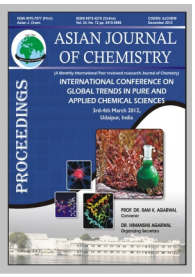
Asian Journal of Chemistry; Vol. 24, No. 12 (2012), 5605-5608

ASIAN JOURNAL OF CHEMISTRY

www.asianjournalofchemistry.co.in



Synthesis, Alkylation Activity and Physico-chemical Evaluation of Benzodiazepine Linked Nitrogen Mustard Agent to Penetrate the Blood-Brain Barrier†

RAJESH K. SINGH[1,*], D.N. PRASAD[1] and T.R. BHARDWAJ[2]

[1]Pharmaceutical Chemistry Division, Shivalik College of Pharmacy, Nangal-140 126, India
[2]Indo-Soviet Friendship College of Pharmacy, Moga-142 001, India

*Corresponding author: E-mail: rksingh244@gmail.com

AJC-11707

The design of drugs for the chemotherapy of tumours of the central nervous system contains numerous challenges. Most of clinical alkylating anticancer agent, such as nitrogen mustard (mustine) is too polar to cross blood-brain barrier. So it is aimed to link nitrogen mustard to CNS active 1,4-benzodiazepine carrier to obtain CNS active benzodiazepine derivative of nitrogen mustard. The benzodiazepine-mustard agent was oily at room temperature and stable when stored at less than 0 ºC. Structures of all the synthesized compounds were confirmed by UV, IR and ^{1}H NMR spectroscopy. The *in vitro* chemical alkylation activity studies (NBP) of benzodiazepine-mustard was comparable to that of *N,N-bis*-(2-chloroethyl)amino moiety as standard alkylating agent. The log P value of benzo-mustard determined experimentally is significantly higher than nordiazepam. Value of polar surface area for the benzo-mustard agent (35.9 A^2) predicts that > 90 % of any amount present in the intestinal tract will be absorbed. The study of some physico-chemical properties calculated by online software such as lipophilicity, log BB (0.295), no. of violation of Lipinski's rule of five (0), number of NH or OH hydrogen bond donors (0) and nON value (4) also indicates that it can be a potential candidate for targeted delivery of nitrogen mustard to the brain for the treatment of brain tumour.

Key Words: 1,4-Benzodiazepine, Nitrogen mustard, Blood-brain barrier, Physico-chemical parameters.

INTRODUCTION

The design of drugs for the chemotherapy of tumours of the central nervous system contains numerous challenges. Various attempts have been made to overcome the limited access of drugs into the brain and consequently, reduce the systemic side effects. The 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. Nitrogen mustard is one of the most active and widely used alkylating anticancer agents. At present nitrogen mustard and its derivatives 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 BBB. One approach to overcome this problem and site specific delivery of nitrogen mustard to brain is to attach this cytotoxic moiety to a suitable carrier, which accumulate in brain tumor tissue. Peripheral benzodiazepine receptors are located on the outer membrane of mitochondria and their density is increased in brain tumors[3]. Thus, they may serve as a unique intracellular and selective target for antineoplastic agents. Moreover recent study reveals that some benzodiazepines derivatives have antiproliferative activities[4]. All these aspects prompted us to link nitrogen mustards to CNS active 1,4-benzodiazepines derivative of nitrogen mustard (Fig. 1).

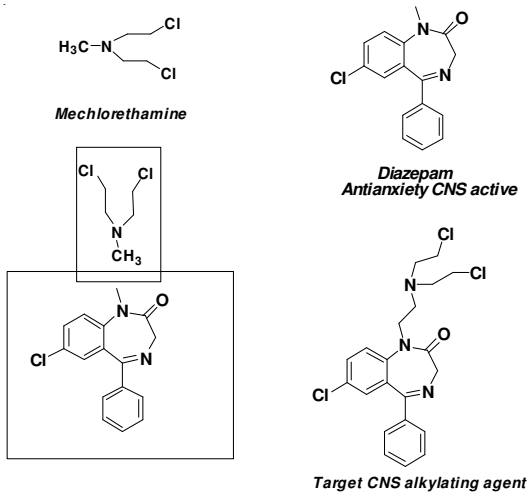


Fig. 1. Design of benzo-mustard agent

†Presented at International Conference on Global Trends in Pure and Applied Chemical Sciences, 3-4 March, 2012; Udaipur, India

EXPERIMENTAL

The melting points were determined on Veego-programmable melting point apparatus (microprocessor based) and are uncorrected. ^{1}H-NMR spectra were obtained using Brucker AC-400 F, 400 MHZ spectrometer and are reported ppm, downfield from tetramethylsilane (TMS) as internal standard. Infrared spectra were obtained with Perkin-Elmer 882 Spectrum and RXI, FT-IR model using a potassium bromide pellets (cm^{-1}). The ultraviolet spectra were recorded on Shimadzu, UV-1800 spectrophotometer. A computational study of titled compounds was performed for prediction of ADME properties. Polar surface area (TPSA), milog P, number of rotatable bonds, molecular volume, number of hydrogen donor and acceptor atoms and violations of Lipinski's rule of five were calculated using Molinspiration online property calculation tool kit.

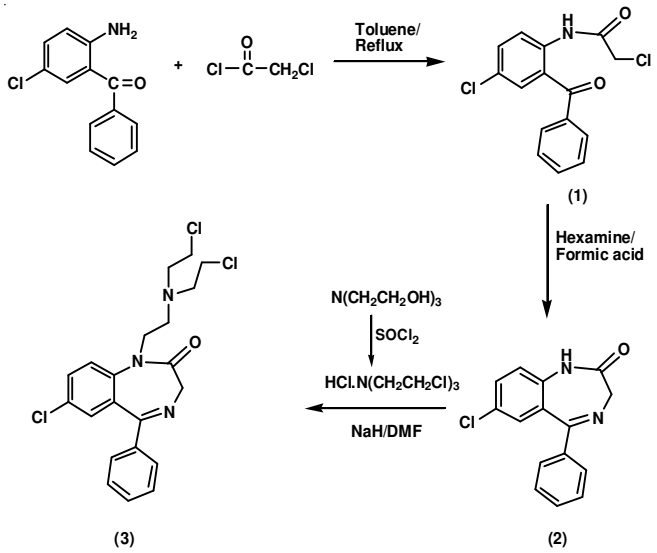


Fig. 2. Scheme for the synthesis of benzo-mustard agent

Synthesis of 7-chloro-1-[2-{*bis*-(2'-chloroethyl) amino}-ethyl]-3-hydro-5-phenyl-benzo-1,4-diazepin-2-one (3) (benzo-mustard): To a solution of 7-chloro-1,3-dihydro-5-phenyl-benzo-1,4-diazepin-2-one[5] (**2**) (2.69 g, 10 mmol) in DMF, sodium hydride (60 % in mineral oil, 0.36 g, 15 mmol) was added and the mixture was stirred for 5 min. *Tris*-(2-chloroethyl) amine hydrochloride (3.07 g, 15 mmol) [previously been synthesized by chlorination of triethanolamine] in DMF was added slowly to the solution under nitrogen at room temperature and stirred for 10 h. The reaction was quenched by adding cold water (10.0 mL), the mixture was extracted with ethyl acetate (10.0 mL × 2) and the organic layer was washed with water (10.0 mL × 3) and dried with sodium sulfate. The solvent was evaporated to get the oily benzo-mustard (**3**), which could not be recrystallized. (yield, 1.08 g, 25 %). Anal.: UV_{max} (MeOH): 340 nm; IR (KBr, ν_{max}, cm^{-1}): 3038 (Aromatic C-H), 2955 (Aliphatic C-H), 1682 (C=O), 1477 (Aromatic C=C), 1178 (C-N) and 699 (C-Cl). ^{1}H NMR ($CDCl_3$): δ 2.73 (m, 6H, -N($CH_2CH_2Cl)_2$ + -CH_2CH_2N), δ 3.33 (t, 4H, -N($CH_2CH_2Cl)_2$), δ 3.8 (t, 2H, -CH_2CH_2N), δ 3.9 (s, 2H, -CO-CH_2-), δ 7.17-7.55 (m, 8H, ArH).

4-(4-Nitro-benzyl)pyridine alkylation assay: The alkylation activity of prepared benzo-mustard was determined as per the given literature procedure[6]. A solution of benzo-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 (1 mL) and acetate buffer (1 mL, 0.25 M, pH 6.0) were incubated at 100 ºC 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 ºC. 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 absorbance in the organic layers were determined (within 2 min. of NaOH addition) at 560 nm. The experiments were carried out in triplicates. The result were expressed in absorbance value (mean ± SEM, n = 3 in all the cases) Table-1.

TABLE-1
DETERMINATION OF CHEMICAL ALKYLATING ACTIVITY EXPRESSED IN ABSORBANCE

Descriptor	Concentration of compounds (μM/mL)	
	50	100
Benzo-mustard	0.54 ± 0.05	0.68 ± 0.03
N-di(2-chloroethyl)amine	0.48 ± 0.07	0.71 ± 0.04
Blank	0.07 ± 0.02	0.08 ± 0.02

Calculation of M log P: M log P was calculated by the method[7] of Moriguchi *et al*. by the formula:

$$M\ log\ P = 1.464(CX)^{0.6}\text{-}1.221(NO)^{0.9} \times 0.653(PRX)\text{-}0.300(UB)^{0.8} + 0.335\ (POL) + 0.726\ (ALK\text{-}0.269\ (RNG)\text{-}1.358)$$

where, 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 log BB: log BB was calculated by the method[8] of Clark 1999 by the formula:

$$log\ BB = -0.0145\ PSA + 0.172\ M\ log\ P + 0.131$$

where, PSA= Polar surface area, M log P = Moriguchi partition coefficient.

Determination of experimental partition coefficient: Partition coefficient was determined in between *n*-octanol and distilled water using modified procedure[9]. Various standard solutions of concentrations 4, 8, 12 and 16 μg/mL of benzo-mustard agent were prepared from stock solution (100 μg/mL) and absorbance was taken at λ_{max} 340 nm. Standard plot of benzo-mustard was prepared between absorbance and concentration in *n*-octanol (Fig. 3). Accurately weighed quantity of compounds (10 mg) was taken in glass stopper tubes containing equal volumes (50 mL) of distilled water and *n*-octanol. The tubes were shaken for 6 h using water bath shaker. After 24 h, organic phase was separated with the help of separating funnel. The absorbance was measured on UV spectrophotometer after making dilution to 10 μg/mL. The concentration of the test compound in the *n*-octanol layer was quantitated by UV spectroscopy as determined from the absorbance *versus* concentration curve. The partition coefficient was also calculated by K_p, where K_p is the test compound concentration in *n*-octanol/total concentration-concentration in *n*-octanol layer.

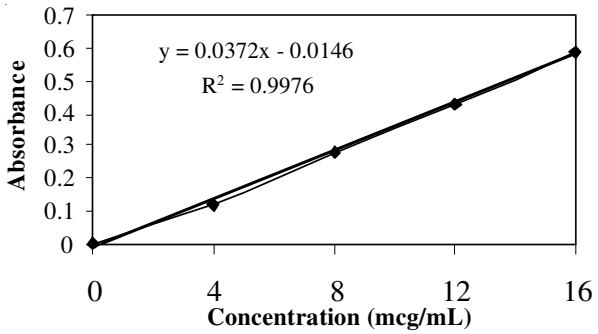


Fig. 3. Standard curve of benzo-mustard agent

RESULTS AND DISCUSSION

Benzo-mustard (**3**) was prepared (Fig. 2) by stirring the mixture of 7-chloro-1,3-dihydro-5-phenyl-benzo-1,4-diazepin-2-one (2), *tris*-(2-chloroethyl)amine hydrochloride and sodium hydride in DMF. The reaction mixture was then processed to get the product. In IR spectrum of the compound C-Cl stretching was found at 699 cm^{-1} and the stretching at 3300 cm^{-1} for N-H disappeared. From NMR spectrum, the final compound (**3**) was confirmed by disappearance of signal of NHCO- at δ 9.7 and appearance of relevant signals of δ 2.73 (m, 6H, $-N(CH_2CH_2Cl)_2$ + $-CH_2CH_2N$), δ 3.33 (t, 4H, $-N(CH_2CH_2Cl)_2$), δ 3.8 (t, 2H, $-CH_2CH_2N$), δ 3.9 (s, 2H, $-CO-CH_2-$), δ 7.17-7.55 (m, 8H, Ar*H*).

Alkylating activity assessment: The final compound was evaluated by its alkylating activity using 4-(4-nitro-benzyl) pyridine as an analytical reagent (Fig. 4). It is hypothesized that there is correlation between the chemical alkylating activity and antitumor activity (Table-1). The benzo-mustard agent proved to be active alkylating activity comparable to that of *N*-di(2-chloroethyl)amine as standard alkylating compound.

Physico-chemical characterization: Since target compound is designed to be CNS active, hence the parameters were selected, which effect blood-brain barrier. Lipophilicity is an important aspect of transport and distribution of drugs in biological systems. Table-2 presents the partition coefficient (log P) values for nordiazepam and benzo-mustard derivative

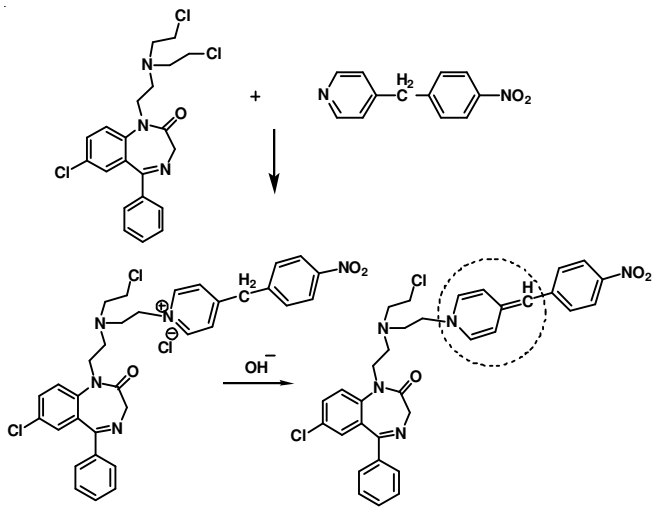


Fig. 4. NBP reacts specifically with alkylating agent to produce chromophore upon basification with sodium hydroxide (see broken oval) giving purple colour and has a strong absorbance peak at 560 nm

TABLE-1
DETERMINATION OF CHEMICAL ALKYLATING ACTIVITY EXPRESSED IN ABSORBANCE

Descriptor	Concentration of compounds (μM/mL)	
	50	100
Benzo-mustard	0.54 ± 0.05	0.68 ± 0.03
N-di(2-chloroethyl) amine	0.48 ± 0.07	0.71 ± 0.04
Blank	0.07 ± 0.02	0.08 ± 0.02

after calculation by various algorithms. All methods show a significant increase of lipophilic nature for the benzo-mustard construct compared with nordiazepam. The increased lipophilicity of the benzo-mustard agent is due to the addition of the mustard substituent (a positive lipophilic substituent constant (LSC) is obtained). Accordingly, it was expected that benzo-mustard by virtue of its enhanced lipid solubility, would cross the BBB.

TABLE-2
COMPARISON OF PARTITION COEFFICIENT FOR NORDIAZEPAM AND BENZO-MUSTARD AGENT

Descriptor	Nordiazepam	Benzo-mustard agent
M log P[1]	2.86	3.98
M log P[2]	2.75	3.84
Exp. log P[3]	-	4.10
Lipophilic substituent constant[4] (LSC)	-	1.12

[1]Calculated by method of Molinspiration; [2]Calculated by method of I. Moriguchi; [3]Experimental log P value determined (see experimental section); [4]LSC= log $P_{(Derivative)}$-log $P_{(Parent)}$ and Partition coefficients of Molinspiration

Other physico-chemical descriptors presented in Table-3 support the clinical potential of this *N*-mustard agent. Polar surface area has become an important and accurate parameter for predicting brain penetration and its numerical value is inversely correlated with BBB penetration[10] *i.e.* as polar surface area increases, BBB penetration decreases. The most active of CNS drug will have polar surface area of less than 70 A^2. Polar surface area of benzo-mustard compound 35.9 A^2, which predicts that greater than 90 % of this agent will be absorbed by the intestine. The endothelial cell linings of the BBB are tightly packed and maintain the homeostasis of the CNS. 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. The parameter BB or C_{brain}/C_{blood} is profoundly increased for the benzo-mustard construct 1.97 relative to nordiazepam 1.05 (approx. 100 % increase). According to the Lipinski's rule of five[11], compounds with number of violations not more than 1 shows good bioavailability and bioactivity. Analysis of molecular structure by Molinspiration showed that both benzo-mustard and nordiazepam have zero violations of the rule of 5 (Table-3). The target compound benzo-mustard has no NH or OH hydrogen bond donors, which show increase solubility in cellular membranes. The target compound has nON value 4 which is < 10 and has molecular weight 438 which is < 500 preferable for compound to be CNS active. All this properties could permit a better penetration of the drug through the blood-brain barrier.

TABLE-3
COMPARISON OF PHYSICOCHEMICAL PARAMETERS OF NORDIAZEPAM AND BENZO-MUSTARD AGENT

Descriptor	Nordiazepam	Benzo-mustard agent
Polar surface area[1]	41.46 A^2	35.9 A^2
Percent intestinal absorption[2] of drug	> 90 %	> 90%
Log BB[3]	0.00283	0.295
MlogP	2.75	3.98
$BB^3 = C_{brain}/C_{blood}$	1.05	1.97
Molecular weight[1]	270.7	438.8
No of Violation[1] of rule of 5	0	0
-NH and –OH[1]	1	0
nON values[1]	3	4

[1]Calculated by method of molinspiration, www.molinspiration.com/cgi-bin/properties
[2]Calculated by correlation of PSA to experimental intestinal absorption; [3]log BB, where $BB = C_{brain}/C_{blood}$, log BB = -0.0145 PSA + 0.172 Mlog P+0.131

Conclusion

Benzodiazepine linked nitrogen mustard was designed in the hope to obtain CNS active antitumor agent. The benzo-mustard agent was solid at room temperature and stable for more than one week when stored at less than 0 ºC. The *in vitro* chemical alkylation activity studies (NBP) of benzo-mustard was comparable to that of *N,N-bis*-(2-chloroethyl)amino moiety as standard alkylating agent. The log P value of benzo-mustard is determined experimentally and compared with other partition coefficient (milog P, Mlog P). All methods show an increase of lipophilic nature for the benzo-mustard construct compared with the parent compound nordiazepam. Value of polar surface area for the benzo-mustard agent (35.9 A^2) predicts that > 90 % of any amount present in the intestinal tract will be absorbed. The study of some other physico-chemical parameter calculated by online software such as log BB, Polar surface area, rule of five, number of NH or OH hydrogen bond donors and nON value also indicates that it can be a potential CNS antitumor agent.

ACKNOWLEDGEMENTS

The authors are thankful to Shivalik College of Pharmacy, Nangal, India for providing partial financial support for this research work. Thanks are also due to SAIF, Panjab University, Chandigarh for spectral analysis.

REFERENCES

1. W.O. Foy and S.K. Sengupta, in eds.: W.O. Foye, T.L. Lemke and D.A. Williams, In Principles of Medicinal Chemistry, Williams and Wilkins USA, pp. 822-840 (1995).
2. W.R. Wilson, M. Tercel, R.F. Anderson and W.A. Denny, *Anti-Cancer Drug Des.*, **13**, 663 (1998).
3. L. Kupczyk-Subotkowska, T.J. Siahaan, A.S. Basile, H.S. Friedman and P.E. Higgins, *J. Med. Chem.*, **40**, 1726 (1997).
4. J. Dourlat, W.-Q. Liu, N. Gresh and C. Garbay, *Bioorg. Med. Chem. Lett.*, **17**, 2527 (2007).
5. L.H. Sternbach, G.A. Archer, J.V. Earley, R.I. Fryer, E. Reeder and N. Wasyliw, *J. Med. Chem.*, **8**, 815 (1965).
6. A. Pen, S. Samanta, S. Dutta, A.K. Saxena, M. Shanmugavel, S. Kampasi, G.N. Quazi and U. Sanyal, *Exp. Oncol.*, **24**, 173 (2002).
7. I. Moriguchi, S. Hirono, Q. Liu, I. Nakagome and Y. Matsushita, *Chem. Pharm. Bull.*, **40**, 127 (1992).
8. D. Clark, *J. Pharm. Sci.*, **8**, 815 (1999).
9. T. Fujita, J. Iwasa and C. Hansch, *J. Am. Chem. Soc.*, **86**, 5175 (1964).
10. J. Kelder, P. Grootenhuis, D. Bayada, L. Delbressine and J. Ploemen, *Pharm. Res.*, **10**, 1514 (1999).
11. C. Lipinski, F. Lombardo, B. Dominy and P. Feeney, *Adv. Drug Del. Rev.*, **23**, 3 (1997).