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Small Molecule Compounds Targeting the Bacterial Cell Wall for Bacterial Growth Inhibition

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Author's contribution

Author RB designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. The author read and approved the final manuscript.

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ABSTRACT

Aims: To determine the influence of molecular properties upon the effectiveness of four peptide compounds to inhibit growth of *Escherichia coli* and show the potential of small molecule peptide drugs.
Study Design: Examine molecular properties and extent of bacterial inhibition. Utilize numerical analysis to identify underlying relationships of molecular properties.
Place and Duration of Study: Department of Chemistry, University of Nebraska, Omaha, Nebraska, between January 2018 and April 2018.
Methodology: After studying the synthesis and evaluation of four compounds having amino acids substituent groups for their antibacterial activity, *in vitro*, the molecular properties were determined and analyzed by various methods of numerical analysis. The numerical methods included correlation, ANOVA, Grubb's test, path analysis, and multiple regression. Two-dimensional plots revealed relationships among trends in molecular properties and bacterial growth inhibition.
Results: Compounds 1 and 2 have –D-alanine-D-alanine substituent covalently bonded to the carbonyl carbons of aspirin and nicotinic acid, respectively. Compounds 3 and 4 have –glycine-D-alanine-D-alanine substituent bonded to the carbonyl carbon of aspirin and ibuprofen, respectively. Rule of 5 indicated that all four compounds have favorable drug-likeness (i.e. zero violations of Rule

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of 5). The bioactivity evaluation indicated compounds 1, 2, 3, and 4 fall within the drug-likeness and biological activity of ion channel modulator, kinase inhibitor, protease inhibitor, GPCR ligand, and enzyme inhibitor. All four compounds showed significant growth inhibition of *Escherichia coli*, *in vitro*. Path analysis indicated that Log P, number of oxygen and nitrogen atoms, and number of rotatable bonds have highest causal relationship to the growth inhibition of bacteria.
Conclusion: Values of bioactivity and Rule of 5 showed that all compounds have favorable drug-likeness. Peptide-type compounds show promise for application in the clinical treatment of bacterial infections. This study provides evidence for which molecular properties are most important for the level of growth inhibition observed.

Keywords: Antimicrobial; bactericidal; cell wall; peptide drugs.

ABBREVIATIONS

GPCR : G-protein-coupled receptors
IUPAC : International Union of Pure and Applied Chemistry
NSAID : Non-steroidal anti-inflammatory drug
PSA : Polar surface area; MW, molecular weight
MV : Molecular volume

1. INTRODUCTION

At the present time, the resistance of bacteria, viruses, parasites, and other disease causing organisms to antimicrobial drugs that are utilized for clinical treatment is a substantial threat to infectious disease management [1]. Antimicrobial resistance incurs burdensome health, economic, and economic costs [1]. The presence of drug resistant microbes is increasing in prevalence and involving many pathogens across different regional areas of the globe [1]. The development of bacterial resistance to antibacterial agents has already become a substantial problem in hospitals as well as communities [1]. The common cause of severe infections acquired in both health care facilities and communities is the drug resistant *Staphylococcus aureus* [1].

Resistance to beta-lactam antibiotics is on the rise among clinical isolates of gram-negative *bacilli* [2]. The beta-lactam antibiotics, which are an important group of medicinal compounds prescribed across the globe, have been made less effective by drug resistance microbes [2]. The beta-lactams antibiotics (penicillins, cephalosporins, monobactams, and carbapenems) interfere with the synthesis of bacterial cell wall by inhibiting the process of peptidoglycan polymerization (although vancomycin combines to the D-Ala-D-Ala, preventing cell wall synthesis) [3]. The combination of certain antibiotics can act synergistically and produce stronger effect than the sum of the effects of the individual drugs, or alternatively antagonistically if one inhibits another [3]. The antagonistic activity of multiple drug use is also a limiter of the efficacy of treatment [3].

It is thought that antibiotic resistance occurs primarily through three types of action: 1) Prevention of drug interaction with the target; 2) Efflux (removal) of the antibiotic from the cell; 3) Destruction/modification of the medicinal compound (i.e. hydrolysis, group transfer, redox reactions) [4,5]. Emergence and prevalence of bacteria resistant drugs is a considerable threat to effective clinical treatment of microbial infections. Novel strategies for identification of new antibacterial drugs are necessary to insure that clinical treatment remains effective [6]. As an example, studies have shown that combinational antibiotic therapies hold great potential for providing alternative treatment options [6]. This study presents the effectiveness and properties of four peptide compounds that have been shown to inhibit the growth of penicillin-resistant *Escherichia coli*. This study will examine the molecular properties making these compounds useful for the clinical treatment of bacterial infection.

2. METHODOLOGY

2.1 Properties and Molecular Modeling

Numerical values of molecular properties (i.e. Log P, polar surface are, molecular weight) for all four compounds were determined through heuristic calculation through Molinspiration Chemical Properties Service (Molinspiration Cheminformatics, Nova ulica 61, SK-900 26 Slovensky Grob, Slovak Republic). Identification of molecular structural components was

accomplished utilizing ACD ChemSketch Modeling v. 12.01 (Advanced Chemistry Development, 110 Yonge Street, Toronto Ontario, M5C 1T4 Canada, http://www.molinspiration.com/services/search.html). Determination of drug-likeness scores for GPCR ligands, ion channel modulators, kinase inhibitors, nuclear receptor ligands, protease inhibitors, and other enzyme targets was determined by Molinspiration Cheminformatics (http://www.molinspiration.com/cgi-bin/properties). Properties of Kow, dermal permeability coefficient, and water solubility were determined using EPISUITE (U.S. EPA version 1.66, Estimation Programs Interface Suite, Washington D.C., USA).

2.2 Statistical Analysis

Statistical analysis of numerical data including molecular properties of the compounds in this study including various descriptive statistics was accomplished by Microsoft EXCEL v. 14.0.6112.5000 (EXCEL Professional plus 2010). ANOVA analysis and Kruskal-Wallis test was accomplished utilizing PAST version 2.06 (copyright Oyvind Hammer, D.A.T. Harper, 2011). Multiple regression analysis of molecular properties was accomplished with Smith's Statistical Package version 2.5 (copyright Gary Smith, 1995 to 2001). Grubb's analysis for outliers was accomplished utilizing online GraphPad (http://www.graphpad.com/quickcalcs/)

2.3 Antibacterial Compounds

All four peptide compounds were prepared and evaluated *in vitro* according to the previous studies [7,8]. The formation of compounds 1 and 2 (both dipeptides), came from the parent compounds aspirin and nicotinic acid, respectively. The carbonyl carbon (-C(=O)-) of aspirin and nicotinic acid was activated by thionyl chloride, which was then followed by careful introduction of D-alanine in solution. This was followed by a second round of thionyl chloride activation of carbonyl carbon and adding D-alanine [7]. Similarly, for peptide compounds 3 and 4 the carbonyl carbon activation using thionyl chloride was followed by addition of glycine. This was then followed by two cycles of carbonyl carbon activation with thionyl chloride, then witth careful addition of D-alanine with covalent bonding [8].

3. RESULTS AND DISCUSSION

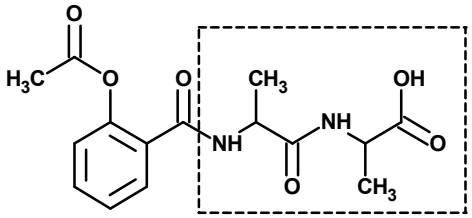
The four peptide drugs presented in this study have been shown to be effective in inhibiting growth of gram-negative bacteria *Escherichia coli* [7,8]. Compounds 1 and 2, utilizing aspirin and nicotinic acid as parent structure, have –D-alanine-D-alanine covalently bonded to the carbonyl carbon [7]. Compounds 3 and 4, utilizing aspirin and ibuprofen as parent structure, have –glycine-D-alanine-D-alanine covalently bonded to the carbonyl carbon [8].

It is known, that gram-negative bacteria (i.e. *Escherichia coli)* have thinner cell walls than that of gram-positive bacteria [9]. In addition, that cell wall is comprised of peptidoglycan layers bound by an outer membrane [9,10]. That peptidoglycan layer consists of two sugar derivatives (N-acetylglucosamine, N-acetylmuramic acid) with various amino acids, with the most common amino acids being D-alanine, D-glutamic acid, and mesodiaminopimelic acid [11,12,13]. The –D-amino acids are generally viewed to be a defense against peptidases [11].

Specifically, in the case of *Escherichia coli*, the peptidoglycan contains a muramic acid subunit and a glycan backbone having two amino sugars and a tetrapeptide consisting of –L-alanine-D-glutamic acid-meso-diaminopimelic acid (DAP)-D-alanine [9]. These particular tetrapeptide units are linked to another with DAP and –D-alanine [9]. Therefore, the D-alanine amino acid is common in *Escherichia coli* cell wall and may be subject to biological action such as that of the penicillins which mimic the D-alanine-D-alanine terminal dipeptide of peptidoglycan and bind at the active site of transpeptidase [10,12].

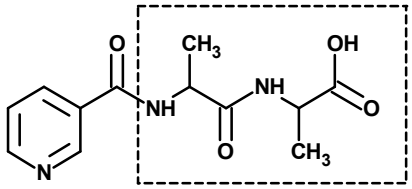
The molecular structures of the compounds presented in this study, the dipeptide compounds 1 and 2, with those of the tripeptide compounds 3 and 4, are presented in (Fig. 1). The amino acid substituents for each compound are indicated in rectangle. The dipeptide compound 1 consists of acetylsalicylic acid having a –D-alanine-D-alanine unit covalently bonded to the carbonyl carbon (-C(=O)-), and thereby forming an amide group (-C(O)-NHn-R). Similarly, the dipeptide compound 2 is the molecule nicotinic acid having –D-alanine-D-alanine unit covalently bonded to the carbonyl carbon (-C(=O)-).

The tripeptide compound 3 begins with the drug acetylsalicylic acid (aspirin), then having –glycine-D-alanine-D-alanine covalently bonded to the carbonyl carbon (-C(=O)-). The tripeptide compound 4 is in reality the non-steroidal anti-inflammatory drug (NSAID) ibuprofen, having the unit –glycine-D-alanine-D-alanine covalently bonded to the carbonyl carbon (-C(=O)-). The growth inhibition of penicillin resistant *Escherichia coli* accomplished by dipeptide compounds 1 and 2 are presented in (Fig. 2). The growth inhibition of penicillin susceptible *Escherichia coli* accomplished by tripeptide compounds 3 and 4 are presented in (Fig. 3).



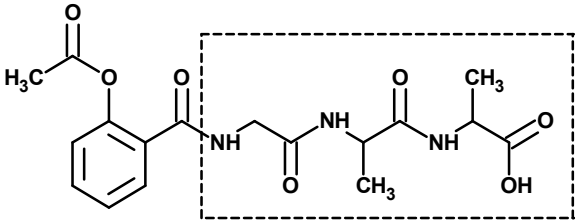
Compound 1

2-{[2-({[2-(acetyloxy)phenyl]carbonyl}amino)propanoyl]amino}propanoic acid



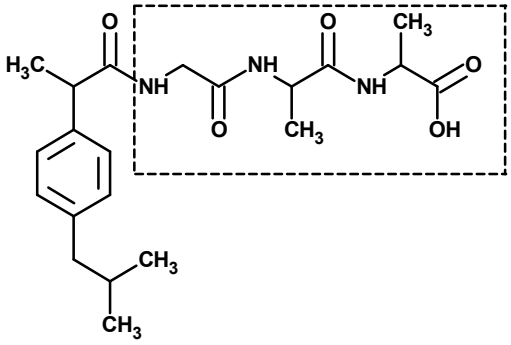
Compound 2

2-({2-[(pyridin-3-ylcarbonyl)amino]propanoyl}amino)propanoic acid



Compound 3

2-[(2-{[({[2-(acetyloxy)phenyl]carbonyl}amino)acetyl]amino}propanoyl) amino]propanoic acid



Compound 4

2-[{{[4-(2-methylpropyl)phenyl]carbonyl}aminoacetyl]amino}propanoyl) amino]propanoic acid

Fig. 1. Molecular structures of peptide compounds with the amino acid substituent

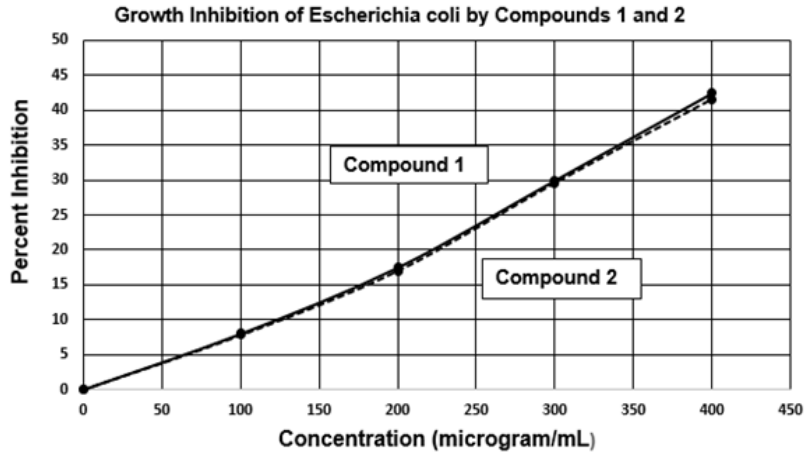


Fig. 2. Percent growth inhibition of bacteria by dipeptide compounds 1 and 2 are presented in the 2-way plot

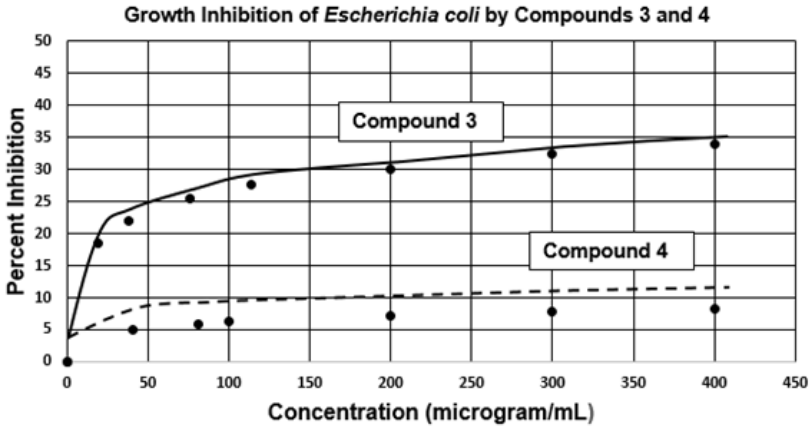


Fig. 3. Percent growth inhibition of bacteria by tripeptide compounds 3, and 4 is presented in the 2-way plot

The percentage of growth inhibition (y-axis) of penicillin resistant *Escherichia coli* by compounds 1 and 2 are highly similar across the concentration range studied (x-axis) (Fig. 2). At about 8% inhibition at 100 microgram/mL, 18% inhibition at 200 microgram/mL, and more than 40% inhibition at 400 microgram/mL. The percent of growth inhibition of bacteria *Escherichia coli* by compounds 3 and 4, are given in (Fig. 3). Observing compound 3 the percent inhibition ranges from 25% at about 75 microgram/mL to as high as 34% at 400 microgram/mL. Observing compound 4, the percent inhibition ranges from about 7.5% at 75 microgram/mL to as high as 9.5% at 400 microgram/mL.

Extent of growth inhibition at approximately 8% inhibition occurs at 100 microgram/mL for both 1 and 2. However, the percentage of growth inhibition substantially increases to 18% inhibition at 200 microgram/mL, for both 1 and 2. This is followed by even greater growth inhibition, that reaches more than 40% inhibition at 400 microgram/mL for both 1 and 2.

For tripeptide compound 3 the percentage growth inhibition ranges from 25% at about 75 microgram/mL, but reaching to as high as 34% at 400 microgram/mL. For tripeptide compound 4 the percentage growth inhibition ranges from about 7.5% at 75 microgram/mL but reaches to as high as 9.5% at 400 microgram/mL. These levels of *Escherichia coli* growth inhibition (both penicillin resistant and penicillin susceptible) are significant levels of growth suppression for this bacteria. Notably, the –D-alanine-D-alanine group jutting from the peptide compounds 1, 2, 3,

and 4; will be identified and have similar activity to that of the penicillin group of antibiotics that successfully mimic the D-alanine-D-alanine terminal dipeptide of peptidoglycan and bind at the active site of transpeptidase [10,12].

Now, the molecular properties will be examined to provide supporting data for the favorable drug-likeness properties of these four compounds. There are nine molecular properties listed in (Table 1) for comparison among these four compounds. Noteworthy in terms of understanding the drug-likeness of these four compounds is that all four show zero violations of the Rule of 5. Previous studies have shown that orally active drugs with favorable bioavailability have no more than one violation of the Rule of 5 [14]. The Rule of 5 states orally active drugs have parameters [14]: 1) No more than 5 hydrogen bond donors; 2) No more than 10 hydrogen bond acceptors; 3) Molecular weight under 500 grams/mole; and 4) Log P values less than 5.

Furthermore, compounds 1, 2, and 4 have polar surface area of less than 140 Angstroms^2 (Table 1). Drugs having polar surface area greater than 140 Angstroms^2 have been shown to be poorly absorbed in the intestinal organs [15,16]. Only compound 3 has polar surface area at 150.90 Angstroms^2. The level of intestinal absorbed assimilation for compounds 1, 2, and 4 would be anticipated to be about 15% to 20% [15,16].

The range in Log P (Molinspiration) values are -1.26, -2.55, -1.65, and 0.49, for compounds 1, 2, 3, and 4, respectively. A negative value for Log P means the compound has a higher affinity for the aqueous phase (i.e. it is more hydrophilic); when Log P = 0 the compound is equally partitioned between the lipid and aqueous phases; a positive value for Log P means a higher concentration of drug in the lipid phase (i.e., the compound is more lipophilic) [10,14,15,16]. The Log P values of the four compounds show a very strong positive correlation to number of atoms, molecular weight, number of rotatable bonds, and molecular volume by Pearson r ($r > 0.7000$). The number of atoms range from smallest 19 (compound 2) having highest affinity for aqueous phase (Log P = -2.55) to largest 29 (compound 4) having highest lipophilic nature (Log P = 0.49). Notably, the dipeptide compounds have the fewer –OH (hydroxyl) and -NHn (amine) groups. Pearson r correlation of Log P to number of atoms, molecular weight, number of rotatable bonds, and molecular volume (Angstroms^3) has a very strong positive relationship (Pearson r greater than 0.7000). Interestingly, the Pearson r values of Log P relationship to polar surface area (Angstroms^2) and number of oxygen and nitrogen atoms, has no relationship (Pearson r less than 0.1900). This suggests that the influence of oxygen and nitrogen atoms is outweighed by the influence of molecular weight, molecular volume, and number of atoms & rotatable bonds on Log P.

Grubb's test for outliers applied toward the properties shown in (Table 1), indicated no outliers among values of Log P, polar surface area, number of atoms, molecular weight, number of oxygen & nitrogen atoms, number of –OH & -NH_n, number of rotatable bonds, and molecular volume (P=.05, two-sided).

A notable trend in the molecular structures of these four peptide compounds is presented in (Fig. 4). That is to say that the number of oxygen atoms, nitrogen atoms, hydroxyl groups, and amine groups have a very high correlation to molecular weight (Fig. 4). The coefficient of determination represents the percentage of variation that can be explained by the regression equation. The coefficient of determination for lines A, B, C, and polynomial fit D, are 0.4068, 0.9998, 0.8317, and 0.9636, respectively. Line D is a polynomial fit with extremely high coefficient of determination. Pearson r correlation of molecular weight to the hydrogen bond acceptors (oxygen and nitrogen), hydrogen bond donors (hydroxyl and amine groups), and rotatable bonds, is 0.6379, 0.9120, and 0.9998, respectively. The relationship of number of oxygen, nitrogen, amine groups, hydroxyl groups, and number of rotatable bonds is highly linear as molecular weight increases. There is even a larger coefficient of determination of molecular weight to Log P, at R^2 = 0.9636. Therefore, for simple polypeptide compounds analogous to those of this study would be expected to have these relationships according to their molecular weight.

Other properties are presented in (Table 2), including dermal permeability coefficient, water solubility, and Log Kow (Episuite v. 1.66). The dermal permeability coefficient (Kp) is an important descriptor for assessing dermal absorption of medicaments utilized for clinical treatment of various dermal accessible diseases.

Table 1. Molecular properties of compounds

Peptide drug	Log P	Polar surface area (A^2)	Number of atoms	Molecular weight (g/mole)	Number of oxygen & nitrogen	Number Of -OH & -NH_n	Violations Of rule of 5	Number of Rotatable Bonds	Molecular Volume (A^3)
1	-1.26	121.8	23	322.32	8	3	0	7	285.12
2	-2.55	108.39	19	265.27	7	3	0	5	236.44
3	-1.65	150.90	27	379.37	10	4	0	9	333.31
4	0.49	124.59	29	405.50	8	4	0	10	388.92

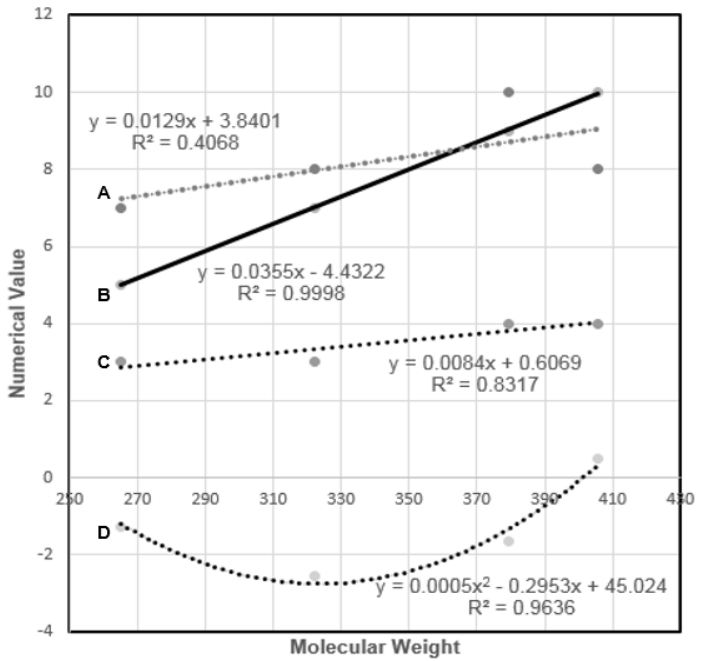


Fig. 4. Comparison of molecular weight to number of oxygen & nitrogen atoms (Line A), number of rotatable bonds (Line B), number of –OH & -NH_n groups (Line C), and Log P (Line D)

Water solubility is an important property when considering bioavailability [14,15,16]. Compound 4 has the highest Log Kow (at 2.23), therefore, highest level of lipophilic attribute consistent with lowest water solubility (935.44 mg/Liter) and highest level of dermal permeability (2.44 x 10^{-4} cm/hour). The Log Kow for compounds 1, 2, and 3 are all below zero and negative in value, thus, making these tree peptide compounds more hydrophilic. This is expected and consistent with their respective values of water solubility being all substantially greater than that for compound 4. In addition, the negative Log Kow values are consistent with a substantially lower dermal permeability for compounds 1, 2, and 3, when compared to that for compound 4 (see Kp values in (Table 2).

The Log P values (Molinspiration) shown in (Table 1) are statistically analogous to Log Kow (Episuite) provided in (Table 2). The Kruskal-Wallis test between these two sets of partition coefficients indicates they have equal medians (*P*=.15). The Kolmogorov-Smirnov test indicates the two sets of partition coefficients are form populations of equal distribution (*P*=.11). The Mann-Whitney tests also indicates these two sets are from populations of equal medians (*P*=.20). Altogether, the two sets of partition coefficients have no outliers among the numerical values (*P*=.05, two-sided). In addition, the two sets of partition coefficients are very strongly positive correlation, Pearson r = 0.9505.

Another screening engine for potential medicinal compounds that can be described utilizing molecular properties and/or several active ligands are compared to GPCR ligands, ion channel modulators, kinase inhibitors, nuclear receptor ligands, protease inhibitors and other enzyme targets (Table 3). Many compounds having favorable drug-likeness with highest chance to become active drugs or pesticides can be identified utilizing Bayesian statistics to compare structures to representative ligands. In (Table 3) indicates the bioactivity scores of compounds 1, 2, 3, and 4, compared to drug classes presented above. Column headers in (Table 3) show the classes of drug-like entities with the favorable drug-like score for each class. Notably, all four compounds show bioactivity scores in these six classes of pharmaceutics that are favorable, and fall within the acceptable score range for drug-like activity in these six classes. Essentially the bioactivity scores support and corroborate the results for Rule of 5, having zero violations of Rule of 5. Namely, all four compounds show favorable drug-likeness based on bioactivity scores.

One-way ANOVA analysis, including all numerical values of all six bioactivity categories comparing all four peptide compounds, indicated that these bioactivity scores have equal means (*P*=.95) [17]. Furthermore, the Kruskal-Wallis test for all six bioactivity categories comparing all four compounds, show their ratings have equal medians [17].

Path analysis is a useful extension of multiple regression. The concept and objective is to provide estimates of the magnitude and significance of potential causal connections between sets of variables (molecular properties) [17,18,19]. The results of such analysis are often represented by utilizing a path diagram [18,19]. Path analysis is considered an extremely effective method for the elucidation of complex interrelationships of a desired project [18,19]. The determined path coefficients are standardized regression coefficients [19]. Applying multiple regression to this end, the regression coefficients (or multipliers) describe the extent of the effect of the independent variables (molecular property) to the dependent variable (percent inhibition for this study) [19].

Utilizing the percent growth inhibition of *Escherichia coli* expressed by each compound as the dependent variable and eight molecular

Table 2. Properties of Log Kow, Kp, and water solubility

Peptide compound	Log Kow	Dermal permeability coefficient Kp (cm/hour)	Water solubility (mg/Liter)
1	-0.22	$1.44x10^{-5}$	683.8
2	-1.00	$8.89x10^{-6}$	6859
3	-1.05	$1.67x10^{-6}$	1578
4	2.23	$2.44x10^{-4}$	35.44

Table 3. Bioactivity of compounds

Peptide compound	GPCR ligand (-0.5 to 1.3)	Ion channel modulator (-0.5 to 1.6)	Kinase inhibitor (-.6 to 1.5)	Nuclear receptor ligand (-.5 to 1.5)	Protease inhibitor (-.5 to 1.6)	Enzyme inhibitor (-1 to 1.5)
1	0.06	-0.07	-0.31	-0.24	0.32	0.02
2	0.15	0.08	-0.19	-0.53	0.38	0.20
3	0.08	-0.14	-0.31	-0.26	0.32	0.0
4	0.25	-0.04	-0.35	-0.25	0.48	0.09

Table 4. Path coefficients for causal activity of inhibition with molecular properties

Molecular property	Path coefficient	Property of causality
Log P	22.6	
Polar Surface Area	1.82	
Number of Atoms	-13.1	
Formula Weight	-1.70	Percent growth inhibition
Number of Oxygen & Nitrogen	10.7	
Number of –OH and $–NH_n$	-20.1	
Number of Rotatable Bonds	20.9	
Molecular Volume	-0.84	

Table 5. Lipophilic substituent constant (π) for derivative compounds 1, 2, 3, and 4

Derivative compound/Standard compound	Log P derivative compound	Log P standard compound	Lipophilic substituent constant for Log P (Derivative – standard compound)
1/aspirin	-1.26	1.43	-2.69
2/nicotinic acid	-2.55	0.27	-2.82
3/aspirin	-1.65	1.43	-3.08
4/ibuprofen	0.49	3.46	-2.97

properties as independent variables, it is possible to estimate the importance of each of these molecular properties for producing the level of bacterial growth inhibition. The outcome of this analysis is presented in (Table 4). The size of the path coefficient for each property provides the amount of effect of that variable on the dependent variable (percent growth inhibition). The sign of the coefficient (positive or negative) provides the direction of their effect on the dependent variable.

Examining the path coefficient for each property, numerical size and sign, it is clear that the properties of Log P, number of oxygen & nitrogen atoms, and number of rotatable bonds have the greatest positive effect for greater effective growth inhibition (Table 4). The path coefficients for these three molecular properties are 22.6, 10.7, and 20.9, respectively. These three properties have the greatest positive contributing effect for percent growth inhibition. All path coefficients have a single head arrow to the property of causality (percent growth inhibition). It follows then, that the remaining properties have some sort of effect on percent inhibition themselves. The greatest negative effect (reducing growth inhibition activity) is caused by the number of –OH and –NHn groups (coefficient is -20.1). The negative effect on growth inhibition induced by these compounds is number of atoms (coefficient is -13.1) and formula weight (coefficient is -1.70). Presumably then, this confirms that the larger the drug (by formula weight and number of atoms) will have a detrimental (lessoning) effect on the percentage of bacterial growth inhibition.

The numerical sign of the lipophilic substituent constants (π) indicates the solubility trend of a compound based on Log P [20]. A negative π value indicates that the π substituent has a lower lipophilicity than hydrogen and the drug favours the aqueous phase (Table 5). A positive π value indicates that the π substituent has a higher lipophilicity than hydrogen and the drug favours the organic phase [20]. The equation for determining the value π between the parent compound and derivative [20], is shown in equation (1):

$$\pi = \text{Log (derivative)} - \text{Log (parent)} \quad (1)$$

Values for π for compounds 1, 2, 3, and 4, are determined and in (Table 5). The Grubb's test for outliers (extreme studentized deviate), showed no outliers among the four values of π (P=.05) [17]. All numerical values of π negative in sign, thereby, all peptide compounds will favour the aqueous phase in solubility.

Multiple regression analysis, can be used when three or more measurement variables exist. One of the measurement variables is considered to be the dependent (Y) variable [17]. The rest of the variables are considered to be the independent (X) variables (that have an effect on the dependent variable (Y)). The purpose of a multiple regression is to find an equation that best predicts the Y variable as a linear function of the X variables [17]. Multiple regressing essentially has two applications [17]: 1) One use of multiple regression is prediction or estimation of the dependent variable (Y) that corresponds to the independent (X) variables. It is possible to predict the molecular weight (MW) of analogous compounds based on running values for Log P, polar surface area (PSA), and molecular volume (MV), by utilizing the equation (2).

$$MW = 29.5869 + \text{Log P}(10.0920) + \text{PSA}(1.0994) + \text{MV}(0.6017) \quad (2)$$

The coefficient of determination R^2 of the equation is 1.000. This indicates that 100% of the proportion of the variance in the dependent variable that is predictable from the independent variable [17]. Therefore, equation (2) should be an effective predictor of molecular weight for similar compounds.

Most therapeutics that are considered peptide drugs are delivered intravenously or subcutaneously for clinical use, in order to circumvent issues with absorption [21,22,23]. The potential and number of peptide based therapeutics have grown significantly [21,24]. Most of the approved peptide based drugs have fewer than 20 amino acids in their molecular structure [21], such as the four compounds presented in this study. Other studies have shown the potential of peptide pharmaceuticals. Recent studies are showing that antimicrobial peptides are increasing in potential and application as sources of novel drugs in the control and treatment of schistosomiasis [25]. Schistosomiasis is an acute and chronic parasitic disease caused by blood flukes (trematode worms) [25]. An important criteria for evaluating antimicrobial peptides is their diverse therapeutic applications, generally thought to occur due to size, properties, and their broad spectrum of activities. Therefore, antimicrobial peptides are attractive candidates in the search for novel

therapeutic agents for control and treatment of schistosomiasis [25]. Clearly then, peptide based drugs have a great potential for clinical treatment of disease.

4. CONCLUSION

Compounds having peptide substituents are showing substantial potential as antimicrobial drugs that are effective for clinical application. Drug development should include peptide-based compounds because of their efficacy as antibacterial agents. Four compounds presented in this study have been examined by way of their molecular properties to determine the causal relationship towards their antibacterial activity. Each of these peptide compounds has demonstrated a significant level of growth inhibition of *Escherichia coli.* Compound 1 and 2 have –D-alanine-D-alanine substituents covalently bonded to aspirin and nicotinic acid, respectively, and compound 3 and 4 have –glycine-D-alanine-D-alanine substituent bonded to aspirin and ibuprofen, respectively. Path analysis showed that Log P, number of oxygen & nitrogen atoms, and number of rotatable bonds have greatest causal relationship to their respective percent inhibition of *Escherichia coli*. Calculation of their bioactivity within six categories of biological constituents (GPCR ligand, ion channel modulator, etc.), showed compounds 1, 2, 3, and 4 to be within the ranges of active molecular functionality. Showing zero violations of the Rule of 5, each compound then demonstrates favourable drug-likeness. Water solubility was higher for compounds 1, 2, and 3, but very substantially lower for compound 4. The lipophilic substituent constant is negative for all compounds, therefore, all four peptide compounds will favour the aqueous phase in solubility. Drugs having peptide substituents show promise as antimicrobial agents for application in the clinical treatment of bacterial infections.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

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COMPETING INTERESTS

Author has declared that no competing interests exist.

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