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Fluorination of an antiepileptic drug: A self supporting transporter by oxygen enrichment mechanism

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ABSTRACT

Drug therapy of seizures involves producing high levels of antiepileptic drugs in the blood. Drug must enter the brain by crossing from the blood into the brain tissue, called a transvascular route (TVR). Even before the drug can reach the brain tissue, factors such as systemic toxicity, macrophage phagocytises and reduction in oxygen content limit the success of this TVR. Encapsulating the drug within a nano scale delivering system, synthesising drugs with low molecular weight are the best mechanisms to deliver the drug to the brain. But through this article, we have explored a possibility of attaching a molecule 4-(trifluoromethyl) benzoic acid (TFMBA), that possess more number of fluorine atom, to benzodiazepine (BDZ) resulting in an ionic salt (*S*)-(+)-2,3-dihydro-1H-pyrrolo[2,1-c][1,4]benzodiazepine5,11(10H,11aH)-dione with 4-(trifluoromethyl)benzoic acid. By this way, reducing the toxicity of BDZ than the conventional anti-epileptic drugs (AEDs), increasing the solubility, reducing the melting point, enriching the TVR with excess oxygen content with the support of fluorine. With all these important prerequisites fulfilled, the drug along with the attached molecule is expected to travel more comfortably through the TVR without any external support than any other conventional AEDs. FTIR, ^{1}H NMR, ^{13}C NMR, HRMS spectroscopy, HRTEM and In vitro cytotoxicity analysis supports this study.

1. Introduction

Currently, drug discovery for central nervous system (CNS) disorders is almost exclusively limited to smaller molecular weight (practically difficult for epilepsy kind of disorders), less toxic, high solubility, lipophilic molecules that can cross Blood Brain Barrier (BBB). This prerequisite eliminates a majority of potential AED molecular candidates early in the drug discovery process. Apart from these prerequisites, the most vulnerable problem for the conventional AEDs is that their inability to travel through the TVR (Sharma and Sharma 1997; Tamargo et al., 2002; Lopez et al., 2007, 2010, 2011). It is not only because of the lacking of the above said prerequisites, but also the lack of oxygen content to a greater extent. Generally, during the abnormal behaviour of the seizures, both the brain and the TVR suffer much with oxygen content. This in turn affects the transport of the drug too through TVR.

Encapsulating the drug within a nano scale delivering system, synthesising drugs with less toxic are the best mechanisms to deliver the drug to the brain (Lopez et al., 2006; Madhusudhan et al., 2007; Bennewitz and Saltzman 2009; Luszczki et al., 2009; Rossi 2012). Direct delivery of drugs using a miniblocks from carbon nano-diamonds composite (NDC) as minicontainers has been developed by Volnova et al. (2013). Their method allows pharmacological substances that do not cross the BBB to be delivered into the CNS.

This article reviews this challenge by coming out with a drug (ionic salt of) (*S*)-(+)-2,3-dihydro-1H-pyrrolo[2,1-c][1,4] benzodiazepine5,11(10H,11aH)-dione with 4-(trifluoromethyl) benzoic acid, possessing more number of fluorine atoms, as self-supporting transporter at BBB and blood–cerebral spinal fluid barrier (BCSFB) that shuttle drug molecule toward and away from neural tissue. This is expected to be made possible because of the tremendous oxygen carrying capacity of fluorine, which is highly electronegative.

Addition of fluorine atom to any drug not only helps to enrich the oxygen content at the active site but also (a) reduces the toxicity of the drug (b) increases the solubility (c) reduces the melting point (d) more adoptive structure and so on. There are good numbers of evidences (Smart, 2001; Schenck et al., 2004; Song et al., 2005; Dolbier, 2005; Obniska et al., 2006) for establishing these credentials of fluorine atoms. Apart from this capacity, addition of one or more number of fluorine atoms in any basic structure could drastically enhance its biological activity.

To suit this specific study, for example, retigabine, a drug that has been synthesised by Gunthorpe et al., (2012) proves to inhibit

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the K+ channel of the BBB only because of the presence of a single fluorine atom in its structure (Fig. 1). All other AEDs inhibit all other channels. It is because of the excellent oxygen carrying capacity and highly electronegative nature of fluorine, retigabine is able to achieve this possibility.

Though there is several transporter mediate access of therapeutic drug molecule to brain, the extent to which this transporter is involved in drug tolerance in human remains unclear (Collins-Gold et al., 1990; Lopez et al., 2009; Heredia-Cervera et al., 2009; Lenkov et al., 2013; Sanchez et al., 1991). Hence, a self-supporting transporter that can have a good drug tolerance in human is much more dependable. This article is designed in such a way that an attempt has been made to synthesise a drug which is capable of deliver and attract more oxygen while it travels through the TVR, so that it is capable of accessing the BBB with relative ease.

The structure of the synthesized compound has been characterized by Fourier transform infrared (FT-IR) spectroscopy, proton NMR (^{1}H), carbon NMR (^{13}C) spectroscopy, high-resolution mass spectral (HRMS) with electron spray ionization (ESI) analysis and high resolution transmission electron microscopy (HRTEM) analysis. The new compound was tested for in vitro cytotoxicity evaluation by MTT assay against breast adenocarcinoma cell line of MCF-7 cells comparable with known reference drugs carbamazepine (CBZ), topiramate (TPM) and benzodiazepine (BDZ).

2. Experimental procedure and spectral data

2.1. General

BDZ, CBZ, TPM and 4-(trifluoromethyl) benzoic acid used were generic 99% pure from Sigma–Aldrich (Steinheim, USA). Dimethylformamide (DMF) was purchased from Merck scientific Inc. (Darmstadt, Germany) and DMF is used as an effective solvent. All the chemicals and solvents were used without further purification.

Melting points (mp) were measured in open capillaries on Nessler digital Auto melting point apparatus and are uncorrected. The FT-IR spectrum of the synthesized compound was recorded as neat liquids or potassium bromide (KBr) and absorptions are reported in cm^{-1}. The ^{1}H NMR spectra were recorded on 300 MHz (Bruker) spectrometer in appropriate solvents using tetra methyl silane (TMS) as an internal standard or the solvent signals as secondary standards and the chemical shifts are reported in δ values (ppm). Coupling constants J are expressed in Hertz (Hz). Signal multiplicities are represented by the following abbreviations: s (singlet), d (doublet), dd (double doublet), t (triplet), q (quartet), and m (multiplet). ^{13}C NMR spectra were recorded on 75 MHz spectrometers. High resolution mass spectra were obtained by using ESI-QTOF mass spectrometry. To monitor the reactions, the purity of the reactants and products was confirmed by analytical thin-layer chromatography (TLC) performed on silica gel GF254 pre-coated plates. After elution, plate was visualized under UV illumination at 254 nm for UV active materials. The HRTEM analysis was performed in a JEM 2100 microscope at the working voltage of 200 kV (with wavelength $\lambda = 0.00251$ nm), with spherical aberration coefficient $C_s = 1$ mm and magnify up to 0.1 nm. The HRTEM imaging magnifications were calibrated using standards of 6H-SiC lattice fringes and commercial cross-line grating replica. Its optimum defocus, Scherzer defocus is $f_{Sch} = -((4/3)\, C_s\, \lambda)^{1/2} = -58$ nm, which gives a point resolution of $r_{Sch} = 0.66\, C^{1/4}\, \lambda^{3/4} = 0.23$ nm.

2.2. General procedure for preparation of ionic salt of (S)-(+)-2,3-dihydro-1H-pyrrolo[2,1-c][1,4]benzodiazepine5,11(10H,11aH)-dione with 4-(trifluoromethyl) benzoic acid (3):

(*S*)-(+)-2,3-dihydro-1H-pyrrol[2,1-c][1,4]benzodiazepine 5,11 (10H,11aH)-dione **1** (0.4 g, 2 mmol) in DMF at 60–80 °C for 30 min was added 4-(trifluoromethyl) benzoic acid **2** (2.4 mmol) to the reaction and stirred for 6 h. After completion (by TLC), the reaction mixture was cooled at ambient temperature and separated in aqueous phase medium, water (50 ml) was added, solid precipitate thus formed was filtered, washed with water and dried to give the ionic salt of (*S*)-(+)-2,3-dihydro-1H-pyrrolo[2,1-c][1,4]benzodiazepine5,11(10H,11aH)-dione with 4-(trifluoromethyl) benzoic acid **3** in 85% yield as pure white salt.

2.3. Ionic salt of (S)-(+)-2,3-dihydro-1H-pyrrolo[2,1-c] [1,4] benzodiazepine 5,11(10H, 11aH)-dione with 4-(trifluoromethyl) benzoic acid (3)

Yield :	85 %
Melting Point (mp) :	150 – 153^{0}C
IR (KBr):	ν_{max} 3326, 3073, 2929, 2852, 2553, 2359, 1698, 1648, 1588, 1428, 1322, 1288, 1241, 1062, 941, 857, 761, 645, 539 cm^{-1}
^{1}H NMR (300 MHz, DMSO-d$_6$):	δ 10.65 (s, 1H), 8.10 (d, *J* = 31.1 Hz, 2H), 7.80 (d, *J* = 7.4 Hz, 2H), 7.63 (dd, *J* = 43.2, 7.4 Hz, 1H), 7.54 (t, J=8.12 Hz, 1H), 7.23 (d, J=8.30 Hz, 1H), 7.17 (d, J= 7.93 Hz, 1H), 4.13 (t, *J* = 6.0 Hz, 1H), 3.59 (dd, J=4.34Hz, J=7.93 Hz, 1H) 3.46 – 3.57 (m, 2H), 1.82 - 1.94 (m, 2H), 1.22 (m, 1H).
^{13}C NMR (75 MHz, CDCl$_3$ + DMSO-d$_6$) :	δ 166.26, 156.85, 153.26, 133.78, 132.71, 129.35, 126.90, 124.80, 124.47, 124.42, 124.37, 124.16, 121.20, 48.88, 47.50, 33.13, 30.83, 29.88, 25.23.
HR-MS (ESI) with Positive and Negative Ionization:	Calculated m/z for Positive ions: 217.2438 ($C_{12}H_{12}NO_2NH^+$), found: 217.1428; Calculated m/z for Negative ions: 189.1114 ($C_8H_4OF_3O^-$), found: 189.0159.