Measuring the Alkylation Kinetics and Drug Likeness of Four Candidate Antineoplastic Compounds

Ronald Bartzatt[1*]

[1]*University of Nebraska, Durham Science Center, 6001 Dodge Street, Omaha, Nebraska 68182, USA.*

Author's contribution

Author Ronald Bartzatt designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript.

Original Research Article

ABSTRACT

Aims: To synthesize small molecule alkylating compounds and analyze the kinetics of the alkylation in aqueous solution. Determine molecular properties and the drug likeness of these four compounds as potential antineoplastic agents and apply statistical analysis to identify interrelationships of properties.
Study Design: Four compounds were synthesized, characterized, and studied for alkylation capability. The alkylation kinetics were elucidated, as well as drug likeness properties. The interrelationships of properties were examined by statistical methodology.
Place and Duration of Study: Department of Chemistry, Durham Science Center, University of Nebraska at Omaha, Omaha NE, from May 2015 to June 2015.
Methodology: Four compounds were modified by the covalent bonding of an alkyl halide substituent or nitrogen mustard group. The four compounds were placed in aqueous solution at pH 7.4 and 37°C to monitor alkylation efficiency that targeted p-chloroaniline. Alkylation was monitored utilizing fluorescamine and measurement at 400 nm. Time and absorbance plots determined whether alkylation step is first-order or second-order. Molecular properties Log P, formula weight,

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Corresponding author: Email: rbartzatt@unomaha.edu;

polar surface area, etc., were determined. Statistical analysis and path analysis revealed which molecular property was most responsible for rate constant values.

Results: Compounds A, B, C, and D showed ranges of Log P, formula weight, and polar surface area of 0.010 to 4.21, 177.59 to 714.77, and 29.64 to 88.63, respectively. All compounds showed a favorable drug likeness, with only compound C showing a violation of the Rule of 5. The Log P values and number of alkylation reactive sites were most responsible for rate constant value.

Conclusion: Small molecule alkylating agents are synthesized, the efficiency of alkylation measured in aqueous solution utilizing fluorescamine at pH 7.4 and 37℃. Rate-order of reactions is determined utilizing fluorescamine assay for surviving primary amine groups. The four compounds showed a favorable drug likeness based on molecular properties.

Keywords: Antineoplastic; nitrogen mustard; alkylating; cancer.

ABBREVIATIONS

Term: *PC, path coefficient; GPCR, G protein–coupled receptors.*

1. INTRODUCTION

Alkylating agents are a class of anticancer drugs that directly damage DNA to prevent the cancer cell from undergoing division [1]. A rapidly dividing cell is most susceptible to the effects of alkylating agents, which in their action to produce a cross-linking of DNA either intra-strand or inter-strand [1]. Nitrogen mustard drugs are an alkylating agent that can cyclize in an aqueous environment to form highly electrophilic immonium ions that can covalently bind to the nucleophilic N-7 nitrogen position on guanine [1]. Examples of alkylating agents are busulfan (utilized for treating chronic myelogenous leukemia), cyclophosphamide (utilized for treating non-Hodgkin's lymphoma, leukemias, ovarian cancer, and breast cancer), and mechlorethamine (utilized for treating Hodgkin's disease) [1]. Monoalkylating agents can react with only one N-7 of a guanine molecule [1].

Classical alkylating agents include melphalan, chlorambucil, nitrogen mustards, nitrosoureas, and alkyl sulfonates [2,3]. In general, anticancer drugs can be classified by the mechanism of action, the groups as follows [1]: Alkylating agents (damage done during S phase or doubling of chromosomal material), heavy metals (e.g. platinum agents), antimetabolites, folic acid antagonists, pyrimidine analogues, purine analogues, cytotoxic antibiotics, spindle poisons (e.g. toxoids), and topoisomerase inhibitors. Additional clinical studies have shown a revived application for alkylating agents. A notable example is an improved outcome/treatment found with chlorambucil treatment of chronic lymphocytic leukemia [4]. The clinical purpose of antineoplastic agents is the elimination of cancer cells without affecting normal tissue [1].

Alkylating agents can cross link with carboxyl, hydroxyl, amino, sulfhydryl, and phosphate groups of bio-macromolecules [5]. This action cause abnormal base paring or scission of the DNA strand [5]. The kill rate of cancer cells can be enhanced with intensive/more frequent treatment or surgical removal with chemotherapy [1].

In the case of alkylating agents targeting DNA at physiological pH, the most reactive nucleophilic sites are N-7 of guanine > N-3 of adenine > N-1 of adenine > N-1 of cytosine [5]. Alkylating agents such as nitrogen mustards are able to undergo either SN1 (unimolecular) or SN2 (bimolecular) type reactions depending on the rate of aziridinium ion (a positive charged ring) formation [5].

Further studies of the alkylation agents will improve effectiveness and clinical outcome for cancer patients. The knowledge of the mechanism of action can enhance understanding of the type of alkylating antineoplastic agents more suitable for specific cancer cases. This study presents four novel alkylating agents, with the characterization of the kinetics of reaction with a targeted nucleophilic amine group.

2. METHODOLOGY

2.1 Chemicals and Instrumentation

Reagents utilized for the study were of HPLC grade and obtained from Aldrich Company

(Aldrich, PO Box 2060, Milwaukee WI 53201). Infrared spectra were obtained from a Mattson Galaxy FTIR in dimethyl-sulfoxide that was previously dried over molecular sieves. The visible wavelength spectroscopy for kinetics was accomplished utilizing Spectronic 21-D instrument with glass cuvettes. For controlled temperature monitoring of alkylation reaction required for rate-order determination a THELCO Model 84 controlled water bath was utilized (Precision Scientific Company, Chicago Illinois 60647 USA).

2.2 Software, Programs, and Molecular Modeling

The molecular properties of these study compounds were determined by utilizing Molinspiration informatics (Molinspiration Cheminformatics, Nova ulica, SK-900 26 Slovensky Grob, Slovak Republic), ChemSketch version 12.01 (copyright 1994-2009, ACD, 90 Adelaide Street West, Toronto Ontario, M5H3V9 Canada), and ALOGPS software [6]. Calculation of water solubility was completed with EPISUITE version 1.66 (Copyright 2000, U.S. Environmental Protection Agency, Washington DC). Summary statistics was performed by PAST Version 0.45 (Copyright Oyvind Hammer, 2001) [7] and Microsoft EXCEL 2013 from Microsoft Office Professional plus 2013. Bioactivity scores for compounds were determined by Molinspiration Cheminformatics. For outliers, Grubb's test was applied with online QuickCalcs Graphpad (http://www.graphpad.com/quickcalcs/).

2.3 Synthesis of Alkylating Compounds

2.3.1 Synthesis of compound (A)

Place 0.000652 moles (0.0750 grams) of (S)-(-)-4-oxo-2-azetidine carboxylic acid into 50.0 mL of acetone that is previously dried over molecular sieves. Force ammonia (NH_3) gas through the mixture for 2 hours then degas with nitrogen flow to remove the residual ammonia. Add 20x excess by moles of $ClCH_2CH_2Cl$ and reflux (mild) for 1 hour. Precipitate the product at -10°C overnight, then filter out by vacuum filtration. Wash with -10°C diethyl ether and the product can be store dry at -10°C.

Infrared analysis of Compound (A) : For C-N, 1350-1000 cm^{-1}; C=O, 1700 cm^{-1}; C-H 3000 cm^{-1} (broad); N-H 3300 cm^{-1} (broad); Beta-lactam ring-1750 to 1800 cm^{-1} (beta-lactam strained ring); Alkyl halogen 720 cm^{-1} (C-Cl). For carbon-13 assignments in sequence (ppm): 22.7, 44.6, 50.9, 69.5, 172.0, and 174.4.

2.3.2 Synthesis of D-alanine nitrogen mustard compound (B)

Place 0.00337 moles (0.300 g) of D-alanine into 40 mL acetonitrile which has been dried over molecular sieves (3 Angstroms). Bubble ammonia gas (NH_3) through the mixture for 30 minutes, then degas with nitrogen flow for at least 30 minutes to remove residual ammonia. Add ½ volume in 1,2-dichloroethane and reflux mildly for 2 to 3 hours. Precipitate product at -10°C overnight, filter out by vacuum filtration, and wash with -10°C diethylether. Keep dry and store over desiccant at -10°C.

Infrared analysis of Compound (B): For ester group 1700 cm^{-1} (C=O stretch); C-N 1350-1000 cm^{-1}; tertiary amine group-1200 cm^{-1} (C-N stretch); Alkyl halogen-720 cm^{-1} (C-Cl); C-H 3000 cm^{-1} (broad). For carbon-13 assignments in sequence (ppm): 14.9, 44.6, 44.7, 53.1, 59.0, 69.5, 172.0.

2.3.3 Synthesis of compound (C)

Place 0.00260 moles (0.500 grams) of citric acid into 50.0 mL of acetonitrile dried over molecular sieves. Add 2.0 mL of thionyl chloride and reflux for 2 hours. Remove excess thionyl chloride by distillation and adding additional acetonitrile to maintain volume. Cool, then add 0.3 mL of triethylamine with 0.0156 moles of triethanolamine (which has been dissolved in minimal dry acetonitrile previously). Reflux mildly for 1 hours. Precipitate product at -10°C overnight, then vacuum filter to isolate product. Wash with -10°C diethyl ether, store the ester of citric acid dry at -10°C. Then place 0.00130 moles of ester of citric acid into 50.0 mL of dry acetonitrile, followed by 5.0 mL of triethylamine. Slowly add 0.00130 moles of thionyl chloride, reflux mildly for 1 hour to 2 hours. Precipitate final product at -10°C overnight, then vacuum filter out the final product. Wash with -10°C acetone, store dry at -10°C.

Infrared analysis of Compound (C): For ester group 1700 cm^{-1} (C=O stretch); C-H 3000 cm^{-1} (broad); C-Cl aliphatic halogen 705 cm^{-1}; tertiary amine group 1180 cm^{-1} (C-N stretch); C-N 1350-1000 cm^{-1}. For carbon-13 assignments in

sequence (ppm): 39.3, 44.7, 52.2, 55.1, 55.9, 66.2, 66.4, 166.4, and 172.0.

2.3.4 Synthesis of octyl methanesulfonate compound (D)

Place 0.0312 moles (3.00 grams) of methanesulfonic acid into 50 mL of acetonitrile dried over molecular sieves (3 Angstroms size of sieve). Add 3 to 5 mL of thionyl chloride into this mixture and reflux for 2 to 3 hours. Distill out excess thionyl chloride while adding additional acetonitrile to maintain volume. Add 0.03590 moles of 1-octanol, 0.1 milliliter of triethylamine, and reflux (mild) for 2 to 3 hours. Distill out solvent to reduce volume and precipitate product at -10°C overnight. Carefully remove supernatant from product and wash several times with -10°C diethyl ether, remove ether supernatant wash from product by vacuum if necessary. The product must be kept dry and store at -10°C until use.

Infrared analysis of Compound (D): For methanesulfonate group 1350-1300 cm^{-1} (S=O); C-O 1300-1000 cm^{-1} (stretch); C(S=O)-C 1050 cm^{-1}; (-CH_2-O-S-) group- 950 to 1075 cm^{-1}; C-H 3000 cm^{-1} (broad). For carbon-13 assignments in sequence (ppm): 14.0, 23.1, 26.2, 30.0, 30.3, 30.7, 32.5, 53.9, and 72.4.

2.4 Measurement and Determination of Rate-Order and Rate Constants

Fluorescamine solution: A stock solution of fluorescamine is made new before each spectroscopy determination. This reagent is made to a concentration of 5.0 milligrams/milliliter of methanol. For 25 milliliters, dissolve 125 milligrams of fluorescamine into 25 milliliters of methanol.

Buffer solution with p-chloroaniline: Into a previous prepared aqueous pH 7.4, 0.100 molar $NaHCO_3$ buffer dissolve one milligram of p-chloroaniline per milliliter. This mixture should be made up fresh prior to use.

Analysis for Rate-Order of Reaction and Rate Constant: Pre-warm a volume of 1.00 milliliter of aqueous pH 7.4, 0.100 molar $NaHCO_3$ that also contains 1.0 milligram of p-chloroaniline. Place 25.0 milligrams of the alkylating compound to be tested into this volume of aqueous buffer and keep at 37°C with the water bath. Start time monitor upon addition of reactant (alkylating

compound and p-chloroaniline). At known time periods withdraw 50 microliters of reaction mixture and place into a glass cuvette, add 50 microliters of fluorescamine (that is 5 mg/mL in methanol), mix well, then add distilled water to 3.00 milliliters total volume. The mixtures should be analyzed by spectrometer quickly, and not stored for later use.

Absorbance of mixtures are read at 400 nanometers wavelength with suitable visible wavelength spectrophotometer (Spec 21 with 1 cm glass cuvettes for this study). To determine the best fit for rate-order of kinetics plot the absorbance data is considered the dependent variable and the time the sample aliquot is withdrawn from the reaction solution is the independent variable. For first-order considered then plot time (independent variable) versus Ln (absorbance). For second-order consideration then plot 1/absorbance versus time. For zero order consideration then plot raw absorbance (dependent variable) versus time.

3. RESULTS AND DISCUSSION

Evaluations of the overall clinical cancer treatment outcome have concluded that many common cancers are not adequately treated by present-day chemotherapy [8]. Crucial is the need for novel drugs to treat childhood cancers and the mechanisms by which the clinical trials can be efficiently conducted [9]. Lead modification of alkylating agents has been consistently shown to be advantageous for this class of anticancer drugs, and has led to expansion of their clinical application [5]. The four compounds presented here demonstrate the potential of covalently bonding alkylation groups onto known functional molecular scaffolding and the measurement of actual alkylating activity to include rate-order determination. The four alkylating compounds presented in Fig. 1, are studied to show alkylation of a primary amine nucleophile (p-chloroaniline), determination of rate-constants, and molecular properties.

The reaction order for nucleophilic substitution depends on the chemical structure of the alkylating agent [5]. An alkylation reaction can proceed by SN1 or SN2 mechanisms [5,10].

Compound A begins with 4-oxoazetidine-2-carboxylic acid that is modified by emplacement of –OCH_2CH_2Cl substituent upon the former carboxyl group (-COOH). The result is an agent
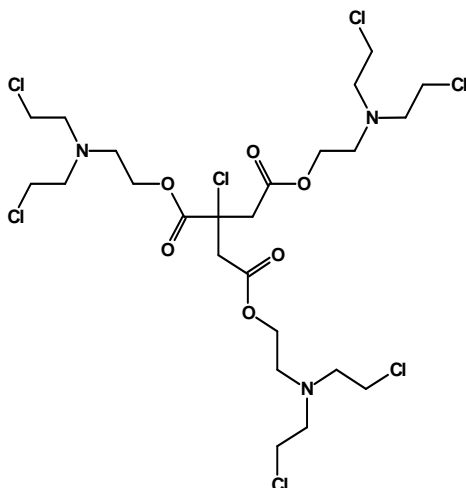
having one site of alkylation. Following with compound B, the resulting structure has potentially three sites of alkylation, to include the nitrogen mustard group ($-N(CH_2CH_2Cl)_2$).
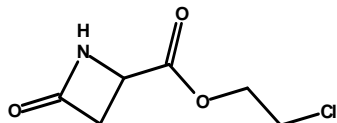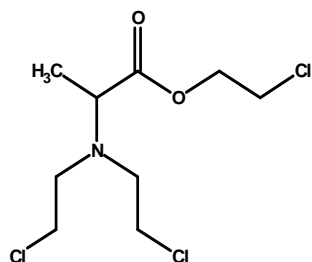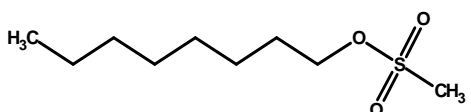

Fig. 1. Molecular structures of four alkylating compounds. Compound A (2-chloroethyl 4-oxoazetidine-2-carboxylate) has one potential alkylating site; Compound B (2-chloroethyl 2-[bis(2-chloroethyl)amino]propanoate) has three potential alkylating sites; Compound C (1,2,3-({2-[bis(2-chloroethyl)amino]ethoxy}carbonyl)-2-chloropropane) has 6 potential alkylating sites; and compound D (octyl methanesulfonate) has one potential alkylating site

Compound C begins with citric acid ($C_6H_8O_7$, 2-hydroxypropane-1, 2, 3-trioic acid) that continues to finally having three nitrogen mustard groups with a potential of six alkylation sites. Compound D, beginning with 1-octanol, has a single methane sulfonate group allowing one alkylation site.

The molecular properties of compounds A, B, C, and D are shown in Table 1. Various molecular properties are known to substantially affect the drug-likeness of a compound, the polar surface area has been shown to be useful in predicting intestinal absorption [11]. Fast and reliable estimation of intestinal absorption can be achieved by determination of polar surface area [11]. Based on polar surface area values for A, B, C, and D, the expected level of intestinal absorption for drug amount present following oral administration would be 89%, 99%, 48%, and 97%, respectively.

Although substantially different in molecular structure, the property numerical values for all four compounds given in Fig. 1 have very strong positive correlation with Pearson r correlation for all four compounds is greater than 0.9900 (very strong positive correlation is r ≥ 0.7000).

One-way ANOVA testing of the Table 1 properties indicates that the mean of the summed values are equal (*P=0.38*) [12]. In addition, by the Kruskal-Wallis test there is no significant difference between sample medians (*P= 0.86*). An interesting finding for these alkylating compounds of different structures.

Following with the Grubb's test for outliers (alpha = 0.05) [12], notably there are no outliers for numerical values of formula weight, number of oxygen & nitrogen atoms, polar surface area, molecular volume, and Log P. The solubility indicated in mole per liter has a strong negative correlation with a formula weight (Pearson correlation r = -0.4505) and molecular volume (Pearson correlation r = -0.5005). That is, as molecular weight and molecular volume increases, the solubility of the compound decreases. This is consistent with previous studies of drug-likeness where drug solubility in general is shown to decrease with increase of molecular size [13].

The Rule of five, also known as the Pfizer's rule of five, is a set of parameters to evaluate drug-likeness and to determine if a chemical compound with a certain pharmacological or

Table 1. Properties of compounds

Property	Compound A	Compound B	Compound C	Compound D
Log P	0.010	2.35	4.21	3.13
Polar surface area (Angstroms^2)	55.40	29.54	88.63	43.38
Number of atoms	11	15	40	13
Formula weight grams/mole	177.59	276.59	714.77	208.32
Number of oxygen & nitrogen atoms	4	3	9	3
Number of –OH & -NH$_n$	1	0	0	0
Violations of the rule of 5	0	0	1	0
Number of rotatable bonds	4	9	29	8
Molecular volume (Angstroms^3)	141.92	228.19	582.18	203.79
Solubility (mole/Liter)	0.295	5.01E-03	6.31E-07	6.17E-04

biological activity has properties that would make it a likely orally active drug in humans [14]. In general, when considering potentially useful compounds for medicinal purposes, then an orally active drug has no more than one violation of the following criteria [14]:

A. No more than 5 hydrogen bond donors (total number of –NH$_n$ and -OH)
B. Not more than 10 hydrogen bond acceptors (all nitrogen or oxygen atoms)
C. A molecular mass less than 500 Daltons
D. An octanol-water partition coefficient log *P* not greater than 5

Shown in Table 1, compounds A, B, and D has zero violations of Rule of 5, while compound C has only one violation. Essentially, by this criteria, all compounds are considered to have suitable oral activity capability. These compounds show a favorable drug-likeness. In addition, previous studies have shown that orally active drugs having polar surface area less than 120 Angstroms^2 are suitable for passive transport by the transcellular route [15], which is a criteria compounds A, B, C, and D fulfill. A drug having polar surface area less than 60 Angstroms^2 are able to penetrate through the brain-blood barrier and into the brain. By this criteria, compounds A, B, and D are suitable for penetrating into the brain.

It is beneficial to have tools to predict and discriminate the pharmacological activity of a given molecular compound so that the study is directed to molecular groups where there is a high probability of finding new compounds with

desired properties [14,15]. Biological activity or pharmacological activity, describes the beneficial or adverse effects of a drug on target tissues. Generally, an activity is considered to be dose-dependent and a compound is considered bioactive if it has interacted with or effect on a target tissue of the human body. By use of Bayesian statistics the four compounds were compared to six highly important drug classes to determine bioactivity scores (see Table 2). With respect to drug-likeness for the classes of GPCR ligand, ion channel modulator, and kinase inhibitor the range of drug-lilke compounds are: -1.50 to 0.50, -2.0 to 0.5, and -1.8 to 0.5, respectively. Note that all four compounds A, B, C, and D have scores that fall within the prospective ranges for identification as drug-like compounds. For the classes of nuclear receptor ligand, protease inhibitor, and enzyme inhibitor, the criteria range of score for drug-like compounds are: -2.0 to 0.5, -1.7 to 0.5, and -1.5 to 0.5, respectively. Note that all four compounds A, B, C, and D have scores that fall within the perspective ranges for drug-like compounds. These results support the beneficial potential of these four compounds. All four compounds have bioactivity scores within the drug-like region for all six important classes of drugs. The molecular structures of the four compounds substantially differ. One-way ANOVA analysis of Table 2 values indicates that the means of the scores determined for A, B, C, and D are not equal (*P= 0.000139*).

For determination of rate-order and rate constants of alkylation reactions, the compounds were placed into buffered aqueous solutions at

physiological pH 7.4 and 37°C. The target of alkylation is the nucleophilic amine group ($-NH_2$) of p-chloroaniline. An alkylation reaction of p-chloroaniline with any of the four compounds will eliminate the primary amine group. The presence, and change in concentration of the primary amine group $-NH_2$ can be accurately assayed by use of fluorescamine. Fluorescamine itself is a spiro compound that does not fluorescent itself, but reacts with primary amines to form a highly fluorescent product. By this means, it has been used as a reagent for the detection of amines and peptides [16]. The reaction of compound A with p-chloroaniline is shown in Fig. 2.

In the reaction solutions, after alkylation, there is left remaining unreacted p-chloroaniline which can be assayed by the highly specific fluorescamine derivatization of the primary amine group and detection at 400 nm wavelength [17,18]. Removing of aliquots at known time and assay at 400 nm allows the monitoring of the course of the alkylation reaction (by a monitor of un-reacted p-chloroaniline). By this approach the elucidation for rate-order of the reaction and the rate constants can be determined. Two examples of these plots, one for second-order (compound A) and one for first-order (compound C) are presented in Fig. 3.

Note that the Pearson correlation coefficient for compound A second-order plot, is r = 0.9947 (extremely high positive correlation). With the Pearson correlation coefficient for compound C first-order plot being r = 0.9399 (very strong positive correlation is r $\geq$ 0.7000).

The reaction order with rate constants for compounds A, B, C, and D are presented in Table 3 for comparison to the number of alkylation sites, formula weight, polar surface area, and Log P. The Grubb's test indicated that there were no outliers among the numerical values of the rate constants.

There is a strong negative relationship of rate constants values to polar surface area (Pearson r= -0.5027) and to Log P (Pearson r = -0.5149) (strong negative correlation is r from -0.4000 to -0.6900). There is a very strong positive relationship of number of alkylation sites to formula weight (Pearson r = 0.9672), but strong positive relationship to polar surface area (Pearson r = 0.6849) and Log P (Pearson r = 0.6949) (strong positive correlation is r from 0.4000 to 0.6900). This suggests interrelationship of rate of reaction to the physiochemical properties of the alkylating compounds.

Table 2. Bioactivity scores for compounds

Biological activity	Compound A	Compound B	Compound C	Compound C
GPCR ligand	-1.07	-0.80	-0.13	-0.56
Ion channel modulator	-0.79	-0.54	-0.48	-0.66
Kinase inhibitor	-1.37	-0.75	-0.17	-0.95
Nuclear receptor ligand	-1.53	-1.00	-0.13	-0.50
Protease inhibitor	-0.93	-0.70	-0.08	-0.39
Enzyme inhibitor	-0.86	-0.61	-0.015	0.34

Table 3. Rate of reaction and rate constant comparison

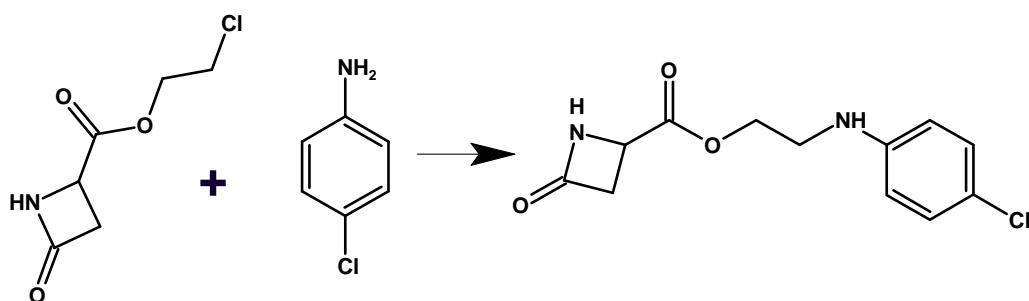| Drug	Number of sites of alkylation	Formula weight (grams/mole)	Polar surface area (A^2)	Log P	Reaction order rate constant
Compound A	1	177.59	55.40	0.010	Second order 0.0184 molar^{-1} minute^{-1}
Compound B	3	276.59	29.54	2.35	First order 0.0258 minute^{-1}
Compound C	6	714.77	88.63	4.21	First order 0.00997 minute^{-1}
Compound D	1	208.32	43.38	3.13	First order 0.00594 minute^{-1}

Fig. 2. Example alkylation reaction of compound A with target p-chloroaniline. The primary amine group of p-chloroaniline is eliminated
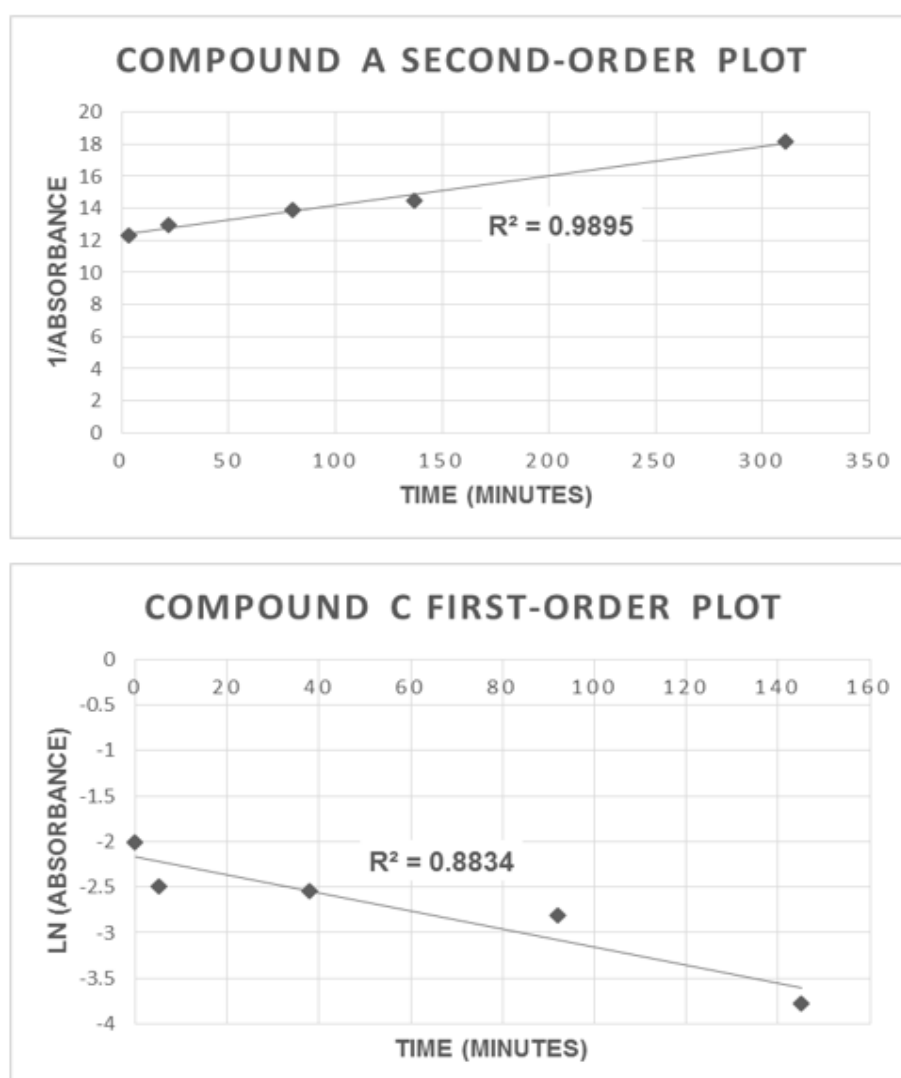

Fig. 3. Example plots for rate-order analysis, for compound A (top) and compound C (bottom). The pearson correlation coefficient for compound A second-order plot, is r = 0.9947 (very strong high positive correlation is r ≥ 0.7000). The pearson correlation coefficient for first-order plot of compound C is a very strong positive correlation r = 0.9399

Further explanation for the causality of the rate of reactions can be determined by use of path analysis. Path analysis is an extension of multiple regression with the aim to provide estimates of the magnitude and significance of hypothesized causal connections between sets of variables [19]. Path analysis is a form of statistical analysis used to evaluate causal models by examining the relationships between a dependent variable and two or more independent variables. There are two main requirements for path analysis [19]: 1) All causal relationships between variables must go in one direction only (i.e. a pair of variables cannot cause each other); 2). The variables must have a clear time-ordering since one variable cannot be said to cause another unless it precedes it in time.

Representation of results of path analysis is achieved by considering a path diagram, which presents the names of the variables and draws arrows from each variable to any other variable we believe that it affects. Associated with the arrows are path coefficients (PC) that are standardized versions of linear regression weights used to examine the possible causal linkage between variables in the modeling approach [19,20].

The rate constants for alkylation reaction of compounds A, B, C, and D with a nucleophile is shown in Table 3. An interesting inquiry is to apply path analysis to elucidate which of the important molecular properties formula weight, polar surface area, Log P, or number of alkylation sites can be identified as causative variable(s) for rate of reaction. The larger the path coefficient, the greater the proposed causative relationship between variables [19,20].

The path diagram outcome of path analysis of Table 3 is presented in Fig. 4. The parameters of the path analysis evaluated the causality of each property as stand-alone to actual rate constants (or the rate of reaction). A striking result is observed, and that is the importance of each property for the measured rate of reaction. Essentially, Log P is found to have great influence (PC = 0.629), followed by number of alkylation sites (PC = 0.435). These two properties exert highest influence, but are followed by the formula weight (PC = 0.351) and polar surface area (PC = 0.261). This provides a means to understand the rate of alkylation of a nucleophile and how that rate is influenced.

The four compounds A, B, C, and D have been shown to efficiently alkylate a nucleophilic primary amine group. All compounds demonstrated favorable drug-likeness and for compounds A, B, and D have the favorable potential of penetrating through the blood-brain barrier, as a consequence of the low polar surface area.
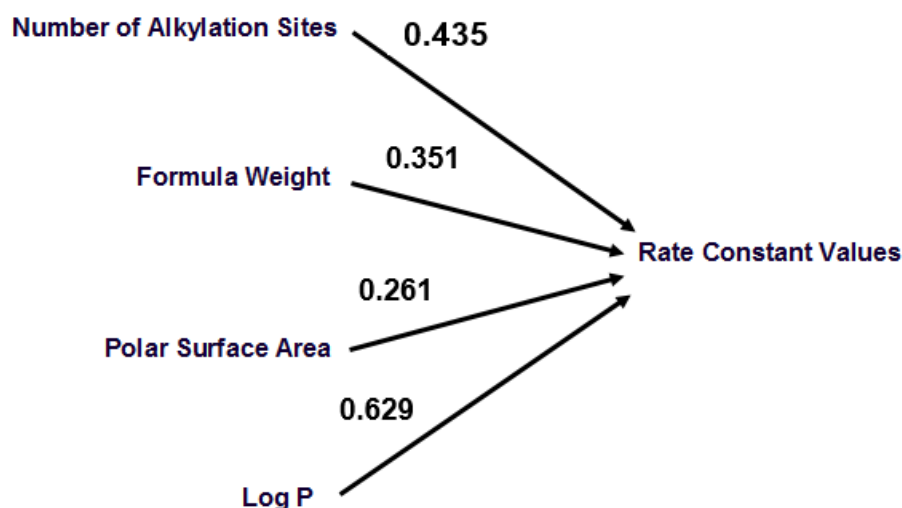
Fig. 4. Path diagrams show variables interconnected and specify causal flow. A path coefficient in this model clearly shows that the casual relationship of property to rate constant values has the following order: Log P (0.629) > number of alkylation sites (0.435) > formula weight (0.351) > polar surface area (0.261)

The study, synthesis, and examination of novel alkylating agents is a very necessary endeavor in the pursuit of useful medicaments for clinical treatment of neoplastic diseases. In this study, four compounds were formed by emplacement of functional groups known to effectuate an alkylation reactivity. The requirement for successful kinetics analysis is that it is imperative to monitor the reaction's progress by following the change in concentration for at least one of its species. These four compounds are successful alkylating agents and have been characterized to authenticate the favorable potential as antineoplastic drugs.

4. CONCLUSION

Compounds A, B, C, and D were synthesized and have one or more alkylating substituents covalently bonded to molecular scaffolding suitable for drug delivery. Molecular properties were determined, finding values of polar surface area to be: 55.40 A^2, 29.54 A^2, 88.63 A^2, and 43.38 A^2, respectively. These values indicate that compounds A, B, and D would penetrate into the central nervous system. Examination of various molecular properties infers all four compounds have a favorable drug-likeness, based on Rule of 5. From these polar surface area values for A, B, C, and D, the anticipated level of intestinal absorption for drug amount present following oral administration would be 89%, 99%, 48%, and 97%, respectively. Monitoring the absorbance of fluorescamine-drug complex at 400 nm allowed determination of rate-order and rate constants for alkylation reactions of these compounds. Rate constants were determined for alkylation reactions and path analysis calculated path coefficients to be Log P (0.629), number of alkylation sites (0.435), formula weight (0.351), and polar surface area (0.0261). Bioactivity scores for six classes showed that all compounds are classified drug-like.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

ACKNOWLEDGEMENTS

COMPETING INTERESTS

Author has declared that no competing interests exist.

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