Research Paper

IN SILICO OPTIMIZED MECHLORETHAMINE BASED DRUG STRUCTURES TARGETING BRAIN AND SPINAL CORD TUMORS

ABSTRACT

Aims: Brain and spinal cord tumors are the third most common type of childhood cancer following leukemia and lymphoma. The treatment of brain tumors for children is usually different than for adults. Mechlorethamine (mustine) itself is a bifunctional alkylating cytotoxic vesicant. Eleven variants of mechlorethamine are presented in this study that possess molecular properties enabling substantial access to tumors of the central nervous system.

Methodology: Following extensive in silico search and identification of potential drug structures, the conclusive set of brain penetrating structures were compiled. Extensive characterization of structure properties was accomplished followed by multivariate numerical analysis utilizing pattern recognition and statistical analysis.

Results: All twelve compounds (including mechlorethamine) exhibited zero violations of Rule of 5, indicating favorable bioavailability. The range in Log P, formula weight, and polar surface area for these compounds are: 1.554 to 3.52, 156.06 to 324.12, and 3.238 to 20.240, respectively. The physicochemical properties of all 12 compounds are very highly correlated (by Pearson r > 0.9900). High resolution hierarchical cluster analysis determined that agent 2

and 6 are most similar to the parent compound mechlorethamine. The average Log P, formula weight, polar surface area, and molecular volume are 2.446, 235.433, 8.58, and 213.8 A^3, respectively. Nonhierarchical K-means cluster analysis indicated that agents 2, 6, and 12 are most similar to mechlorethamine and multiple regression analysis of multivariate data set of molecular properties produced a model that will enable the design of similar alkylating agents. Conclusion: These eleven drug designs possess attributes that effectuate high permeation into the central nervous system.

Key words: brain tumors, astrocytomas, glioma, mechlorethamine, mustine

Abbreviations: PSA, polar surface area; CNS, central nervous system; MV, molecular volume; NO, number of nitrogen and oxygen atoms; BB, logarithm value of brain to plasma concentration ratio; Log(BB), log value of BB.

1. INTRODUCTION

Brain and spinal cord tumors are the third most common type of childhood cancer, with only leukemia and lymphoma in greater occurrence. The cancers occurring in the central nervous system (CNS) can be primary (tumors that begin in the CNS) and metastatic (tumors formed from cancer cells beginning in other parts of the body). Types of childhood brain and spinal cord tumors include: astrocytomas, atypical teratoid tumor, brain stem glioma, CNS embryonal tumor, CNS germ cell tumor, craniopharyngioma, ependymoma, medulloblastoma, spinal cord tumors, and supratentorial primitive neuro ectodermal tumors.

Mechlorethamine (mustine, mustargen, chlorethazine, chlormethine) is a

nitrogen mustard agent having antineoplastic and immunosuppressive activity. Mechlorethamine alkylates DNA, especially at the 7-Nitrogen site of guanine, which induces interstrand crosslinking and inhibition of DNA repair. It is a light yellow brownish, hygroscopic, crystalline powder that is very soluble in water and alcohol. There are approximately 20,000 new cases of primary CNS tumors in the United States every year [1]. Growth of the CNS tumors is at the considerable expense of other structure contained within the CNS, therefore symptoms depend on the location of tumor [1]. Symptoms include: confusion, headache, nausea, vomiting papilledema, seizures, and cognitive impairment [1].

Metastases based tumors are the most common type of cancer of the CNS, which appear to be on increase [2]. The pathophysiology of brain metastases is very important and influences the efficacious of therapies to target brain tumor growth [2]. Studies conducted in Korea have shown females to be more inclined to CNS tumors (1.43:1) with the most common tumor to be meningioma (31.2%) followed by glioblastoma (30.7%) and malignant primary tumors (19.3%) [3]. Patients of less than 19 years of age will most commonly have germ cell tumors and embryonal/medulloblastoma [3].

While breast cancer is the most common malignancy within women in the United States, the total incidence of brain metastases of breast cancer is a significant 30% [4]. The incidence of brain metastases is on the increase with breast cancer patients [4]. The development of CNS metastases with breast cancer depends on prognostic factors including age and negative hormone

receptor status [5]. However, patients having breast cancer with intramedullary spinal cord metastases tend to improve better than other case types of cancer [6].

Interestingly, nearly half of patients with advanced melanoma develop metastases of the CNS, with up to 20% of these patients incur CNS metastases as the first site of relapse [7]. These incidents of CNS metastases rarely benefit from systemic therapy due to lack of penetration into the CNS by the applied chemotherapeutics [7]. The pursuit of novel drugs to treat melanoma is focused on those agents having useful antitumor activity in addition to the capability of crossing the blood-brain barrier of the CNS [7]. Autopsy have shown that up to two thirds of cases of metastatic melanoma do have CNS involvement [8].

Left sided primary colon tumors are predominant in cases of brain metastases associated colorectal cancer (CRC), however these cases arise in only 2.3% of total CRC [9]. Greater survival of CRC is also associated with increased survival of the brain metastases [9]. Patients with primary rectal versus primary colon cancer are more likely to develop bone metastases which has an association to brain metastases as well [10]. Bone metastases among CRC patients is more common with increased numbers of active systematic agents received by the patient [10].

These outcomes of clinical studies clearly reveal the need for novel antitumor agents that have effective antineoplastic activity but with molecular properties enabling the penetration of the CNS. Albeit the difficulties of CNS penetration

is substantial due to the blood brain barrier, the design of molecular structures that can effectuate CNS infiltration are crucial for the treatment of pediatric brain tumors.

2. MATERIALS AND METHODS

2.1 MOLECULAR MODELING

Molecular properties and modeling was accomplished by utilizing ACD/ChemSketch modeling v. 10.00 (Advanced Chemistry Development, 110 Yonge Street, Toronto Ontario, M5C 1T4 Canada). Various properties; polar surface area, violations of Rule of 5, molecular volume, number of oxygens, nitrogens, amines, hydroxyls, etc were determined using Molinspiration (Molinspiration Chemiformatics, Nova ulica 61, SK-900 26 Slovensky Grob, Slovak Republic). In silico structure search for substituent replacement was accomplished using Chemical substructure and similarity search with MolCart Chemical Data Base (Molsoft L.L.C. 3366 North Torrey Pines Court, Suite 300, La Jolla, CA 92037 U S A).

2.2 PATTERN RECOGNITION

To identify underlying associations/patterns within the multivariate data set required the use of various pattern recognition techniques. Included in the analysis is hierarchical cluster analysis accomplished by KyPlot v. 2.0 Beta 15 (copyright Koichi Yoshioka 1997-2001). ANOSIM (analysis of similarity), 95% ellipses, and non-hierarchical K-means cluster analysis were performed by PAST v. 2.04 (copyright Oyvind Hammer, D.A.T. Harper 1999-2008).

2.3 NUMERICAL ANALYSIS

Statistical analysis of all numerical data including correlation analysis by Pearson r was performed by Microsoft EXCEL (EXCEL 2003, copyright 1985-2003). Multiple regression analysis of molecular properties was accomplished by GraphPad Instat v. 3.00 for Windows 95 (GraphPad Software, San Diego California USA).

3. RESULTS & DISCUSSION

With the appearance of brain metastases occurring in up to 40% of cancer patients (with increasing frequency) [11] the investigation of new cytotoxic agents is definitely warranted. Lung cancer, breast cancer, and skin melanoma are the commonest sources of brain metastases [11]. While whole brain radiotherapy (WBRT), with or without surgery, and systemic chemotherapy have levels of success, the later neurotoxicity of WBRT treatment is not insignificant [11,12]. Several clinical outcomes applying chemotherapy for childhood brain tumors are now presented to substantiate the elucidation of small molecule antitumor agents to enhance/improve clinical outcome. Nitrogen mustard (mechlorethamine), in addition with procarbazine, vincristine, and prednisone, when delivered over a 12 year period resulted in halted disease progression in 73% of children with medulloblastoma (some with complete remission for > 10 years) [13]. This regimen resulted in 50% long term survival with children having anaplastic glioma but < 30% response with brain stem gliomas [13].

Mechlorethamine, vincristine, procarbazine, and prednisone (MOPP) for treatment of childhood brain tumors has been shown to be well tolerated

and improves neurodevelopmental outcome [14] and postpones the debilitating consequences of radiotherapy [15]. Clinical evidence supportive for mechlorethamine (nitrogen mustard) type constructs for targeting tumors include the following: promising response in adult high grade glioma [16], successful treatment of child Hodgkin disease [17], and effective response for mycosis fungoides [18,19,20]. Utilizing mechlorethamine as parent construct for design of similar compounds with analogous properties appears advantageous.

The mechlorethamine sort (see structure 1 Figure 1), is a bifunctional alkylating nitrogen mustard agent having a small formula weight (156.06) and a single methyl group (-CH_3) covalently bonded to the nitrogen atom. Variation of this structure is accomplished by substituent search through in silico structure search (for substituent replacement) using chemical substructure and similarity mining by MolCart Chemical Data Base. Screening for small formula weight moieties and minimizing polar surface area (the surface sum over all polar atoms, oxygen and nitrogen, also any attached hydrogen atoms) the population of agent 2 to 12 is filtered (see Figure 1). Although restricted to analogy of the mechlorethamine molecule, there is considerable diversity in structural substituents within 2 to 12. Notably there is aromatic ring (agent 4, 10, and 11), aliphatic carbon chains (agent 5, 6, 7), amine groups (agent 5, 9), and other substructure. Beginning with mechlorethamine but building a diverse variety of substituted substituents will be shown to enable a multifariousness in pharmaceutical properties. Measured as molecular properties (or descriptors) the alteration of druglikeness presents a credible group of designs that will

permeate the CNS.

Molecular properties have been utilized to enhance filtering of drug candidates by druglikeness and pharmacodynamics to stymie specific physiological abnormalities. For evaluation of bioavailability and measurement of CNS permeation various molecular properties are shown in Table 1, that include Log P (measurement of lipophilic activity), molecular weight, polar surface area (PSA), and violations of Rule of 5. Values of Log P have a strong positive correlation with molecular weight (Pearson r = 0.4551) and molecular volume (Pearson r = 0.5615). Molecular weight has a very strong positive correlation to molecular volume (Pearson r = 0.9607) and strong positive correlation to number of oxygen and nitrogen atoms (Pearson r = 0.6365). Polar surface area has a strong positive correlation to molecular volume (Pearson r = 0.4070).

Molecular polar surface area is a property that has been shown to correlate well with passive molecular transport through cellular membranes, allowing prediction of transport properties of drugs [21]. Examining PSA values for this group of structures confers the capacity that 1 through 12 (see Figure 1) will be more than 85% absorbed via the intestinal tract following oral administration [21]. Previous investigations have shown that PSA can be effectively utilized to discriminate poorly from highly absorbed drugs [22]. In addition, those studies concluded that drugs have PSA less than 60 Angstroms^2 are completely absorbed by the intestinal tract [22]. Notably all nitrogen mustard agents 1 through 12 have PSA attributes well below 60 Anstroms^2 (the maximum value is 22.24 Angstroms^2 of agent 10).

The Rule of Five is developed to evaluate druglikeness (a chemical compound with a certain pharmacological or biological activity), and properties that would make it a likely orally active drug in humans [23]. Druglikeness is a qualitative measure of the extent of drug-like action of a substance. Drugs that are administered orally must pass through the intestinal lining and be transported in aqueous blood, followed by penetration of the lipid cellular membrane to reach the inside of a cell for pharmaceutical activity. The Rule of Five states that an orally active drug will have [23]: 1) Not more than 5 hydrogen bond donors (-OH and $–NH_n$ groups); 2) Not more than 10 hydrogen bond acceptors (notably N and O atoms); 3) A molecular weight under 500 g/mol; and 4) A partition coefficient log P less than 5. Structures 1 to 12 have zero violations of Rule of 5, indicating favorable bioavailability for targeting CNS tumors.

Cluster analysis is the elucidation of a set of observations into subsets so that objects in the same cluster are most similar within the multivariate data set. Clustering is a method of unsupervised learning, a common method for statistical data analysis. The multivariate data set (Table 1) can be examined to illuminate underlying relations through hierarchical cluster analysis, which will group (cluster) agents 1 to 12 according to highest similarity. The vertical dendrogram of Figure 2 shows that compounds 2 and 6 are most similar to mechlorethamine (agent 1) and are linked at node C. Node D linking agents 3, 11, and 9 are determined to be most similar and are connected to 1, 2, and 6 at node A. Compounds 4 and 10 are most similar by properties, joined at node E. Node F links agents 5, 12, 7, and 8 (mutually similar), which are linked with 4 and

10 at node B. Clearly the data set of Table 1 show descriptors of 1 through 12 to have similar numerical values, however higher resolution distinguishes 2 and 6 to be the closest to mechorethamine. K-means nonhierarchical cluster analysis will likewise organize objects into clusters in which members have highest similarity, however the number of clusters are predetermined. Outcome of K-means determined that mechlorethamine (1) is similar to agent 2, 6 and 12; with 3, 5, 9, and 11clustered; lastly are agents 4, 7, 8, and 10. Proficient ordination can resolve which structures would have similarity in clinical activity and patient response.

Extraordinary challenges remain with childhood brain tumors and advances need to be pursued in devising therapies having less long-term sequelae. Sequelae of brain trauma include headache and dizziness, anxiety, apathy, depression, aggression, cognitive impairments (including visual and semantic memory, attention, and motor coordination), personality changes, mania, and psychosis.

The degree of blood brain barrier (BBB) penetration is commonly assessed as the ratio of the steady-state concentrations of the drug in the blood and brain, expressed as Log (C_{brain}/C_{blood}), or Log (BB) (where BB is concentration of drug in the brain ÷ concentration of drug in blood) [24]. The determination of Log (BB) and BB has been achieved for drugs 1 to 12 which are presented in Table 2. Notably the values of BB are high, all values of BB are greater than one which indicates drugs 1 to 12 will likely have greater partitioning within the CNS than the blood. The relationship to predict this complex mechanism has been

shown in previous studies to be systematically predicted by the model [24]: Log (BB) = -0.0148(PSA) + 0.152(Clog P) + 0.139, where PSA is polar surface area and CLog P is calculated partition coefficient Log P. Drugs that have Log (BB) values greater than 0.3 are shown to readily cross the BBB [24]. Note that nine of the 12 agents of Figure 1 have Log (BB) greater than 0.3, they are 1 (mechlorethamine), 2, 3, 5, 6, 7, 8, 9, and 11. Log (BB) values for the remaining agents are also high (agents 4, 10, and 12). These relationships are determined to valid for passive diffusion consideration [24].

Orally active drugs expected to transport passively by transcellular route should not have PSA exceeding 120 Angstroms^2 [25]. For purposes of crossing the BBB into the CNS, then PSA should be less than 60 to 70 Anstroms^2 [25]. Notably all drugs 1 to 12 have PSA far less that 60 Angstroms^2 (range is from 3.238 Angstroms^2 to 22.24 Angstroms^2), so by this criteria agents 1 to 12 will pierce the BBB to target tumors of the CNS, see Table 1.

The partition coefficient Log P is a property which is a composite of components that include polarity, molecular size, polarizability, and hydrogen bonding. Previous studies have shown distinctly that small molecules penetrate the blood brain barrier [26]. Investigators have determined that optimal penetration through the BBB is achievable for molecules having a Log P between 1 to 4 in value, a formula weight less than 400, and polar surface area less than 90 Angstroms^2 [27]. For drugs 1 to 12, see Table 1, the Log P values range from 1.554 to 3.52, the formula weights are all below 400, and the polar surface areas are far less than 90 Angstroms^2. Therefore all molecules 1 to 12 are determined

to have highly efficient access to the central nervous system. Structures 7 and 8 have been described previously, which established the identical conclusions concerning their effectiveness in CNS penetration for targeting neoplastic tissue [28]. Structures 7 and 8 are two members of a homologous series (homologous series vary by an extra (-CH_2 -) from the previous compound) of nitrogen mustard agents and with each addition of (–CH_2 -) comes a variation of molecular properties [28]. The synthesis and other features of this group of nitrogen mustard agents are described previously [28].

Two functions of multiple regression analysis are: 1) explanation of relationship among multiple independent variables, and 2) prediction by utilizing multiple independent variables. By applying the molecular properties presented in Table 1, the multiple regression model appears as follows for prediction of formula weight for additional analogous compounds (FW= formula weight, PSA= polar surface area, MV= molecular volume, NO=number of oxygen and nitrogen atoms):

FW = 1.756- (2.113)(Log P) – (0.9156)(PSA) + (1.005)(MV) + (21.254)(NO)

The R^2 value of 0.9436 indicates at this model explains 94.36% of the model variance. The formula weight of additional similar structures can be estimated by selection of four physicochemical values. Outcome of in silico search and identification of structures falls within a substantially rigid and tight zone of acceptability as indicated by the 95% ellipses (see Figure 3) of Log P and formula weight (i.e. values of 12 agents fall well within 95% confidence region). Analysis of similarities (ANOSIM) provides a way to test statistically whether

there is a significant difference between two or more groups of sampling units. The ANOSIM result for descriptors shown in Table 1 is R= 1.0 or a large positive R (up to 1) signifying significant dissimilarity among these agents based on their physicochemical values [29].

Any type of brain tumor is inherently serious and life-threatening due to an infiltrative proliferation. The threat level is consistent with aspects of size, location, type, and extent of development. The investigation of novel treatment methods should continue and accompanying presentation of new drug designs that present credible advantages in clinical response.

CONCLUSION

In summation, a set of eleven novel drug structures are elucidated by in silico optimized substituent search that is founded on the successful anticancer nitrogen mustard scaffold of mechlorethamine. Brain metastases has been linked to breast cancer, advanced melanoma, and colorectal cancer. Various molecular properties that enable the transition from blood to CNS have been identified and found to be optimal for the twelve agents reported here. The Log P numerical values fall between 1.554 to 3.52 which is a range well within the BBB piercing range of 1.0 to 4.00. In addition the values of PSA range from 3.238 to 22.24 Angstroms^2, a range well below the upper limit for effective CNS penetration at 90 Angstroms^2. Importantly all twelve agents present zero violations of the Rule of 5, indicating a high level of druglikeness and favorable bioavailability. The success rate of in silico search and identification of suitable CNS targeting antineoplastic structures was less

than ten percent. Various attributes recounting the inherit potential of small molecules applied as chemotherapeutic agents in the treatment of CNS tumors have been presented in this study.

COMPETING INTERESTS: There are no competing interests.

LEGENDS TO FIGURES

Figure 1: The scaffold of mechlorethamine (1) serves as source construct for substituent optimization producing 11 daughter compounds 2 to 12. The outcome includes a diverse type of substituent types: aromatic, aliphatic, amine, and carbonyl. Resultant molecular properties further substantiate the in silico search method and provide a set of compounds that clearly possess efficaciousness in the clinical treatment of CNS tumors.

Figure 2: Hierarchical cluster analysis (Euclidean distance and single linkage cluster parameters) of molecular properties (see Table 1) show with high resolution the assimilation by mutual similarity. Albeit the molecular properties (see Table 1) indicate very high numerical correlation, the underlying relationships indicate that agents 2 and 6 are most similar to mechlorethamine. Other aggregation of similarity are: 3, 11, and 9; 4 and 10; 5, 13, 7, and 8. Compounds 1, 2, 6, 3, 11, and 9 are joined at node A. Agents 4 and 10 are joined at node E and joined to 5, 12, 7, and 8 at node B.

Figure 3: Two-way plot of Log P and molecular weight indicates complete inclusion into 95% ellipses. Thus indicating relationship of lipophilicity to molecular weight is inclusive within 95% confidence.

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FIGURE 1

Molecular Structures of Anticancer Drugs

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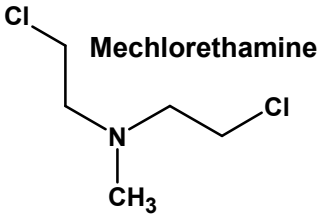

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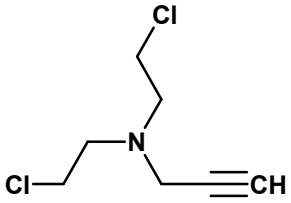

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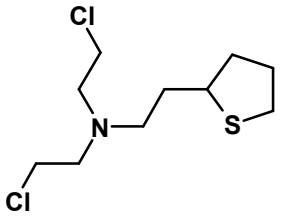

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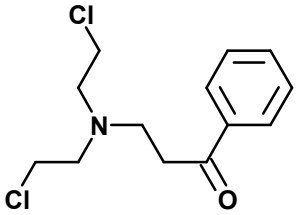

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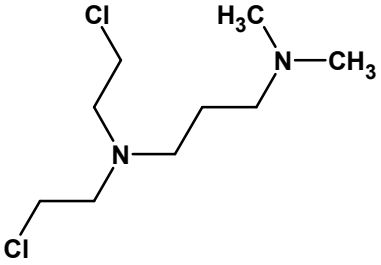

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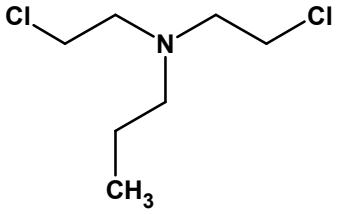

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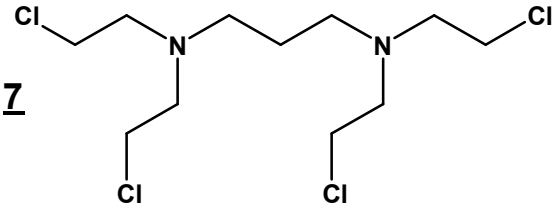

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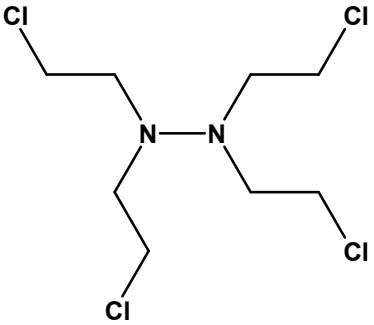

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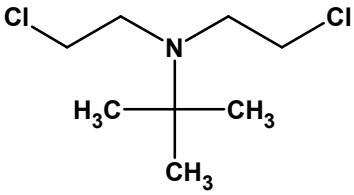

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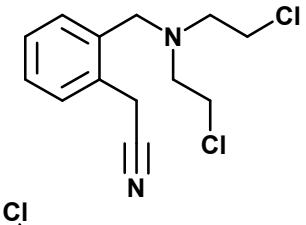

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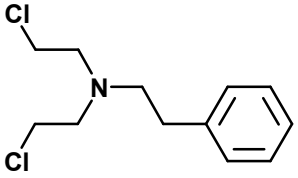

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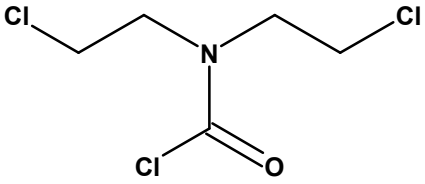

FIGURE 2

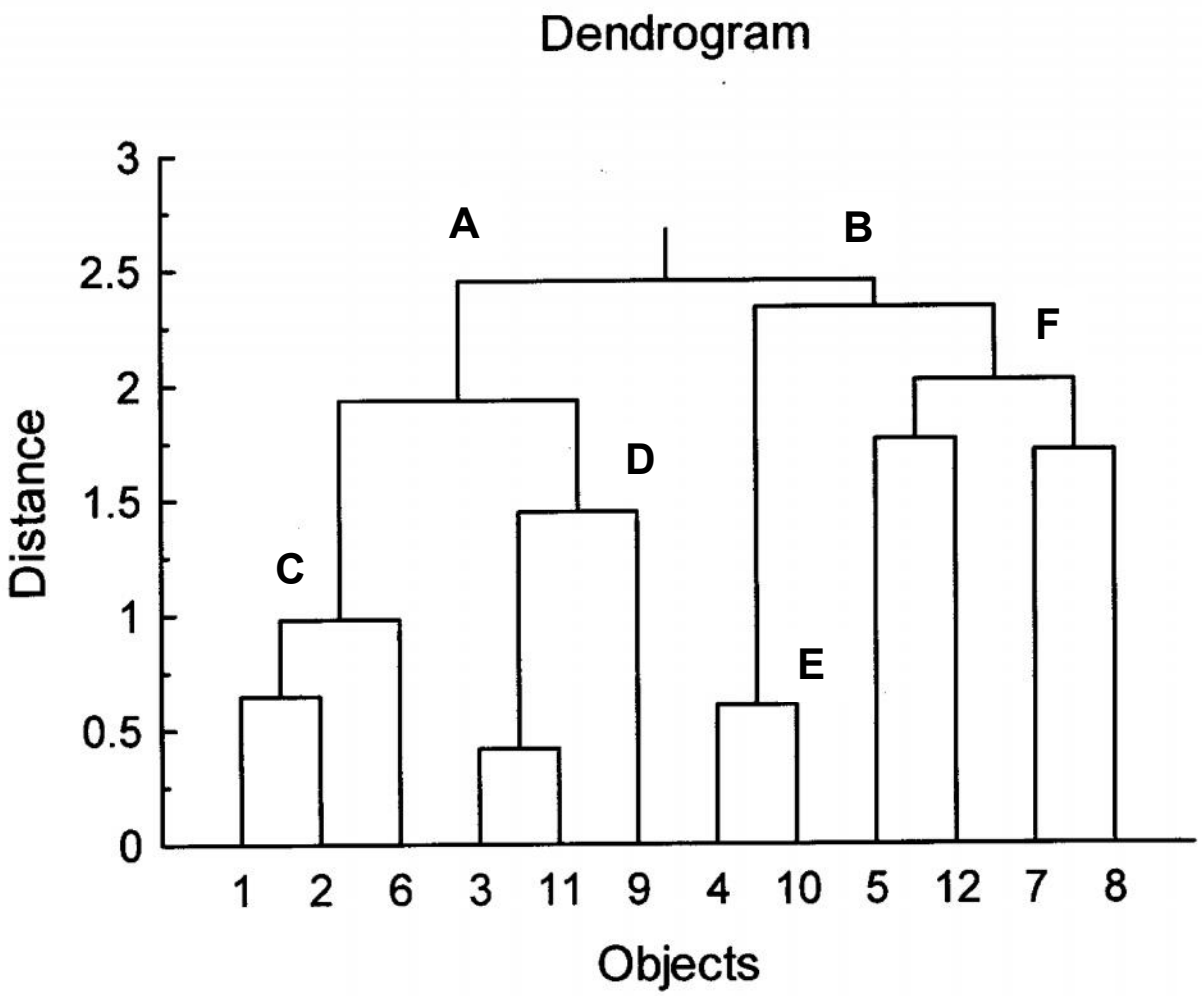

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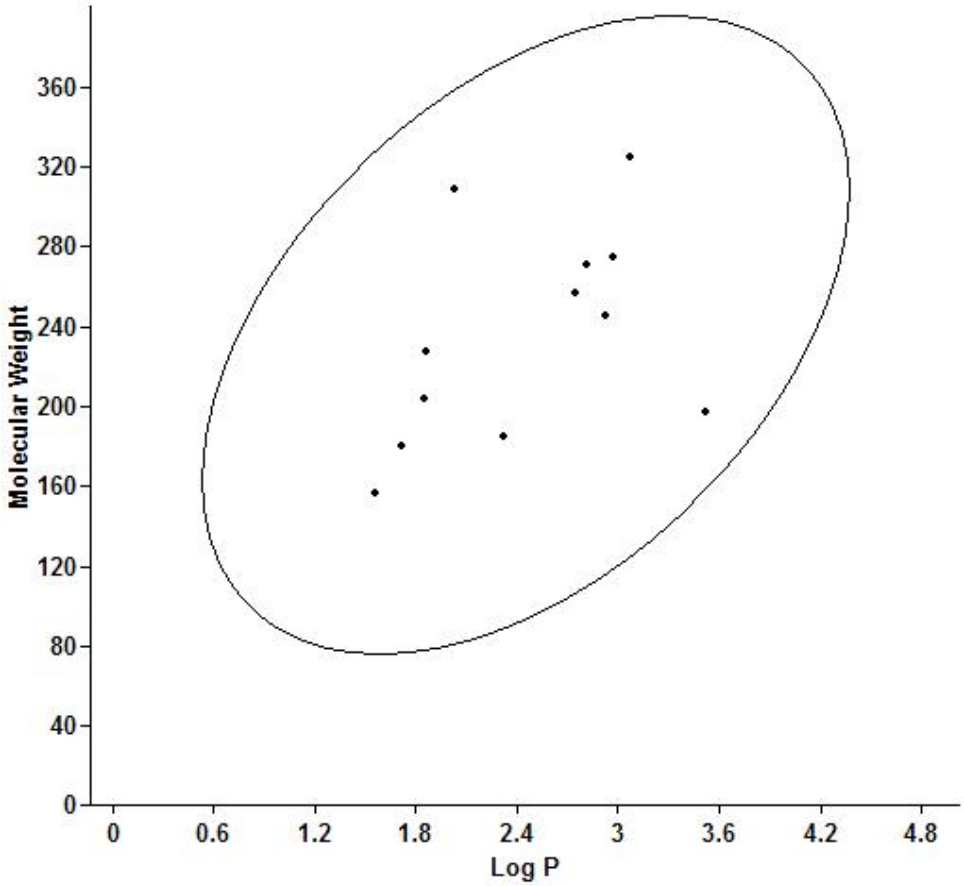
FIGURE 3



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783 # Table 1. Molecular Properties

Drug	Log P	Molecular Weight	Polar Surface Area (Angstroms^2)	Molecular Volume (Angstroms^3)	Nitrogen & Oxygen Atoms	Violations Rule of Five
1, Mechlorethamine	1.554	156.06	3.238	140.05	1	0
2	1.713	180.078	3.238	158.758	1	0
3	2.739	256.242	3.238	227.821	1	0
4	2.972	274.191	20.309	243.693	2	0
5	1.854	227.18	6.476	216.01	2	0
6	2.32	184.11	3.238	169.86	1	0
7	3.07	324.12	6.476	277.166	2	0
8	2.03	308.04	9.5	274.26	2	0
9	3.52	197.07	5.14	200.53	1	0
10	2.81	270.07	22.24	266.03	2	0
11	2.92	245.07	4.73	230.4	1	0
12	1.85	202.97	15.14	161.33	2	0

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789 ## TABLE 2 Numerical Values of Log(BB) and BB

Drug	Log (BB)	BB C_{brain}/C_{blood}
1, Mechlorethamine	0.327	2.12
2	0.351	2.24
3	0.507	3.21
4	0.290	1.95
5	0.325	2.11
6	0.444	2.78
7	0.509	3.23
8	0.307	2.03
9	0.598	3.96
10	0.237	1.73
11	0.513	3.26
12	0.196	1.57

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