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Synthesis, alkylating activity and physicochemical study of nitrogen mustard agent for brain delivery

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Research Article

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Abstract

On the structural pattern of nikethamide which is a potent central nervous system (CNS) stimulant, it was designed to link *N,N*-bis(2-chloroethyl)amino moiety as alkylating agent to nicotinic acid by an amide bond in the hope to obtain a CNS antitumor agent (Nicotinic-mustard). The Nicotinic-mustard agent was solid at room temperature and stable for more than one week when stored at less than 0^{o}C. The *in vitro* chemical alkylation activity (NBP) of Nicotinic-mustard was comparable to that of N,N-bis(2-chloroethyl)amine as standard alkylating agent. The log P value of Nicotinic-mustard was analyzed (miLogP and MlogP) and found to be increased lipophilicity for the Nicotinic-mustard construct compared with the parent compound nicotinic acid. Value of polar surface area for the Nicotinic-mustard agent (33 A^2) predicts that >90% of any amount present in the intestinal tract will be absorbed. The physicochemical potential of this compound to penetrate the BBB such as Log BB, Polar surface area, rule of five, number of NH or OH hydrogen bond donors and no value was computed through online software program and the values obtained for the Nicotinic-mustard suggest a good brain penetration.

Keywords: *Nicotinic acid, Nitrogen mustard, Blood-brain barrier, NBP assay, Physicochemical parameters.*

Introduction

Nitrogen mustard (Mustine) containing the established anticancer moiety [N,N$^{\circ}$-bis(2-chloroethyl)amino] is the one of the most active and widely used alkylating anticancer agents. At present nitrogen mustard and its derivatives (mechlorethamine,chlorambucil, cyclophosphamide, melphalan) are commonly used for the treatment of all types of cancer including cervix, breast and prostate cancer [1,2]. But nitrogen mustard is too polar to cross the highly lipophilic blood-brain barrier (BBB). The BBB comprises the endothelial lining of the microvessels in the brain, pericytes and astrocytes, and is the main barrier to drug transport into the CNS especially for labile hydrophilic compounds. In fact, it is estimated that only about 2% of potential CNS compounds can penetrate the BBB[3]. The physicochemical requirements for a small molecule to diffuse passively across the BBB have been well studied, and guidelines for these properties have been postulated. In general, the BBB will not allow compounds to pass into the CNS that are highly polar, have a large number of hydrogen bond donor groups, or that carries several charges, unless they are specifically taken up by an active transport system. This means that a great many potential drug with high efficacy in *vitro* has little or no activity *in vivo* [4].

Various attempts have been made to overcome the limited access of drugs into the brain and consequently, reduce the systemic side effects. Some of this attempt is to link the active agent to a brain-specific carrier, which delivers the drug specifically into the brain, where it is cleaved enzymatically from the carrier.

The principle of using a carrier for nitrogen mustard, is not new and thiosemicarbazone and hydrazone compounds[5], benzaldeyde[6] aromatic rings[7,8], natural products[9], heterocyclic rings such as phenytoin[10], naphthalimides[11], barbiturates[12], acridines[13] and uracils[14] have already been used as a carrier for nitrogen mustard. Apart of this, sugars, proteins and steroids[15,16] have also been exploited for nitrogen mustard for targeted delivery.

Nicotinic acid is a vitamin B that is water soluble. It is required for the synthesis of co-enzymes used by dehydrogenase in tissue respiration. Ronald L. Bartzatt[8] studied the nitrogen mustard agent linked to nicotinic acid as a carrier through an ester linkage in which 2-[bis(2-chloroethyl)amino]ethanol was the active moiety released. The study of nikethamide in which nictonic acid is amidated with diethylamine shows a CNS stimulant activity. On the continuation

of our work for the synthesis of CNS active agents[17-23] and on the same structural pattern of nikethamide, we designed and synthesized nicotinic acid mustard in which bis(2-chloroethyl)amine) as active alkylating moiety is directly attached to nicotinic acid by amide linkage in the hope to obtain a CNS active anticancer agent. **Fig 1.**

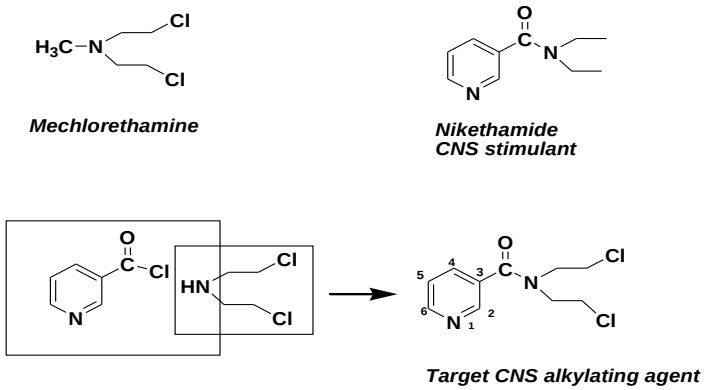

Fig 1. Design of Nicotinic-mustard agent

Material and Method

Experimental Section

This study was carried out from 10th September 2009 to 15th November 2009 in the Pharmaceutical Chemistry Division of Shivalik College of Pharmacy, Nangal. All reagents and chemical were obtained from Himedia and CDH Chemical Laboratory. All the solvents were dried and freshly distilled prior to use according to standard procedure. All glass apparatus used for the preparation of the amide was dried at 120° C for 24 hours.

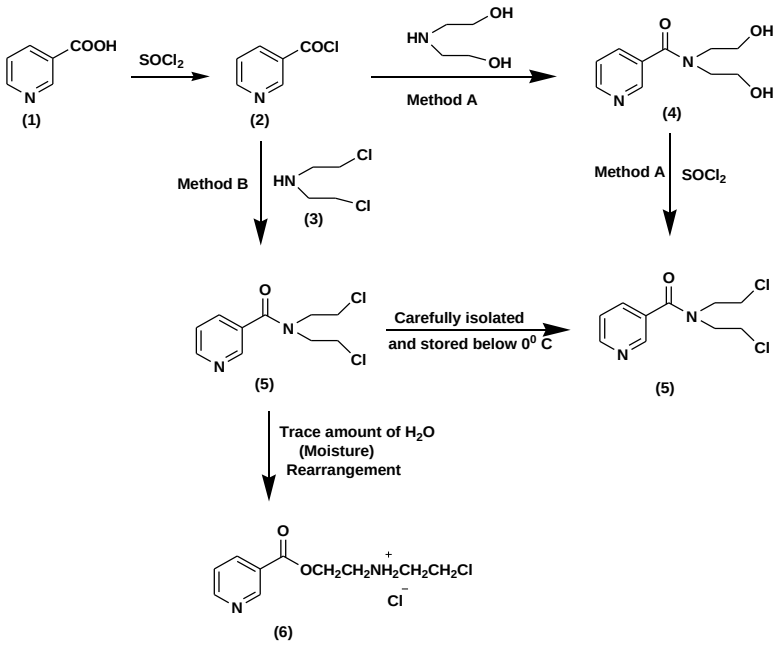

Fig 2. Scheme for the synthesis of Nicotinic-mustard agent

Synthesis of N, N-bis(2-chloroethyl)nicotinamide (5)

The synthesis of N, N-bis(2-chloroethyl)nicotinamide **(5)** its rearranged product N-[2-(2-chloroethylamino)ethoxycarbonyl]pyridine hydrochloride **(6)** have been previously described and characterized[24].

NBP alkylation assay

The alkylation activity of prepared Nicotinic-mustard was determined as per the given literature procedure[11]. Thus a solution of Nicotinic-mustard or N-di(2-chloroethyl amine)[-NH($CH_2CH_2Cl)_2$] in different concentration as indicated in **Table 1** in acetone (1 ml), distilled water (1ml) and acetate buffer (1 ml, 0.25 M, pH 6.0) were incubated at 100 °C for 20 min with a solution of 4-(4-nitro-benzyl) pyridine (NBP) (5% w/v) in acetone (0.4 ml) and cooled to 25 °C. After the addition of acetone (2 ml), ethyl acetate (5 ml) and sodium hydroxide solution (0.25 M, 1.5 ml), the reaction mixture was vortexed and allowed to stand to separate the organic layers. The absorabance in the organic layers were determined (within 2 min of NaOH addition) at 545 nm. The experiments were carried out in triplicates. The result was expressed in absorbance value (mean±S. E. M., n=3 in all the cases) **Table 1.**

Table 1. Determination of Chemical Alkylating Activity Expressed in Absorbance

Descriptor	Concentration of Compounds (µmol/ml)	
	0.50	1.0
Nicotinic-mustard	0.52±0.05	0.74±0.03
N-di(2-chloro ethyl)amine	0.46 ± 0.07	0.67±0.04
Blank	0.07 ± 0.02	0.08±0.02

Calculation of MlogP

MlogP was calculated by the the method of Moriguchi *et al.,* 1992[24] by the formula:

MlogP=1.464(CX)$^{0.6}$-1.221(NO)$^{0.9}$x0.653(PRX)-0.300(UB)$^{0.8}$+0.335(POL)+0.726(ALK-0.269(RNG)-1.358)

CX = summation of cabon and halogen atoms:

NO= total number of nitrogen and oxygem atoms

PRX = proximity effect

UB= total number of unsaturated bonds

POL= number of polar substituents

ALK= alkane, alkene, cycloalkene, cycloalkane dummy variable

RNG= ring structures

Calculation of Lipophilic Substituent Constant (LSC)

Also known as hydrophobic substituent constant, calculated utilizing the following algorithm:

LSC= π=Log P_D-Log P_P

Where Log P_D is the partition coefficient of the Nicotinic-mustard agent and Log P_P is the partition coefficient of the nicotinic acid (parent compound).

Calculation of Log BB

Log BB was calculated by the method of (Clark 1999)[25] by the formula:

Log BB = -0.0145 PSA + 0.172 Mlog P+0.131

(PSA= Polar surface area)

(Mlog P= Moriguchi partition coefficient)

Results and Discussion

Chemistry and spectral characterization

The synthesis and characterization of the title compounds by two different routes have been described earlier[20]. The Nicotinic-mustard **(5)** formed was very hygroscopic, so isolation of pure drug was most favorable during period of low laboratory humidity. It was sensitive to trace amounts of water and readily rearranged to 2-(2ó-chloroethylaminoethyl)esters **(6)**. This observation is like amide-ester rearrangement reaction as observed by Pettit et al, 1963[26]. The mechanism of the rearrangement is probably similar for the *N*-bis(2-chloroethyl)amides as shown in **fig 4** and is applicable to both the esters and the carboxylic acid. The latter transformation may generally provide useful route to stable derivatives of nitrogen mustard amides. The Nicotinic-mustard agent was solid at room temperature and stable for more than one week when stored at less than 0°C.

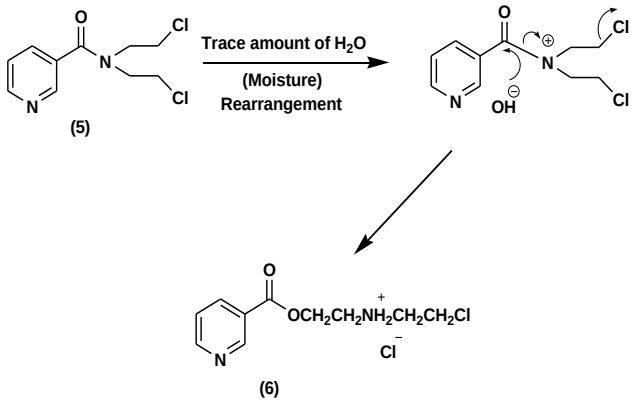

Fig 4 Mechanism of rearrangement

Alkylating activity assessment

The final compound was evaluated by its alkylating activity using 4-(4-nitro-benzyl) pyridine (NBP) as an analytical reagent by reported procedure[11] using spectrophotometric quantitation. The 4-(4-nitro-benzyl)pyridine reacts with alkylating agents and gives a purple color upon basification (Fig 5). The intensity of the color produced is directly proportional to the degree of alkylation. It is hypothesized that there is a correlation between the chemical alkylating activity and anti-tumor activity. The Nicotinic-mustard agent proved to be an active alkylating activity comparable to that of *N*-di(2-chloroethyl)amine as standard alkylating compound.

Physicochemical characterization

Since the target compound is designed to be CNS active, hence the parameter were selected which effect BBB.

Lipophilicity is one of the most important factors in controlling the interaction of drugs with biological system. Lipophilicity was the first of the descriptors to be identified as important for CNS penetration. The value of various partition coefficients of Nicotinic-mustard (miLogP, MLogP) is determined (**Table 4).** All methods show a significant increase of the lipophilic nature of the Nicotinic-mustard construct compared with the parent compound nicotinic acid. The increased lipophilicity of the Nicotinic-mustard agent is due to the addition of the Nicotinic-mustard substituent (a positive lipophilic substituent constant (LSC) is obtained). Accordingly, it was expected that Nicotinic-mustard by virtue of its enhanced lipid solubility, would cross the BBB.

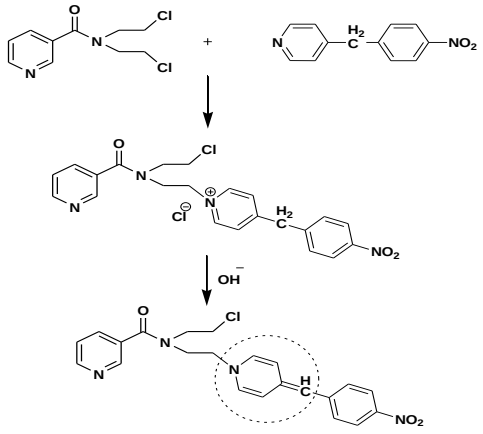

Fig 5**.** NBP reacts specifically with alkylating agent to produce chromophore upon basification with sodium hydroxide (see broken oval) giving purple color and has a strong absorbance peak at 545 nm

Table 4. Comparison of partition coefficient for nicotinic acid and Nicotinic-mustard Agent

Descriptor	Nicotinic acid	Nicotinic-mustard agent
miLog Pa	0.273	0.879
MLog P[b]	0.200	1.06
Lipophilic Substituent Constant[c] (LSC)	-	0.606

[a]Calculated by method of Molinspiration. www.molinspiration.com/cgi-bin/properties.

[b]Calculated by method of I. Moriguchi.

[c]LSC= Log $P_{(Derivative)}$-Log $P_{(Parent)}$, and Partition coefficients of Molinspiration

Other physicochemical descriptors presented in **Table 5** support the clinical potential of this N-

mustard agent. PSA has become an important and accurate parameter for predicting brain penetration and its numerical value is inversely correlated with BBB penetration[27-28] that is, as PSA increases, BBB penetration decreases. Most acts of CNS drug will have a PSA of less than 70 A^2. PSA of our Nicotinic-mustard compound 33 A^2, which predicts that greater than 90% of this agent will be absorbed by the intestine.

Partitioning of drugs between blood and CNS can be expressed in concentration terms C_{brain}/C_{blood} (or BB), which can be utilized as Log BB similar to Log P. Equation estimating Log BB include the following [24].

Log BB = -0.0145 PSA + 0.172 Mlog P+0.131

(Mlog P= Moriguchi partition coefficient)

The parameter BB or C_{brain}/C_{blood} is increased for the Nicotinic-mustard agent 0.683 relative to nicotinic acid of 0.329 (more than 100% increase).

In accordance with Lipkinski's rule of 5[29], the molecular weights of compounds should have <500 Daltons, <5 hydrgen bond donors, <10 hydrogen bond acceptors and logP of <5. Compounds that satisfy these rules are considered drug-like. According to this rule, compounds with the number of violations not more than 1 shows good bioavailability and bioactivity. Highest drug bioavailability and bioactivity is achieved when there are no violations of the following rules. Analysis of molecular structure by Molinspiration showed that both Nicotinic-mustard and its parent compound have zero violations of the rule of 5 (**Table 5**). The target compound Nicotinic-mustard has no NH or OH hydrogen bond donors which show increased solubility in cellular membranes. The target compound has nON value 3 which is <10 and has molecular weight 247 which is <500 preferable for the compound to be CNS active. All these properties could permit a better penetration of the drug through the BBB.

Table 5. Comparison of physicochemical parameters of nicotinic acid and nicotinic-mustard agent

Descriptors	Nicotinic acid	Nicotinic-mustard agent
Polar surface area[a]	50.19 A^2	33.20 A^2
Percent intestinal absorption[b] of drug	>90%	>90%
Log BB[c]	-0.5625	-0.1655
Lipophilic substituent Constant[d]	-	0.606
BB[c]= C_{brain}/C_{blood}	0.329	0.683
Molecular weight[a]	123.1	247.12
No of Violation[a] of rule of 5		0
-NH and –OH[a]	1	0
nON values[a]	3	3

[a]Calculated by method of molinspiration Molinspiration Cheminformatics, Bratislava, Slovak Republic, Available from: http://www.molinspiration.com/servies/properties.html

[b]Calculated by correlation of PSA to experimental intestinal absorption.

[c]Log BB, where BB= C_{brain}/C_{blood} , Log BB= Log BB = -0.0145 PSA + 0.172 Mlog P+0.131

[d]LSC= Log $P_{(Derivative)}$-Log $P_{(Parent)}$, and Partition coefficients of Molinspiration

Conclusion

Nicotinic acid linked nitrogen mustard was designed and synthesized as CNS active antitumor agent. The *in vitro* chemical alkylating activity (NBP) and physicochemical studies supported the applicability of this agent as CNS antitumor agent. There is need to synthesize various substituted derivatives to enhance the stability of this compound and evaluated further for the *in vitro* cytotoxicity studies.

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AUTHORS' CONTRIBUTIONS

Authors contributed equally to all aspects of the study.

PEER REVIEW

Not commissioned; externally peer reviewed.

CONFLICTS OF INTEREST

The authors declare that they have no competing interests.