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Design, synthesis and computational evaluation of a novel intermediate salt of *N*-cyclohexyl-*N*-(cyclohexylcarbamoyl)-4-(trifluoromethyl) benzamide as potential potassium channel blocker in epileptic paroxysmal seizures

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ABSTRACT

The narrow therapeutic range and limited pharmacokinetics of available Antiepileptic drugs (AEDs) have raised serious concerns in the proper management of epilepsy. To overcome this, the present study attempts to identify a candidate molecule targeting voltage gated potassium channels anticipated to have superior pharmacological than existing potassium channel blockers. The compound was synthesized by reacting (S)-(+)-2,3-dihydro-1H-pyrrolo[2,1-c][1,4] benzodiazepine5,11(10H,11aH)-dione with 4-(Trifluoromethyl) benzoic acid ($C_8H_5F_3O_2$) in DMF and *N,N′*-dicyclohexylcarbodiimide (DCC) which lead to the formation of an intermediate salt of *N*-cyclohexyl-*N*-(cyclohexylcarbamoyl)-4-(trifluoromethyl) benzamide with a perfect crystalline structure. The structure of the compound was characterized by FTIR, ^{1}H NMR and ^{13}C NMR analysis. The crystal structure is confirmed by single crystal X-ray diffraction analysis. The Structure-Activity Relationship (SAR) studies revealed that substituent of fluoro or trifluoromethyl moiety into the compound had a great effect on the biological activity in comparison to clinically used drugs. Employing computational approaches the compound was further tested for its affinity against potassium protein structure by molecular docking in addition, bioactivity and ADMET properties were predicted through computer aided programs.

1. Introduction

International League Against Epilepsy [ILAE] defines epilepsy as a disease condition characterized by an enduring predisposition to generate seizures by neurobiological, cognitive, psychological and social consequences (McNamara, 1999; Aird et al., 1989). The worldwide prevalence of active epilepsy ranges from 4 to 10 per thousand populations (Fisher et al., 2005) and in India almost 5.59 per thousand suffers epileptic attack once in their life time (Banerjee et al., 2010). The electrogenic property of an individual neuron forms an important marker for hyper excitability of neuronal circuits which are dependent on the functional properties of ion channels like Na^+, K^+, and Ca^{2+} in the membranes. Voltage-Gated Potassium Channels in particular, are the mediators of intrinsic neuronal excitability and are central to most important determinants of pathophysiology of epileptic seizures and execute initiation of action potentials, synaptic transmission and neurotransmitter release.

The major role of potassium channels in epileptic pathogenesis comes from the compelling evidence wherein mutations in the potassium channel were strongly associated with circumscribed seizure syndrome (BFNC). The stupendous role of potassium channels became more apparent when families were diagnosed with combination of generalized epilepsy and paroxysmal non-kinesigenic dyskinesia (PNKD) had potassium channel mutations with autosomal dominant pedigree (Tinel et al., 1998). Sixteen affected members were reported: of these, four had epilepsy alone, seven developed PNKD and five had both. Interestingly, they harbour a mutation which segregates with disease status in the subunit of the BK (maxi-K) potassium channel *KCNMA1*, on chromosome 10q22. Expression studies indicated that the mutant BK channel exhibited increased sensitivity to ambient calcium ion concentration, resulting in an increase in BK channel open probability: a gain of channel function. It is proposed that

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enhanced BK channel activity *in vivo* may lead to increased neuronal excitability by inducing rapid repolarization, thereby facilitating fast neuronal firing, which may underlie the seizures and PNKD (Du et al., 2005).

In recent years, several antiepileptic drugs (AEDs) like carbamazepine, benzodiazepine, topiramate, lamotrigine have been used for therapeutic agents against epilepsy. However, around 30% of the patients still continue to have uncontrolled seizures (drug resistance) and drug toxicity which may be due to structural or functional change at the site of drug action or alteration in the drug pharmacodynamics (Falip et al., 2013; Jerome, 2003; Piplani et al., 2016). In addition, daily dosing of AEDs with long term treatment has been associated with severe side effects like renal impairment, hyperkalaemia, drug cough, skin rashes in one-third of subjects (Sisodiya et al., 2002; Browne and Holmes, 2001; Kramer, 2001; Kwan and Brodie, 2000; Navale et al., 2013; Izzo et al., 2011). Hence, development of novel AED's with an optimal efficacy that can enhance an improved clinical outcome is undoubtedly an important medical demand.

Fluorinated compounds are the focus of much interest in modern pharmaceutical chemistry, and the incorporation of fluoro or fluoroalkyl substituents plays a significant role in development of anticonvulsant active molecules (Dolbier, 2005; Schenck et al., 2004). Design and development of fluorinated compounds as potential drugs have steadfastly increased in pharmaceutical industries. Due to its small size, combined with the high electronegativity that modulates electronic, lipophilic and steric parameters, fluorinated derivatives exhibit commendable biological activity (Elliott, 1995; Smart, 2001). Enhanced activity of fluoro derivatives can be further attributed for its excellent oxygen carrying potential, offering least toxic effects (Welch and Eswarakrishnan, 1991; Song et al., 2005). They offer promising and amazing chemical diversity, there by inspiring the development of structurally diverse new molecules to play a vital role in drug discovery (Shoichet, 2013; Obniska et al., 2006).

In view of this background, in the present study, we designed and synthesized a new intermediate salt of *N*-cyclohexyl-*N*-(cyclohexylcarbamoyl)-4-(trifluoromethyl)benzamide using chemical reaction scheme. The new compound was investigated for anticonvulsant screening through the correlation of structure-activity relationships of widely, clinically used drugs. The crystallographic studies of new compound were carried out to validate the structure conformation with their potenation points. Employing computational approaches the compound was further tested for its affinity against potassium protein structure by molecular docking further, bioactivity and ADMET properties were predicted through computer aided programs.

2. Materials and methods

2.1. Chemicals and reagents

Benzodiazepine (BDZ) and 4-(Trifluoromethyl) benzoic acid used were generic 99% pure from Sigma-Aldrich (Steinheim, USA). *N*,*N*′-dicyclohexylcarbodiimide (DCC), dimethylformamide (DMF) were 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. 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. Silica gel finer than 200 mesh was used for column chromatography.

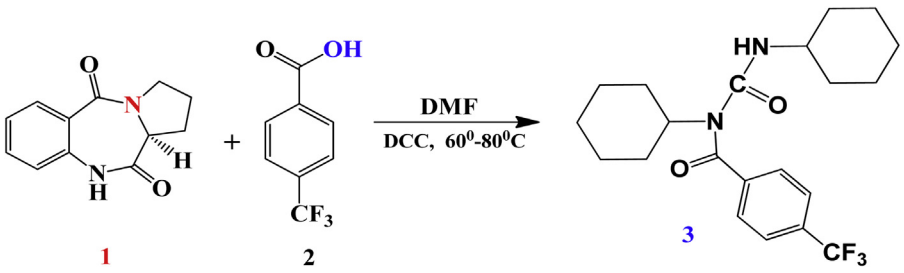
2.2. Synthesis of the compound

Synthetic approach for the preparation of intermediate salt of *N*-cyclohexyl-*N*-(cyclohexylcarbamoyl)-4-(trifluoromethyl) benzamide **3** was presented in Scheme 1.

Commercially available (S)-(+)-2,3-dihydro-1H-pyrrolo[2,1-c][1,4] benzodiazepine 5.11 (10H, 11aH)-dione **1** was taken as starting material. The starting material **1** was reacted with 4-(Trifluoromethyl) benzoic acid **2** in the presence of *N*,*N*′-dicyclohexylcarbodiimide (DCC) in DMF afforded intermediate salt of *N*-cyclohexyl-*N*-(cyclohexylcarbamoyl)-4-(trifluoromethyl) benzamide **3** in 80% excellent yields. The compound **3** was fully characterized by FT-IR, ^{1}H NMR, ^{13}C NMR analysis (Figs. 1–3). Further single crystal X-ray diffraction analysis of compound **3** unambiguously confirmed the structure and stereochemistry (Fig. 4a and b).

2.2.1. Preparation of intermediate salt of N-cyclohexyl-N-(cyclohexylcarbamoyl)-4-(trifluoromethyl) benzamide (3)

(S)-(+)-2,3-dihydro-1H-pyrrol [2,1-c][1,4]benzodiazepine5,11 (10H,11aH)-dione **1** (0.4 g, 2.0 mmol) and *N*,*N*′-



Scheme 1. Synthesis of intermediate salt of *N*-cyclohexyl-*N*-(cyclohexylcarbamoyl)-4-(trifluoromethyl)benzamide **3**.