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ORIGINAL ARTICLE

Design, synthesis and *in vitro* cytotoxicity study of benzodiazepine-mustard conjugates as potential brain anticancer agents

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Log *P*;
NBP assay;
MTT assay

Abstract The combination of two pharmacological entities in a single compound has been utilized as a promising drug design strategy for site-specificity. So two nitrogen mustard agents were synthesized by conjugating mustard with the benzodiazepine nucleus in the hope to obtain central nervous system (CNS) antitumor agents. The benzodiazepine part is aimed to serve as a CNS active carrier enabling the alkylating moiety to cross the BBB by altering its physicochemical properties. Structures of all the synthesized compounds were confirmed by IR, NMR (^{1}H & ^{13}C), mass spectral and elemental studies. The benzodiazepine-mustard conjugates are oily at room temperature and quite stable when stored in refrigerator for 2 months. Both compounds when evaluated for NBP alkylating activity against chlorambucil, proved to be active alkylating agents. The compounds were markedly active when subjected to *in vitro* biological evaluation using an MTT colorimetric assay against four human cancer cell lines (A-549, COLO 205, U-87 MG and IMR-32). The physicochemical ADME studies were also analyzed using Qikprop 2.5 tools of Schodinger software which further indicates that both compounds can be potential candidates for the treatment of brain tumor.

1. Introduction

There are more than two lakhs of population affected by CNS tumor every year [7]. A major difficulty in treating tumors of the CNS is the drug delivery into the CNS. One of the major obstacles is the blood–brain barrier (BBB) which is a primary reason for the non-penetration of the drugs. Nitrogen mustard is the one of the most active and widely used alkylating anticancer agents for the treatment of all types of cancer including cervix, breast and prostate cancer. One of the main demerits of

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mustard drug is its hydrophilicity which does not allow the molecule to cross the BBB and therefore ineffective as brain antitumor agent [9]. One approach to overcome this problem and site specific delivery to the brain is to attach this cytotoxic moiety to a suitable carrier, which accumulates in brain tumor tissue [12,20,4]. Various lipophilic 1,4-benzodiazepine derivatives have been known for their therapeutic CNS activity which is due to their action on peripheral benzodiazepine receptors (PBRs). These receptors are located in the outer membrane of mitochondria, and their density increases in brain tumors. Thus, they may serve as a unique intracellular and selective target for antineoplastic agents [15]. Moreover a recent study reveals that some benzodiazepine derivatives have antiproliferative activities [8,30,5,10].

The conjugating two pharmacophoric entities in a single molecule could be considered as a promising drug design strategy for site-specificity. So on the continuation of our work for the synthesis of CNS therapeutic agents [23–28], and the structure pattern of benzodiazepines having potential CNS activity, it was designed to link *N, N*-bis(2-chloroethyl)amino moiety as alkylating agent with ethyl as linker for targeted delivery of mustard across the brain by synthetic methodology (Fig. 1).

2. Experimental

2.1. Chemistry

The melting points were determined on Veego-programmable melting point apparatus (microprocessor-based). NMR (^{1}H & ^{13}C) spectra were measured using Bruker Avance-II, 400 MHz spectrometer and are reported in parts per million (ppm) and TMS as an internal standard. Infrared (IR) spectra were recorded on Perkin Elmer model 1600 FT-IR RX-I spectrometer as KBR disks. The ultraviolet spectra were recorded on Shimadzu, UV-1800 spectrophotometer. The TOF-MS-ES$^+$ spectra of the compounds were recorded on Waters micromass-LCT Mass spectrometer. Elemental analyses were performed on Elementar Vario EL III. All the intermediates and final compounds obtained were oily in nature and cannot be further purified hence elemental analysis values are off (error > 0.4%). The chromatography was carried out using the Merck silica gel 60 (230–400 mesh). A computational study of titled compounds was performed for prediction of ADME properties by QikProp 3.2 tools available in Schödinger 9.0 version developed by Professor William L. Jorgensen and Molinspiration online property calculation toolkit [18]. Tris(2-chloroethylamine)hydrochloride was prepared by thionyl chloride assisted chlorination of triethanolamine as per procedure [11]. The synthesis of **1**(**a**–**b**) and **2**(**a**–**b**) was carried out by literature methods [29,2], respectively.

2.1.1. General method for the synthesis of 1-[2-{bis(2'-chloro ethyl)amino}ethyl-7-chloro/nitro-5-phenyl-1H-benzo-1,4-diazepin-2(3H)-one (Benzodiazepine-mustard) (**4a**–**b**)

Method A: To a solution of 7-chloro/nitro-1,3-dihydro-5-phenyl-benzo-1,4-diazepin-2-one (**2a**–**b**) (10 mmol) in DMF, sodium hydride (60% in mineral oil, 15 mmol) was added and the mixture was stirred for 5 min. 1-Bromo-2-chloro ethane (15 mmol) 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 benzodiazepine-mustard (**4a**–**b**) as dark brown oil.

*2.1.1.1. 1-[2-{Bis(2'-chloroethyl)amino}ethyl-7-chloro-5-phenyl-1H-benzo-1,4-diazepin-2(3H)-one (3) (Benzodiazepine-mustard) (**4a**).* IR KBr (cm^{-1}): 3038 (Ar–H), 2955 (C–H), 1682 (C═O), 1477 (C═C), 1178 (C–N), 769 (C–Cl); ^{1}H-NMR ($CDCl_3$) δ (ppm): 2.70–2.80 (m, 6H, –N($CH_2CH_2Cl)_2$ + –CH_2CH_2N), 3.33 (t, 4H, J = 7.3, –N($CH_2CH_2Cl)_2$), 3.8 (t, 2H, J = 7.0, –CH_2CH_2N), 4.5 (s, 2H, –COCH_2–), 7.17–7.55 (m, 8H, Ar*H*); ^{13}C-NMR ($CDCl_3$) δ (ppm): 43.5 (2×C–Cl), 47.8 (N–C), 51.0 (C–N), 56.4 (2×C–N), 58.0 (CH_2), 124.4, 127.7, 128.0, 128.8, 129.2, 130.6, 131.4, 132.2, 138.6, 140.0 (Aromatic), 167.2 (—C═N), 171.4 (C═O); TOF MS ES$^+$ m/z: [M$^+$] 438 (100%); Anal calcd for $C_{21}H_{22}N_3OCl_3$: C% 57.48, H% 5.05, N% 9.58; Found: C% 56.04, H% 5.78, N% 9.26.

*2.1.1.2. 1-[2-{Bis(2'-chloroethyl)amino}ethyl-7-nitro-5-phenyl-1H-benzo-1,4-diazepin-2(3H)-one (3) (Benzodiazepine-mustard) (**4b**).* IR KBr (cm^{-1}): 3061 (Ar–H), 2972 (C–H), 1684 (C═O), 1477 (C═C), 1529 (Assymetric –NO_2), 1177–1316

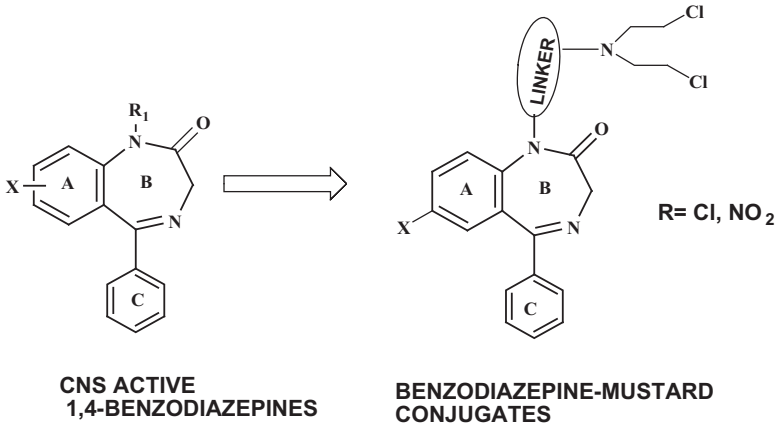

Figure 1 Design of benzodiazepine-mustard agent.