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

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## ABSTRACT

**Aims:** Brain and spinal cord tumors are the third most common type of childhood cancer following leukemia and lymphoma. Mechlorethamine (or mustine) is a nitrogen mustard antineoplastic drug. Eleven variants of mechlorethamine are presented that possess molecular properties enabling substantial access to tumors of the central nervous system.

**Study design:**An extensive in silico search within a data library of molecular structures identifieddrug scaffolds suitable for targeting brain tumors.

**Place and Duration of Study:**University of Nebraska, Durham Science Center, Department of Chemistry, Omaha, Nebraska 68182 USA, between July 2012 to December 2012.

**Methodology:**Following extensive in silico search and identification of potential drug structures, a 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 $A^2$to 22.24$A^2$, respectively. 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 $A^2$, and 213.8 $A^3$, respectively.

**Conclusion:** These eleven drug designs possess attributes that effectuate high permeation into the central nervous system.

*Keywords: brain tumors, astrocytomas, glioma, mechlorethamine, mustine*

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## 1. INTRODUCTION

Brain and spinal cord tumors are the third most common type of childhoodcancer, with only leukemia and lymphoma havinggreater frequency. Cancers that occur in the central nervous system (CNS) can be primary (tumors thatbegin in the CNS) and metastatic (tumors formed from cancer cells beginningin other parts of the body). The various 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 neuroectodermal tumors.

There are about 20,000 new cases of primary central nervous system tumors in the United States every year [1]. The growth of the tumors located in the central nervous system cause considerable strain on other structures, therefore any observed symptoms depend on the location of tumor itself [1]. The symptoms can vary but include: confusion, headache, nausea, vomiting papilledema, seizures, and cognitive impairment [1].

Metastases based tumors are the most common type of cancers of the CNSand theyappear to be on theincrease [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 of women in theUnited States, the total incidence of brain metastases from breast cancer isa significant 30% [4]. In addition, the incidence of brain metastases is on the increasewith breast cancer patients [4]. The development of CNS metastases with breastcancer depends on prognostic factors that include age and negative hormonereceptor status [5]. However, patients having breast cancer with intramedullaryspinal 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 incurring CNS metastasesas the first site of relapse [7]. These incidents of CNS metastases rarelybenefit from systemic therapy due to lack of penetration into the CNS by theapplied chemotherapeutics [7]. The pursuit of novel drugs for treatment ofmelanoma is focused on those agents having effective antitumor activity inaddition to the capability of crossing the blood-brain barrier of the CNS [7].Autopsy resultshave shown that up to two thirds of allcases of metastatic melanoma do have CNS involvement [8].

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

These outcomes of clinical studies clearly reveal the need for novel antitumoragents that have effective antineoplastic activity but with molecular propertiesenabling the penetration of the CNS. Albeit the difficulties of CNS penetrationis substantial due to the blood brain barrier, the design of molecular structuresthat can effectuate CNS infiltration are crucial for the treatment of pediatricbrain tumors.

## 2. MATERIAL 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; polarsurface area, violations of Rule of 5, molecular volume, number of oxygens, nitrogens, amines, hydroxyls, etc were determined using Molinspiration (MolinspirationChemiformatics, Nova ulica 61, SK-900 26 SlovenskyGrob, Slovak Republic; http://www.molinspiration.com/cgi-bin/properties). In silicostructure 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 USA; http://molsoft.com/cgi-bin/msearch.cgi).

### 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 GraphPadInstat v. 3.00 for Windows 95 (GraphPad Software Copyright 1992-1998, San Diego California USA;www.graphpad.com).

## 3. RESULTS AND DISCUSSION

With the appearance of brain metastases occurring in up to 40% of cancerpatients (this frequency increasing) [11], the investigation of new cytotoxicagents is clearly warranted. Lung cancer, breast cancer, and skin melanoma are the commonest sources of brain metastases [11]. While whole brainradiotherapy (WBRT), with or without surgery, and systemic chemotherapyhave levels of success, the later neurotoxicity of WBRT treatment is notinsignificant [11,12]. The prompt elimination of tumors by using multiple drugs that are given concurrently reduces the likelihood of the emergence of resistant clones [13]. As survival increases the impact of long-term treatment-related morbidity and mortality increases dramatically and it is imperative to keep alkylator type drugs and radiation therapy doses as low as possible without sacrificing efficacy [13].

Mechlorethamine, vincristine, procarbazine, and prednisone (MOPP) fortreatment of childhood brain tumors has been shown to be well toleratedand improves neurodevelopmental outcome [14] and postpones thedebilitating consequences of radiotherapy [15]. Clinical evidence supportivefor 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]. Therefore utilizing mechlorethamine as the parentstructure for thedesign of similar compounds having analogous molecularproperties would be advantageous.

The compound mechlorethamine is also known as mustine or mustargen (see structure 1, Figure 1) and is a bifunctional alkylating nitrogen mustard agent having antineoplastic as well as immunosuppressive activity [16].It has 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 silicostructure 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 out(see Figure 1).

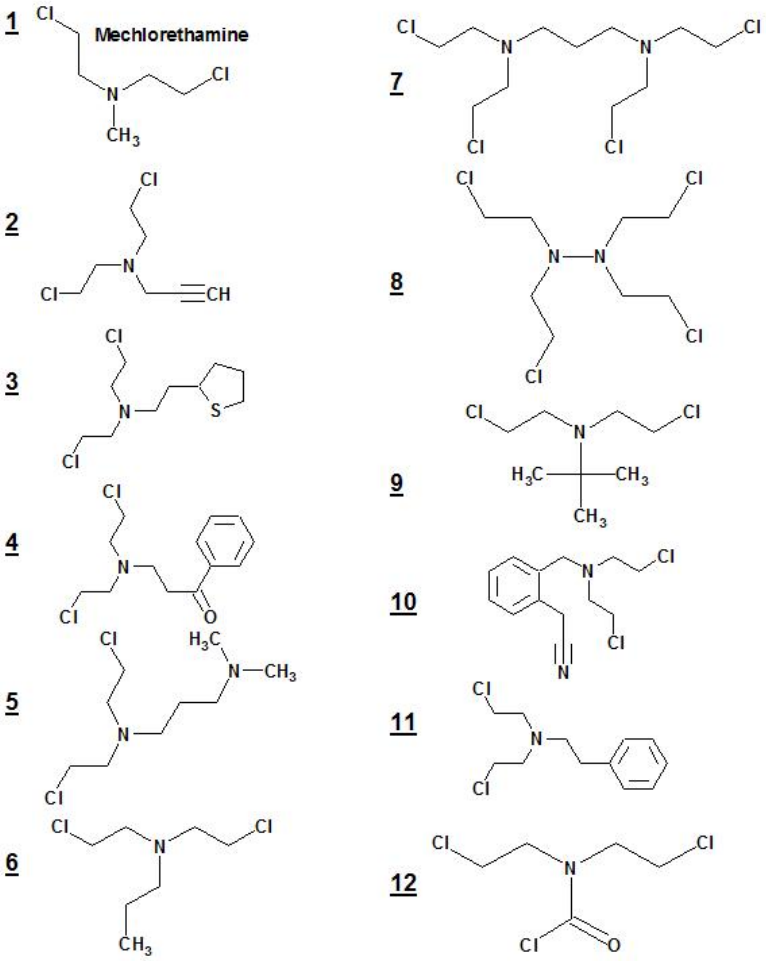


**Fig. 1. The scaffold of mechlorethamine (1) serves as source construct for substituent optimization producing 11 daughter compounds 2 to 12. The compoundshave diverse types of substituents: aromatic, aliphatic, amine, and carbonyl. Resultant molecular properties further substantiate the in silico search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

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 as well asother substructures. 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 drugdesigns that will permeate the CNS.

Molecular properties have been utilized to enhance filtering of drug candidatesby druglikeness and pharmacodynamics to stymie specific physiological abnormalities. For evaluation of bioavailability and measurement of CNSpermeation various molecular properties are shown in Table 1, that includeLog P (measurement of lipophilic activity), molecular weight, polar surfacearea (PSA), and violations of Rule of 5. Values of Log P have a strong positivecorrelation with molecular weight (Pearson r = 0.4551) and molecular volume(Pearson r = 0.4429). Molecular weight has a very strong positive correlationto molecular volume (Pearson r = 0.9492) and strong positive correlation tonumber of oxygen and nitrogen atoms (Pearson r = 0.6365). Polar surfacearea has a verystrong positive correlation to molecular volume (Pearson r = 0.7272).

**Table 1. Molecular Properties**

| Drug | Log P | Molecular Weight | Polar Surface Area(Angstroms$^2$) | Molecular Volume (Angstroms$^3$) | Nitrogen &Oxygen Atoms | Violations of the 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 |

Molecular polar surface area is a property that has been shown to correlate well with passive molecular transport through cellular membranes, allowingprediction 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 studiesconcluded 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 valueis 22.24 Angstroms$^2$ of agent 10).

The Rule of Five is developed to evaluate drug-likeness (a chemical compound with a certain pharmacological or biologicalactivity), and properties that would make it a likely orally activedrug in humans [23]. Drug-likenessis 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 Ruleof 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 statisticaldata 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.

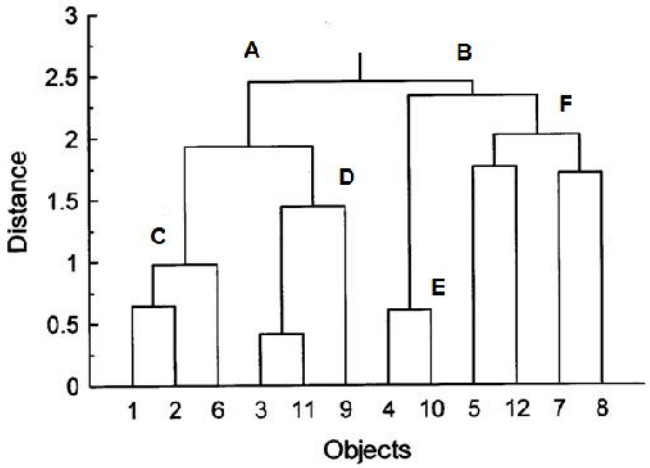


**Fig. 2.DENDROGRAM. Hierarchical cluster analysis (Euclidean distance and single linkage cluster parameters) of molecular properties (see Table 1) show with high resolution the assimilation by mutual similarity. Albeit the molecular properties (see Table 1) indicate very high numerical correlation,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

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. These types of pattern recognition analysis bring about more proficientordination that 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[11,12].

The degree of blood-brain barrier (BBB) penetration is commonly assessedas 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 drugin the brain ÷ concentration of drug in blood) [24]. The determinations of Log (BB) and BB for drugs 1 to 12 are presented in Table 2.

**Table 2. Numerical Values of Log(BB) and BB**

| | | BB |
|---|---|---|
| **Drug** | **Log (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 |

Notably the values of BB are high, all values of BB are greater than one whichindicates drugs 1 to 12 will likely have greater partitioning within the CNS thanthe 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 haveLog (BB) values greater than 0.3 are shown to readily cross the BBB [24]. Notethat nine of the 12 agents of Figure 1 have Log (BB) greater than 0.3, they are1 (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 relationshipsare determined to valid for passive diffusion consideration [24].

Orally active drugs expected to transport passively by transcellular routeshould not have PSA exceeding 120 Angstroms$^2$ [25]. For purposes of crossingthe 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 componentsthat include polarity, molecular size, polarizability, and hydrogen bonding. Previous studies have shown distinctly that small molecules penetrate theblood 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 areasare far less than 90 Angstroms$^2$. Therefore all molecules 1 to 12 are determinedto have highly efficient access to the central nervous system. Structures 7and 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 homologousseries (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 otherfeatures of this group of nitrogen mustard agents are described previously [28].

Two functions of multiple regression analysis are: 1) explanation of relationshipamong multiple independent variables, and 2) prediction by utilizing multipleindependent variables. By applying the molecular properties presented inTable 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 modelvariance. The formula weight of additional similar structures can be estimatedby 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= 1.00 or a large positive R (up to 1) signifying significant dissimilarity among these agents based on their physicochemical values [29].

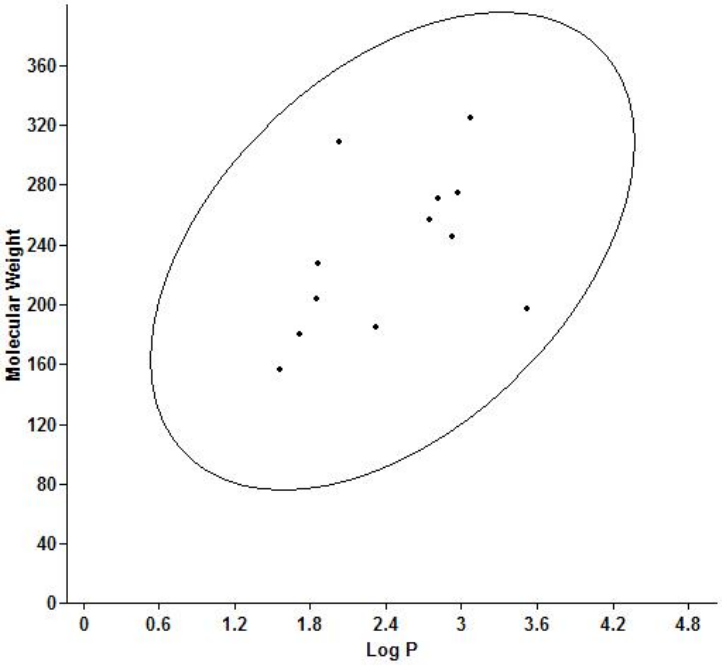


**Fig. 3. Two-way plot of Log P and molecular weight indicates complete inclusion into 95% ellipses. Thus indicating relationship of lipophilicity to molecular weight is inclusive within 95% confidence.**

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

## 4. CONCLUSION

In summation, a set of eleven novel drug structures are elucidated byin silico optimized substituent search that is founded on the successful anticancer nitrogen mustard scaffold of mechlorethamine. Brain metastaseshas been linked to breast cancer, advanced melanoma, and colorectal cancer.Various molecular properties that enable the transition from blood to CNShave been identified and found to be optimal for the twelve agents reportedhere. The Log P numerical values fall between 1.554 to 3.52 which is arange well within the BBB piercing range of 1.0 to 4.00. In addition the values ofPSA range from 3.238 to 22.24 Angstroms$^2$, which isa range well below the upperlimit for effective CNS penetration at 90 Angstroms$^2$. Importantly all twelveagents present zero violations of the Rule of 5, indicating a high level ofdrug-likeness and favorable bioavailability. The success rate of in silico searchand identification of suitable CNS targeting antineoplastic structures was lessthan ten percent. Various attributes recounting the inherit potential of small molecules applied as chemotherapeutic agents in the treatment of CNS tumors

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## COMPETING INTERESTS

The authors declares no competing interests.

## AUTHOR CONTRIBUTIONS

The author Dr. Ronald Bartzatt performed the study and prepared the manuscript according to required directions.

## CONSENT

Not applicable.

## ETHICAL APPROVAL

Not applicable.

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## ABBREVIATIONS

PSA, polar surface area; CNS, central nervous system; MV, molecular volume; NO, number of nitrogen and oxygen atoms; BBB, blood-brain barrier; BB, value of brain over plasma concentration ratio; Log(BB), logarithmic value of BB; CRC, colorectal cancer.