures or dihydropyridine structures are placed in identical clusters.

TABLE II

Kp (cm/hour)

Kp (cm/hour)

Nicotinic Acid Structures	Parent Antibiotic	Dihydropyridine Structures
1.05E-05	Methicillin,	1.87E-05
4.88E-06	Oxacillin	0.000195
1.12E-05	Benzylpenicillin	2E-05
1.63E-05	Penicillin F	2.92E-05
2.25E-05	Dihydro F	4.03E-05
2.76E-05	Propicillin	4.92E-05
5.38E-06	Carbenicillin	9.63E-06
7.57E-05	Penicillin K	0.000135
4.08E-06	Penicillin X	7.29E-06
4.75E-06	Ampicillin	8.5E-06

Analysis of Water Solubility (mg/mL) Values by Grubb's Test

Row	Nicotinic Acid Structures			Dihydropyridine Structures		
	Value	Z	Significant Outlier	Value	Z	Significant Outlier
1	0.0347900	0.329043437		0.11640	0.3457398	
2	0.0048770	0.551575770		0.01632	0.5703164	
3	0.449600	2.756860260	Significant outlier. P < 0.05	1.50200	2.7635062	Significant outlier. P < 0.05
4	0.0045250	0.554194410		0.01511	0.5730316	
5	0.0471200	0.237316640		0.15700	0.2546346	
6	0.0745700	0.033107348		0.25670	0.0309107	
7	0.0004212	0.584723886		0.05918	0.4741398	
8	0.0582200	0.154740205		0.19430	0.1709345	
9	0.0146800	0.478648131		0.04914	0.4966693	

Further evidence of similarity between nicotinic acid and dihydropyridine structures is elucidated in Figure 3. The skin permeability coefficient Kp values (see Table II) for dihydropyridine compounds is plotted (x-axis) versus the corresponding Kp values of their counter part nicotinic acid structures (y-axis) with the 95% confidence interval (see inset arrows) in the top plot (see Figure 3). All values fall clearly within the 95% confidence interval except for penicillin K (see oval indicating this data point). However, a line drawn from the linear data values near the origin clearly includes the penicillin K data point (see broken line crossing the oval point value) and leaves out the data point encased in the inset rectangle (oxacillin).

A Deming Regression Plot shows linear relationships of data values with the assumptions that the values of the independent variable (x-axis) have error. A Deming regression analysis for the water solubility values (these being important because the drug must cross the aqueous layer of the epidermis) shown in Table II is presented as the middle plot in Figure 3. The formula weight of all nicotinic acid and dihydropyridine structures are plotted as the independent variable (x-axis) versus the water solubility (mg/mL) as the dependent variable (y-axis). Note that all 20 compounds do fall within a linear fashion according to the least squares line (see Figure 3) and only one compound (the benzyl-penicillin nicotinic acid, see inset arrow) is radically an outlier from the otherwise linear body of the data. Therefore, the Deming regression analysis clearly demonstrates the similarity of all water solubility values for all nicotinic acid and dihydropyridine structures (high correlation of water solubility to formula weight).

A polar graph is utilized to demonstrate similarities between data values or groups of data values. A polar graph is shown (lower left, Figure 3) having Log Kow values for the dihydropyridine structures indicated by (X) and nicotinic acid structures indicated by triangles ($\triangle$) . Log Kow values indicate the dispersion of the drug between an organic solvent layer and an aqueous layer while assuming no charge species of the drug exist. Log Kow is an indicator of the ability of a drug to penetrate lipid by-layers and hence cellular membranes. Values of Log Kow are an important influence on the pharmacokinetic and pharmacodynamic aspect of the drug. The polar graph shows the Log Kow values of the dihydropyridine structures are generally larger that those of the nicotinic acid structures but corresponding values are clearly in close proximity.

In the lower right hand corner of Figure 3, a connected box plot for Kp values of dihydropyridine structures (var 2) are shown compared to Kp values of the nicotinic acid structures (var 3). In the connected box plot the individual data points are plotted normalized to the average value for that specific group of Kp values. Values for var 2 (dihydropyridine structures) have no outliers and the majority of Kp values are tightly clustered around the mean value (see Connected Box Plot Figure 3, var 2). Values for var 3 (nicotinic acid structures) show one outlier (see var 3 furthest point from origin) which is penicillin K while the remaining members of the group are tightly clustered around the mean value (see Figure 3 Connected Box Plot, var 3).

The operation referred to as factor analysis encompasses both component analysis and common factor analysis. Principal component analysis (PCA) is considered simpler. The central concept behind PCA is representation and summarization. The action of PCA is to replace a large set of variables by a smaller set which best summarizes the larger set. The results of PCA on the Kp values of all dihydropyridine and nicotinic acid structures, which form PCA 1 (dihydropyridine structures) and PCA 2 (nicotinic acid structures) are
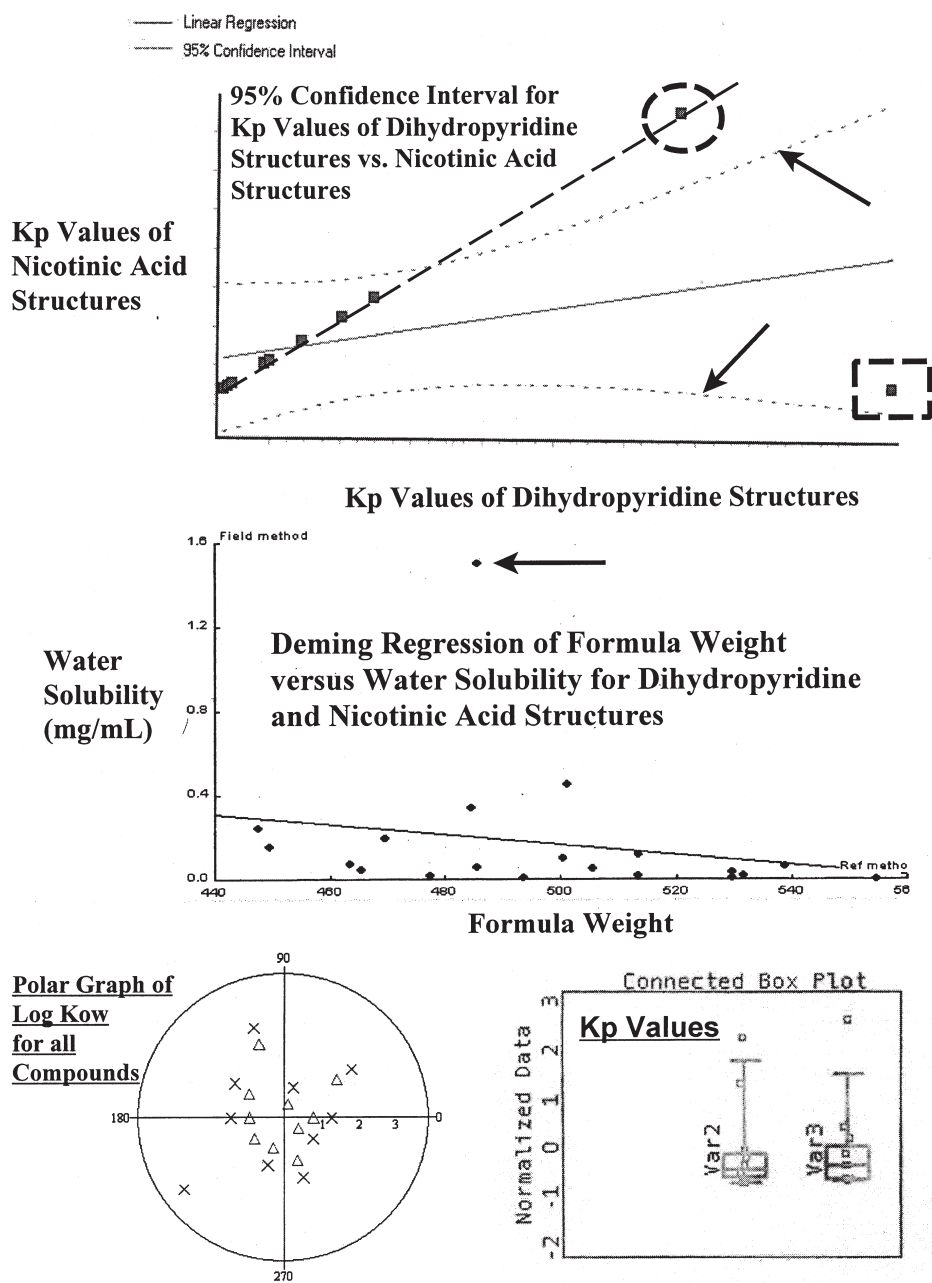
FIGURE 3. Comparison of important properties of the nicotinic acid group of antibiotics to the dihydropyridine group show similarities that support the potential for clinical application of the nicotinic acid derivatives. Top Graph: Linear regression plot of Kp values of all compounds show the majority of derivatives fall together within a 95% confidence interval (indicated by inset arrows) with only one outlier (inset circle). If a line is formed for linear members only, then again most compounds are inclusive with only one outlier (see rectangle). Middle Graph: Deming regression analysis of FW versus water solubility shows clearly the tight linearity of 19 derivatives with only one outlier (see inset arrow). Polar graph shows consistency and similar trend of Log Kow values for all 20 compounds (X= dihydropyridine structures, triangles (△)= nicotinic acid structures). Connected box plots show tight clustering of Kp values about the mean value for each group (var2= dihydropyridines, var3= nicotinic acids).

seen in Figure 4. A PCA 1 versus PCA 2 plot indicates that 80% of all 20 compounds are similar and fall within a tight cluster at about the origin of the graph (see inset oval). This cluster includes the nicotinic acid and dihydropyridine structures of methicillin, benzylpenicillin, penicillin F, dihydropenicillin F, propicillin, carbenicillin, penicillin X, and ampicillin. The outliers are oxacillin (see inset arrow) and penicillin K (see inset rectangle). Therefore, PCA shows that 80% of all 20 structures are similar.

Cluster analysis (CA) is a multivariate method that acts to organize data about variables in which homogeneous groups or clusters are formed. The clusters which are formed should have high internal homogeneity (members similar to each other) and highly external heterogeneity (members dissimilar to each other). CA can be applied to a wide variety of input data. The primary result of CA is the dendogram or tree diagram. In Figure 4 is seen a dendogram of CA on Kp values for all 20 agents. Note that in each case of the compound name found in the inset key it represents both the nicotinic acid and dihydropyridine structures (i.e., for 1) Methicillin, it means both the nicotinic acid and dihydropyridine form of methicillin). Using single linkage and standard euclidean the following clusters are formed (see dendogram of Figure 4): Cluster 1 and 3 (methicillin and benzylpenicillin); Cluster 4 (penicillin F); Cluster 7, 10, and 9 (carbenicillin, ampicillin, and penicillin K); Cluster 5 and 6 (dihydropenicillin F and propicillin); Cluster 2 (oxacillin); Cluster 8 (penicillin K). Therefore, CA indicates that 70% of all 20 structures will have similarities with other members of this group.

Factor analysis is utilized to study patterns of relationships among many dependent variables with the actual goal of discerning characteristics of the independent variables that affect them (the independent variables are not measured directly). Figure 5 shows the plot of FA 1 versus FA 2 after factor analysis of Kp values for all nicotinic acid and dihydropyridine structures. Parameters of factor analysis were maximum likelihood and Joereskog's Formula. Note that the majority (80%) of all 20 antibiotic structures examined here fall within an individual clustering and close proximity (thus indicating similarity). Similar compounds that fall within a super cluster include methicillin, benzylpenicillin, penicillin F, dihydropenicillin F, propicillin, carbenicillin, penicillin X, and ampicillin (this includes both the nicotinic acid and dihydropyridine structures of these antibiotics). Outliers not demonstrating similarity with the super cluster are oxacillin (see inset rectangle) and penicillin K (see inset arrow). Therefore it is found that factor analysis also shows that 80% of both forms of the ten types of antibiotics are similar.

Discriminant analysis (DA) is utilized to determine which variables can be shown to discriminate between two or more occurring groups. Computationally, DA is very similar to analysis of variance. DA can be used to determine which variables (descriptors) can best predict the differing attributes of a particular group. This may also be stated as an analysis to assess the relative importance of the independent variables in classifying the dependent variable and to infer meaning of any dimensions that distinguish groups. To accomplish DA on the antibiotic structures that are the focus of this study, three molecular properties of the compounds were selected (formula weight, molar refractivity, and molar volume) and their numerical values for the dihydropyridine structures placed in Group 1 with those for the nicotinic acid structures placed into Group 2. Discriminant analysis of Group 1 versus Group 2 produces output referred to as DA 1 and DA 2, respectively. The plot of DA 1 versus DA 2 is presented in Figure 5 in which both the DA 1 score (x-axis) and DA 2 score (y-axis) can differentiate three super clusters of these compounds. The

Principal Components Analysis (PCA)

PCA 1= Kp Values of Dihydropyridine Structures
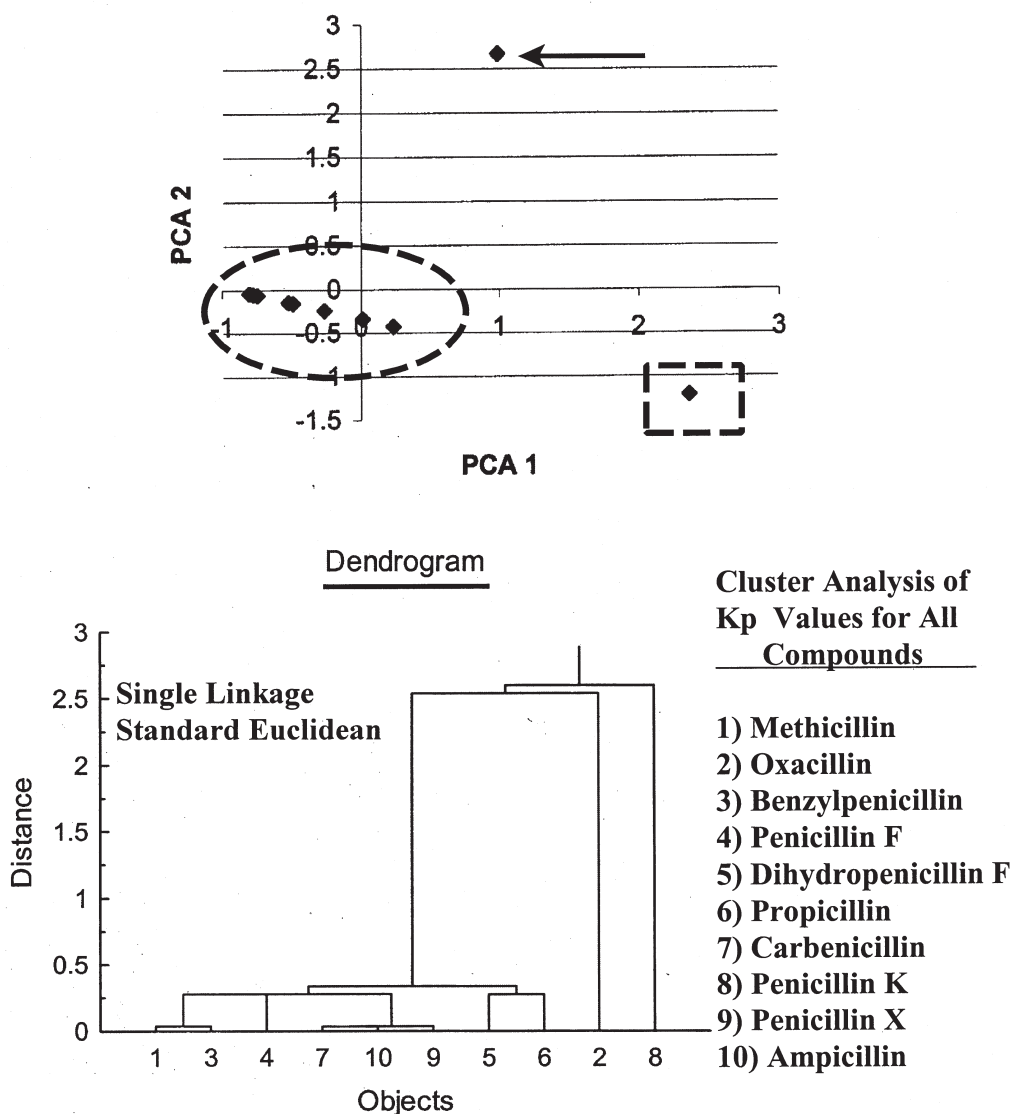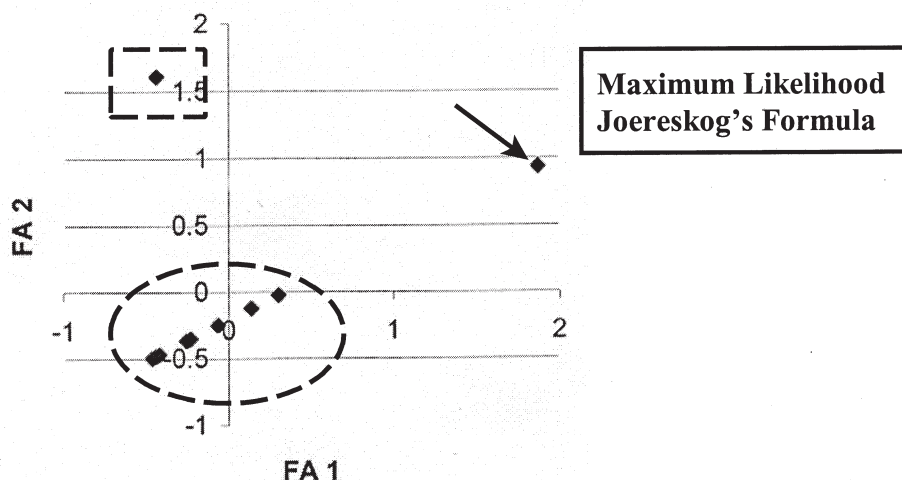## PCA 2= Kp Values of Nicotinic Acid Structures

Cluster Analysis of Kp Values for All Compounds

1) Methicillin
2) Oxacillin
3) Benzylpenicillin
4) Penicillin F
5) Dihydropenicillin F
6) Propicillin
7) Carbenicillin
8) Penicillin K
9) Penicillin X
10) Ampicillin

FIGURE 4. Principal component analysis of Kp values show tight clustering of 80% of all 20 compounds about the origin (see inset oval). Cluster analysis by single linkage and standard euclidean shows clusters of similar compounds: Cluster 1 and 3 (methicillin and benzylpenicillin); Cluster 4 (penicillin F); Cluster 7, 10, 9 (carbenicillin, ampicillin, and penicillin X); Cluster 5 and 6 (dihydropenicillin F and propicillin); Cluster 2 (oxacillin); Cluster 8 (penicillin K).

Factor Analysis of Kp Values

FA 1= Kp Values of Dihydropyridine Structures
FA 2= Kp Values of Nicotinic Acid Structures

Discriminant Analysis of Kp Values

DA 1= Dihydropyridine Structures (Formula Weight, Molar Refractivity, And Molar Volume)
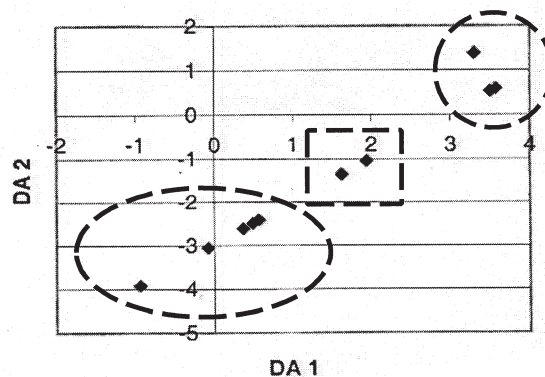## DA 2= Nicotinic Acid Structures (Formula Weight, Molar Refractivity, And Molar Volume)

FIGURE 5. Factor analysis shows the strong similarities of nicotinic acid structures with dihydropyridine structures. 80% of all 20 compounds are included within the super cluster about the origin (see inset oval). Discriminant analysis shows that three properties (formula weight, molar refractivity, and molar volume) may be used to show similarities and distinctions of dihydropyridine and nicotinic acid derivatives of antibiotics. Distinctions obtained by DA 1 are analogous to those obtained from DA 2 (both utilizing formula weight, molar refractivity, and molar volume).

super cluster encased within the inset circle includes oxacillin, penicillin K, and propicillin (i.e., these three compounds are similar). Super cluster encased within the inset square encompasses ampicillin and benzylpenicillin (i.e., these two compounds are similar). The super cluster encased within the inset oval contains the compounds methicillin, penicillin F, dihydropenicillin F, penicillin X, and carbenicillin (i.e., these five compounds are similar). Remember that each data point in the DA graph designates the effects from both the dihydropyridine and the nicotinic acid forms of the same compound. Therefore, it is shown that DA will incorporate all 20 compounds studied here within three super clusters that have distinction from each other. However, members within each group are thereby similar to each other.

AcslXtreme is program software which generates continuous numerical results from system models that incorporate mathematical algorithms designed within a continuous non-discrete framework. The operator designs a model with mathematical functions that can represent a real world paradigm. The output of such a model is a continuous numerical representation of the functions comprising the model. Three such models are presented in Figure 6, each representing some aspect of the application of the antibiotics studied in this work as topical agents. The first model represents the penetration of a drug in centimeters as a function of its Kp (skin permeability coefficient) and time in hours. The output is a 2-dimensional plot of hours (independent variable) versus distance (dependent variable) having equation of line as: $y = (2.597\text{E-04 cm/hour})x - (2.60\text{E-4 cm})$, where slope = 2.597E-04 cm/hour. The second model variance of Kp as a function of Log Kow and formula weight (see Materials and Methods). The output of such a model is linear having formula weight as the independent variable and Log Kow as the dependent variable. The equation of the line is: $y = (-0.7071 \text{ amu}^{-1})x - 2.036l$, where the slope = -0.7071 amu^{-1}. The third model represents the evaluation of diffusivity constant D as a function of formula weight of the drug and depth of stratum corneum (taken here to be 0.004 cm of normal skin). The output is a 2-dimensional plot having formula weight as the independent variable and values of Log D as the dependent variable. The equation of the line that formed is (x axis in hour, y axis in cm^2/hour): $y = (-0.00607 \text{ cm}^2/\text{hour}^2)x - 5.112 \text{ cm}^2/\text{hour}$, where slope = $-0.00607 \text{ cm}^2/\text{hour}^2$. These equations and the output of continuous modeling can be utilized to predict important parameters of the topical application of drugs. These models present the continuous mathematical expression of the parameters, however discrete values may be calculated from the equations for each of diffusivity constant D, Log D, Kaq, and Kpol from values of formula weight and stratum corneum depth in centimeters.

The "lag time" of a drug is time required for a topically applied drug to reach the state in which permeation can be described by Kp, Kpol, Fick's Law, and other functions of the drugs activity. Lag time is a function of skin diffusivity constant D, and length of stratum corneum (see Materials and Methods). Other important parameters for drug dispersion in skin are the Permeation Coefficient of Protein Layer, Kpol; and Permeation Coefficient of Aqueous Layer, Kaq. These relationships describe the movement of a drug through the protein layer and aqueous layer of the epidermis (see Figure 2). Numerical values of both are a function of formula weight of the drug. Therefore, as formula weight increases the value of Kpol decreases, as does the value of Kaq. The relationship with formula weight can be expressed as an equation of a line for Kpol as follows: $y = (-3.175\text{E-08})x + 1.455\text{E-05}$, $r = -0.9701$ and $r^2 = 0.9412$. Similarly, the line formed for Kaq becomes:

ACSLXTREME CONTINUOUS SYSTEM ANALYSIS FOR SKIN PENETRATION PARAMETERS

Penetration of Drug (cm) From Kp x Time(hours)
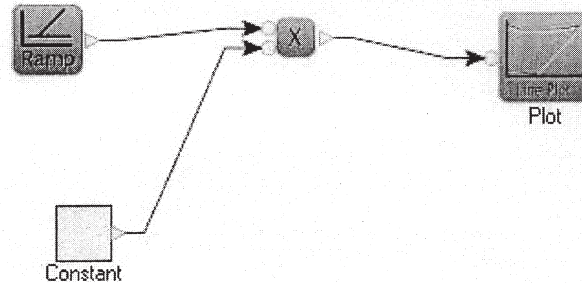
Variance of Kp Values

as a Function of Log Kow
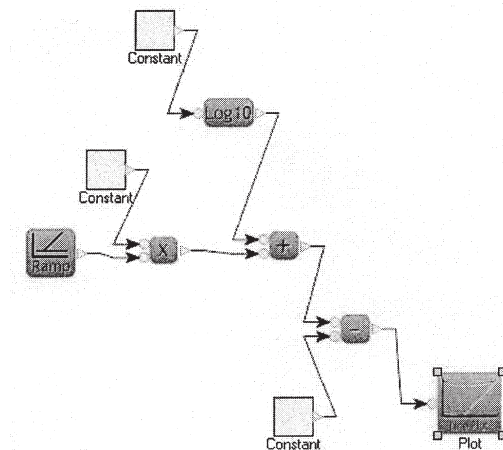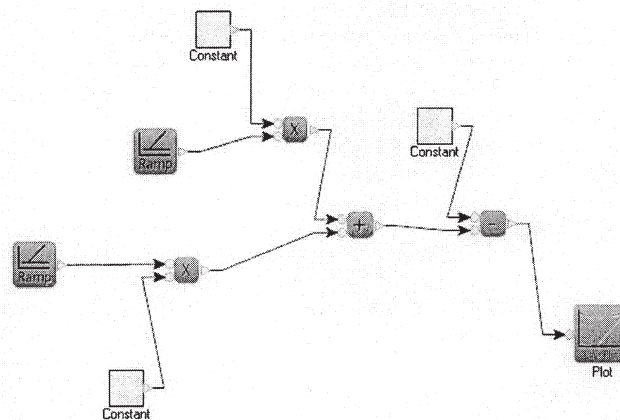
and Formula Weight

Variance of Log D Values

as a Function of Formula Weight

and Log(depth of stratum corneum)

> **Depth of stratum corneum assigned a value of 0.004 cm of normal skin.**

FIGURE 6. Continuous system analysis by acslExtreme can be utilized to obtain these three models of drug penetration, variance of Kp, and variance of Log D values as a function of time, Kp, Log Kow, formula weight, and depth of stratum corneum.

$y = (-4.495\text{E-}04)x + 0.2060$, $r = -0.9699$ and $r^2 = 0.9407$. Therefore, as these β-lactam antibiotics are converted to their respective nicotinic acid of dihydropyridine derivatives, the formula weight increases but the values of Kpol and Kaq decrease (i.e., movement of drug decreases).

In summation, it is shown that normal skin can be represented by a hierarchical tree diagram showing epidermis, dermis, and hypodermis. The carrier groups nicotinic acid and dihydropyridine are attached to the β-lactam antibiotic by a (-O-CH$_2$-) linker and affect Log P, water solubility, and other properties of these antibiotics. The Kp values for the nicotinic acid derivatives of antibiotics are competitive with those of the currently utilized dihydropyridine derivatives. The nicotinic acid derivatives can by synthesized in the same manner as the dihydropyridine derivatives, thus posing no obstacle for their manufacture and testing. Analysis of molecular properties by cluster analysis, principal component analysis, factor analysis, and discriminant analysis shows clearly that the antibiotic derivatives which have a nicotinic acid or dihydropyridine carrier group attached are similar to each other. Properties, such as formula weight, molar volume, molar refractivity, water solubility, for nicotinic acid derivatives are similar and overlap numerically (by standard deviation) with those of the dihydropyridine derivatives. Therefore, the nicotinic acid derivatives of β-lactam antibiotics are comparable and analogous to the current clinically applied dihydropyridine derivatives and have a high probability of effective and beneficial usage in similar clinical applications.

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