

Original Research Article

Design, synthesis and biological activities of 5H-dibenzo[b,f]azepine-5-carboxamide derivatives; Targeted hippocampal trypsin inhibition as a novel approach to treat epileptogenesis

Taha Iqbal, Mohsin Abbas Khan, Irshad Ahmad*, Fahad Mehmood Khan

Department of Pharmaceutical Chemistry, Faculty of Pharmacy, The Islamia University of Bahawalpur, Pakistan

*For correspondence: **Email:** drirshad.iub@gmail.com; **Tel:** 0092-3006800703

Sent for review: 6 September 2021

Revised accepted: 5 February 2022

Abstract

Purpose: To synthesize anticonvulsant drug derivatives that target protease-activated receptor generated epileptic seizures.

Method: Varieties of carbamazepine-based Schiff bases were designed with different aldehydes and ketones, and evaluated for *in silico* computer-aided drug design prediction of absorption, distribution, metabolism and excretion (ADME), and potential drug targets. The resultant compounds were synthesized and characterized by various spectroscopic techniques, including FTIR, ¹H-NMR and ¹³C-NMR, analysis. Thereafter, they were screened for antimicrobial, antioxidant and anticonvulsant potential.

Results: Prominent anti-protease potential was shown by C7 and C3 compounds and the order of activity was C7 > C3 > C5 > C2 > C6 > C4 > C2 > C1 (*p* < 0.05). The anticonvulsant activity of C7 and C5 was comparable with the standard drug; C3, C4, C6 and C8 had mild activity while C1 and C4 showed the least activity. The synthesized compounds exhibited significant (*p* < 0.05) antioxidant potential (rank order: C3 > C4 > C5 > C7 > C8 > C6 > C1 > C2) and antimicrobial activity against *S. aureus* and *B. bronchiseptica* (rank order: C5 > C2 > C8 > C1 > C4 > C3 > C7).

Conclusion: Synthesized derivatives retained their potential for anticonvulsant and antitrypsin activity, unlike their mother moiety, i.e., carbamazepine. The additional antibacterial activity effectively treats neurological disorders associated with bacterial infections

Keywords: Carbamazepine, Epilepsy, Antibacterial, Proteases

This is an Open Access article that uses a funding model which does not charge readers or their institutions for access and distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/4.0>) and the Budapest Open Access Initiative (<http://www.budapestopenaccessinitiative.org/read>), which permit unrestricted use, distribution, and reproduction in any medium, provided the original work is properly credited.

Tropical Journal of Pharmaceutical Research is indexed by Science Citation Index (SciSearch), Scopus, International Pharmaceutical Abstract, Chemical Abstracts, Embase, Index Copernicus, EBSCO, African Index Medicus, JournalSeek, Journal Citation Reports/Science Edition, Directory of Open Access Journals (DOAJ), African Journal Online, Bioline International, Open-J-Gate and Pharmacy Abstracts

INTRODUCTION

Epilepsy is one of the most common central nervous system disorders characterized by recurrent convulsions, affecting almost 1 % of the world's population [1]. Present therapies cannot

facilitate about 30 % of epileptic patients with-satisfactory therapeutic outcomes [2]. Many of the currently used antiepileptic drugs cause serious adverse effects including herpetological, hematological and dermatological disorders [3]. So, there is a need to develop new

anticonvulsants having known mechanisms of action aside from conventional ion channel blockers.

Some studies have shown the role of inflammatory processes in epileptogenic responses [4]. The trypsin receptor such as the protease-activated receptor (PAR₂) is involved in inflammatory processes despite having cytoprotective activity in many organs like airways, pancreas, and gut [4]. Extracellular proteolysis is the triggering factor in stress-related neuronal plasticity yielding trypsin that activates PAR₂, resulting in epilepsy [5]. Trypsin inhibitors limit the PAR₂ role in the induction of convulsions.

Proteinase inhibitors also have their significance in inflammatory ailments, especially arthritis, activity of proteinase inhibitors and proteolytic ability of neutrophils [6]. Carbamazepine is chemically an iminodibenzyl derivative. Treatment with carbamazepine is effective, but 30 – 40 % of patients do not respond to its treatment due to adverse effects like sedation, ataxia and disturbance in the level of androgens. Moreover, its active metabolite carbamazepine epoxide possesses an anticonvulsant effect, which is why its derivatives are needed to be designed at the same functional group in search of better and safe therapeutic outcomes [7].

Schiff bases are chemical compounds possessing azomethine as a functional group (-CH=N-) and are named Schiff bases after the person who first synthesized them, Hugo Schiff [8].

Schiff base derivatives of drugs are used in search of moieties with lesser side effects or altogether distinctive pharmacological effects that may help improve therapeutic procedures. The Schiff bases of drugs have shown antibacterial, anticancer, and antifungal activities [9]. The objective of this study was to synthesize the Schiff bases of carbamazepine with different aldehydes and ketones and investigate their targeted inhibition of protease-activated receptor to prevent epileptic seizures.

EXPERIMENTAL

Reagents

Carbamazepine, (99.9 %) was a gift by Novartis Pvt. Ltd Pakistan. Salicylaldehyde, benzaldehyde, acetophenone, cinnamaldehyde, 4-(dimethyl amino) benzaldehyde, n-n dimethyl formamide, formaldehyde, methyl ethyl ketone and acetic acid were purchased from Sigma

Aldrich, Germany. Ethanol of analytical grade was purchased from VWR, USA.

Animals

After approval from Ethical Committee of Islamia University of Bahawalpur, the guidelines of the National Research Council for the use of animals were followed in all activities. Swiss mice weighing from 25 – 30 g of either sex were used. The mice were kept at the animal house of the Department of Pharmacy, Islamia University Bahawalpur in cages (polycarbonate material) at room temperature of 25 ± 2 °C under alternating light and dark cycle of 12 h.

Derivatization and spectroscopic studies

To investigate the effect of derivatization of 5H-dibenzo[b,f]azepine-5-carboxamide, it was reacted with various aldehydes and ketones with classical condensation reactions for synthesizing Schiff base derivatives. The reaction was carried out using acetic acid and hydrochloric acid as catalysts, and the reaction conditions were optimized through varying reaction solvents. Ethanol proved to be the solvent of choice for rapid reaction and higher yields. The scope of the process was enhanced by the use of a variety of substituted aryl rings containing aldehydes.

The physical properties, i.e., solubility and melting points of the synthesized drug derivatives were determined using HTW sonicator Ela E 30H, D- 78224 Singe and Galen Kamp melting points apparatus, respectively. The FTIR spectra were obtained from Bruker TENSOR 27 FTIR spectrophotometer, while ¹H-NMR and ¹³C-NMR spectral evaluation of drug derivatives was done using Bruker 400-spectrophotometer.

These derivatives were subjected to *in silico* studies for the prediction of their molecular, binding, and pharmacokinetic properties using ADME prediction. Molecular docking studies were done for anti-enzymatic potential indicated by drug binding target prediction. Protein database was used for the receptor site. All the compounds were screened for their *in vitro* biological activities, i.e., antiprotease potential [10], antioxidant activity and antimicrobial potential through *in vitro* activity [11].

In vitro evaluation of trypsin (proteinase) inhibitory activity

Proteinase inhibitory potential was measured by azocasein assay [12]. In this method, the mixture had equal concentration (1:1 v/v) of the enzyme

(trypsin) and its inhibitor. The total volume of the mixture was made up to 200 μ L with a reagent containing 0.2 M glycine and NaOH solution of pH 10. Then the reaction mixture was incubated at 37 °C for 30 min. It was poured into pre-incubated 200 μ L azocasein (0.25 %) solution prepared in 0.1 M Glycine NaOH buffer pH 10. The mixture was kept at 37 °C for 30 min.

The enzyme inhibition reaction was terminated by the addition of 5 % trichloroacetic acid (TCA) (0.3 mL). Controls for each sample were made by adding TCA to the azocasein solution. The mixture was incubated for 30 min at 37 °C. After that, tubes containing reaction mixtures were centrifuged at 1,500 rpm for 10 min. Sodium hydroxide 1.0 N solution (0.6 mL) was then added to the supernatant of the mixture and the inhibitory activity was measured at 450 nm by using trypsin as the standard protease enzyme [10].

Assessment of antioxidant activity

Antioxidant activity was measured by using 2,2-diphenyl-1-picrylhydrazyl (DPPH) in methanol solutions (0.2 mg/mL) of DPPH (2.0 mL) test material solution (2.0 mL) in a reaction tube. They were kept at room temperature for 30 min [13]. The scavenging of the DPPH radical is indicated by the change in color of the reaction mixture. Then, using a UV-Visible spectrophotometer, the absorbance of blank solution (2.0 mL of DPPH solution and 1.0 mL of methanol) sample and ascorbic acid solution was measured at 517 nm. Inhibition (H) was calculated as in Eq 1.

$$H (\%) = \frac{(A_0 - A_t)}{A_0} \times 100 \dots\dots\dots (1)$$

where A_0 and A_t are absorbance of blank and test solutions.

Determination of antimicrobial activity

The synthesized compounds were tested for their antimicrobial potential against *Staphylococcus aureus* (*S. aureus*), *Staphylococcus epidermidis* (*S. epidermidis*), *Micrococcus luteus* (*M. luteus*), *Bacillus pumilis* (*B. pumilis*), *Escherichia coli* (*E. coli*), *Bacillus subtilis* (*B. subtilis*) and *Bordetella bronchiseptica* (*B. bronchiseptica*) by well diffusion method. The stock solutions of the compounds were prepared as 1 mg/mL by dissolving 20 mg of test compound and standard in 20 mL of DMSO. The DMSO was taken as blank. The bacteria were cultured in Mueller-Hinton agar and 30 μ L of sample solution, standard drug (cefixime) and blank were poured in the wells. The petri dishes were kept at 37 °C

for 24 h in an incubator. The diameter (mean \pm SD) of the zone of inhibition for all replicates was measured using Vernier caliper.

Evaluation of anticonvulsant activity

Albino mice with weight range (25 – 30 g) of either sex were selected. Then the mice were grouped into sets of 10. Each test substance was suspended in gum acacia as a carrier at 1 % (w/v). In this study, 90 mg/kg of pentylenetetrazole (PTZ) successfully induced convulsions and the mortality rate was 100 % within 24 h. All of the compounds were administered to the various groups at the dose of 100 mg/kg intra-peritoneally. After 4 h of the test compound administration, the albino mice were then subcutaneously injected with pentylenetetrazole (90 mg/kg). Spasms that lasted at least 5 sec throughout a 1-hour observation period was accounted to be the most humane endpoint. Mice were given 8 mL of warm saline (SC) and returned to their housings if they did not have seizures at the end of the observation period. The absence of a 5 sec spasm episode was taken as protection. Following local standard protocols, all dead mice were disposed of in bio-safety containers.

An episode of spasms that retained for at least 5 sec was noted as a threshold convulsion. Tremulousness and intermittent jerks were not counted. The test animals not having the threshold convulsions during 60 min were noted as protected from seizures. The number of test animals protected in each group was recorded, and then the anticonvulsant activity was presented in terms of percentage protection. No protection from convulsions was recorded in test animals of control group. Test animals were kept under observation for 24 h and their mortality was recorded.

In silico ADME studies, potential drug target prediction and docking studies

Drug design is a gradual process of evaluating the drug regarding its outcomes during its trials. For this purpose, the designed molecule is desirable for its pharmacokinetics prediction and drug-binding capability through *in silico* screening of the molecule designed; by available databases and computer-aided drug designing tools. The designed molecules were evaluated for their ADME properties using *in silico* ADME software to evaluate pharmacokinetic properties such as BBB crossing ability, water solubility, absorption through GIT, skin permeability, bioavailability, drug likeliness and synthetic accessibility [15]. Such tools help to identify the lead compounds

before putting the efforts into their synthesis and avoiding the conventional hit and trial method of synthesizing drug derivatives [15].

Potential drug target prediction in terms of biomolecules in *H. sapiens* has made it easy to identify the compounds as responsible for the desired activity. The compounds passing this screening were investigated for their docking ability through the use of the database (RCSB protein data bank) [16]. The crystal structure of human trypsin was used as ligand, designed drug derivatives were used for evaluating the docking score and binding capacity to analyze the potential target lead compounds for further *in vitro* and *in vivo* screening thereby saving cost and time spent in screening all possible derivatives [17].

Scheme for synthesis

The Schiff bases were synthetically prepared by the condensation reaction of carbamazepine with an equimolar ratio of aromatic aldehydes and ketones. The reaction mixture was refluxed for 3 h using ethanol as solvent and acid as catalyst. The reaction mixture was filtered, and the product was recrystallized with ethanol as shown in Figure 1. A total of eight products were synthesized with respect to side groups R₁ and R₂, as stated in Table 1.

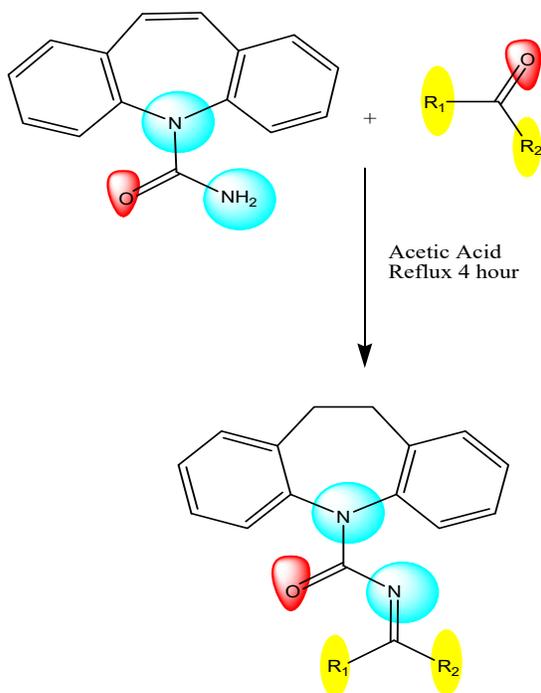


Figure 1: General reaction of carbamazepine with aromatic aldehyde or ketones

Table 1: Synthesized compounds

Compound	Aldehyde/Ketone	R ₁	R ₂
C1	Salicylaldehyde	C ₆ H ₅ O	H
C2	Benzaldehyde	C ₆ H ₅	H
C3	Acetophenone	C ₆ H ₅	CH ₃
C4	Cinnamaldehyde	C ₈ H ₇	H
C5	4-(Dimethyl amino)benzaldehyde	C ₈ H ₁₀ N	H
C6	n-n dimethyl formamide	C ₂ H ₇ N	H
C7	Formaldehyde	H	H
C8	Methyl ethyl ketone	C ₂ H ₅	CH ₃

RESULTS

Spectral properties

Spectral characterization yielded the following data:

C1: Off white crystals; Yield 76 % ; m. p. 179 – 181 °C; with molecular formula C₂₂H₁₆N₂O₂ and molecular weight: 340.38, elemental analysis (calculated) for C₂₂H₁₆N₂O₂; C, 77.63; H, 4.74; N, 8.23; (found) C, 76.93; N, 7.93; H, 4.12; FTIR (cm⁻¹): 2778, (C-H), 1715 (C=O), 1683, 1668 (C=N), 1473 (C=C), 3648 (O-H), 1338 (C-N), 1277 (C-O); ¹H NMR (DMSO-d₆, ppm); 6.89 - 6.90 (2H, m, =C-H), 6.91 - 6.92 (2H, dd=C-H), 6.94 - 6.95 (2H, t, =C-H), 7.10 - 7.11, (1H, t =C-H), 7.28 - 7.29 (2H, t, =C-H) 7.31 - 7.32 (1H, m, =C-H), 7.50 - 7.51 (2H, dd, =C-H), 7.60 - 7.61 (2H, dd, =C-H), 8.90 (1H, s, =C-H), 4.91 (1H, s, OH); ¹³C NMR (DMSO-d₆, ppm); 127.3 (C1/10), 119.0 (C2/9), 128.6 (C3/8) 119.5 (C4/7), 140.1 (C5/6), 126.9 (C11/14), 126.8 (C12/13), 156.7 (C15), 161.4 (C16), 119.6 (C17), 160.7 (C18), 117.6 (C19), 132.8 (C20), 126.8 (C21), 132.1 (C22).

C2: Brown crystals; Yield 70 %; m. p. 178 – 180 °C; with molecular formula C₂₂H₁₆N₂O and molecular weight: 324.38, elemental analysis (calculated) for C₂₂H₁₆N₂O; C, 81.46; H, 8.64, H, 4.97; (found) C, 81.12; H, 4.35; N, 8.04; FTIR (cm⁻¹): 2970 (C-H), 1700 (C=O), 1642.41 (C=N), 1558 (C=C), 1278 (C-N), ¹H NMR (DMSO-d₆, ppm); 7.10 - 7.11 (4H, m (=C-H), 7.31 - 7.35 (3H, m (=C-H), 7.38 - 7.40 (2H, d, =C-H), 7.43 - 7.45 (2H, dd, =C-H), 7.60 - 7.7.61 (2H, dd, =C-H), 7.86 - 7.87 (2H, m, =C-H), 8.87 (1H, s, =C-H); ¹³C NMR (DMSO-d₆, ppm); 127.3 (C1/10), 126.8 (C2/9), 128.1 (C3/8) 126.9 (C4/7), 140.1 (C5/6), 126.9 (C11/14), 126.8 (C12/13), 156.9 (C15), 162.5 (C16), 133.4 (C17), 130.9 (C18,22), 129.2 (C19,21), 130.8 (C20).

C3: Dark Brown crystals; Yield 79 %; m. p. 175 – 177 °C ; with molecular formula C₂₃H₁₈N₂O and molecular weight: 338.4, elemental analysis

(calculated) for $C_{23}H_{18}N_2O$; C, 81.63; N, 8.28; H, 5.36; (found) C, 81.13; H, 4.96; N, 8.18; FTIR (cm^{-1}): 1716, 1698 (C=O), 1652, 1636 (C=N), 1558, 1472 (C=C), 1386 (C-N); 1H NMR (DMSO- d_6 , ppm); 7.10 - 7.11 (4H, m (=C-H)), 7.31 - 7.34 (3H, m (=C-H)), 7.40 - 7.42 (2H, d, =C-H), 7.43 - 7.46 (2H, dd, =C-H), 7.73 - 7.74 (2H, dd, =C-H), 7.75 - 7.77 (2H, m, =C-H), 0.91 (3H, s, CH_3), ^{13}C NMR (DMSO- d_6 , ppm); 127.3 (C1/10), 126.8 (C2/9), 128.1 (C3/8), 126.9 (C4/7), 140.2 (C5/6), 127.0 (C11/14), 126.8 (C12/13), 154.7 (C15), 168.7 (C16), 134.7 (C17), 130.8 (C18,22), 129.3 (C19,21), 130.8 (C20). 21.5 (C-23).

C4: Dark brown solid mass; Yield 68 %; m. p. 192 – 194 °C; with molecular formula $C_{24}H_{18}N_2O$ and molecular weight: 350.4, elemental analysis (calculated) for $C_{24}H_{18}N_2O$; C, 82.26; N, 7.99; H, 5.18; (found) C, 82.15; H, 4.96; N, 7.55; FTIR (cm^{-1}): 3058, 3026, 2815, 2742, (C-H), 1666, 1623 (C=N), 1595, 1486 (C=C), 1385 (C-N), 1205 (C-C); 1H NMR (DMSO- d_6 , ppm); 6.99 - 7.02 (2H, m (=C-H)), 7.04 - 7.05 (2H, m (=C-H)), 7.09 - 7.10 (2H, d, =C-H), 7.40 - 7.43 (2H, dd, =C-H), 7.49 - 7.51 (2H, dd, =C-H), 7.44 - 7.45 (1H, d, =C-H), 5.21-5.22 (1H, d, CH), 6.31 - 6.32 (1H, d, CH), 7.31 - 7.34 (2H, m, =C-H), 7.26 - 7.28 (2H, m, =C-H), 7.21 - 7.24 (1H, m, =C-H), ^{13}C NMR (DMSO- d_6 , ppm): 127.3 (C1/10), 125.6 (C2/9), 128.3 (C3/8), 126.9 (C4/7), 140.2 (C5/6), 128.8 (C11/14), 126.8 (C12,13), 157.9 (C15), 161.7 (C16), 125.6 (C17), 136.9 (C18), 130.8 (C19), 126.2 (C20,24), 129.3 (C21,23), 128.3 (C22).

C5: Black Shiny crystals: Yield 80 %; m. p. 224 – 226 °C with molecular formula $C_{24}H_{21}N_3O$ and molecular weight: 367.45, elemental analysis (calculated) for $C_{24}H_{21}N_3O$; C, 78.45; N, 11.44; H, 5.76; (found) C, 78.45; H, 5.24; N, 11.21; FTIR (cm^{-1}): 3037, 2839 (C-H), 1630 (C=N), 1594, 1487 (C=C), 1333 (C-N), 1234 (C-C); 1H NMR (DMSO- d_6 , ppm); 6.71 - 6.73 (2H, d, =C-H), 7.04 - 7.05 (2H, m, =C-H), 7.09 - 7.10 (2H, d, =C-H), 7.40 - 7.43 (2H, dd, =C-H), 7.41 - 7.43 (2H, dd, =C-H), 7.31 - 7.34 (2H, m, =C-H), 7.58 - 7.60 (2H, m, =C-H), 2.82 (6H, s, CH_3), 8.01 (1H, s=H) ^{13}C NMR (DMSO- d_6 , ppm); 127.3 (C1/10), 125.1 (C2/9), 128.7 (C3/8), 126.8 (C4/7), 140.1 (C5/6), 129.2 (C11/14), 125.1 (C12,13), 156.3 (C15), 162.4 (C16), 125.0 (C17), 130.9 (C18,22), 111.8 (C19,21), 151.2 (C20), 40.3 (C23,24).

C6: Yellow amorphous powder: Yield 75 %; m. p. 200 – 202 °C with molecular formula $C_{18}H_{17}N_3O$ and molecular weight: 291.35, elemental analysis (calculated) for $C_{18}H_{17}N_3O$; C, 74.20; H, 5.88; N, 14.42; (found) C, 74.04; H, 5.25; N, 13.92; FTIR (cm^{-1}): 1652 (C=N), 1589, 1486 (C=C); 1H NMR

(DMSO- d_6 , ppm); 7.10 - 7.11 (2H, dd(=C-H)), 7.31 - 7.34 (2H, t (=C-H)), 7.40 - 7.43 (2H, t, =C-H), 7.49 - 7.51 (2H, d, =C-H), 8.50 - 8.51 (2H, d, =C-H), 7.46 (1H, s, =C-H), 3.08 (6H, s, CH_3); ^{13}C NMR (DMSO- d_6 , ppm), 128.4 (C1/10), 126.8 (C2/9), 128.9 (C3/8), 128.3 (C4/7), 140.0 (C5/6), 129.5 (C11/14), 129.7 (C12/13), 157.2 (C15), 154.3 (C16), 38.2 (C17, 18).

C7: Black crystals: Yield 76 %; m. p. 185 – 187 °C with molecular formula $C_{16}H_{12}N_2O$ and molecular weight: 248.29, elemental analysis (calculated) for $C_{16}H_{12}N_2O$; C, 77.40; N, 11.28; H, 4.87; (found) C, 76.40; H, 4.19; N, 11.11; FTIR (cm^{-1}): 2981, 2843 (C-H), 1653 (C=N), 1585, 1487 (C=C), 1331 (C-N), 1061 (C-C); 1H NMR (DMSO- d_6 , ppm); 6.05, 6.30 (2H, s, (=CH₂)), 7.10 - 7.11 (2H, dd(=C-H)), 7.31 - 7.34 (2H, t (=C-H)), 7.40 - 7.42 (2H, t, =C-H), 7.43 - 7.44 (2H, d, =C-H), 7.46 - 7.49 (2H, dd, =C-H), ^{13}C NMR (DMSO- d_6 , ppm), 128.7 (C1/10), 127.0 (C2/9), 129.2 (C3/8), 127.3 (C4/7), 139.7 (C5/6), 130.9 (C11/14), 129.7 (C12/13), 157.2 (C15), 162.3 (C16).

C8: Dark green crystals: Yield 82 %; m. p. 188 – 190 °C with molecular formula $C_{19}H_{18}N_2O$ and molecular weight: 290.37, elemental analysis (calculated) for $C_{19}H_{18}N_2O$; C, 78.59; N, 11.28; H, 6.25; (found) C, 78.18; H, 5.96; N, 9.20; FTIR (cm^{-1}): 3016 (C-H), 1714 (C=O), 1653 (C=N), 1472 (C=C); 1H NMR (DMSO- d_6 , ppm); 1.09 (3H, s, CH_3), 1.06 - 1.07 (3H, t, CH_3), 2.27 (2H, m, CH_2), 7.10 - 7.11 (2H, dd (=C-H)), 7.31 - 7.34 (2H, t (=C-H)), 7.40 - 7.42 (2H, t, =C-H), 7.43 - 7.45 (2H, d, =C-H), 7.49 - 7.51 (2H, dd, =C-H), ^{13}C NMR (DMSO- d_6 , ppm), 128.6 (C1/10), 126.8 (C2/9), 129.2 (C3/8), 127.3 (C4/7), 140.2 (C5/6), 130.9 (C11/14), 129.2 (C12/13), 154.5 (C15), 174.2 (C16), 31.0 (C17), 10.3 (C18), 21.1 (C19).

***In silico* ADME, potential drug target prediction and docking**

Designed derivatives after evaluation from *in silico* ADME studies exhibited drug-like properties, good absorption through the GIT, blood-brain barrier crossing ability and high synthetic accessibility. Complete data-sheet ADME properties for C1 are shown in Table 2, while related properties for all derivatives are shown in Table 3 using the ADME tool [18].

As the parent molecule, i.e., carbamazepine, the derivatives can also cross BBB, making them CNS active agents and in *H. sapiens*, C1 derivative exhibited maximum ability to cross the BBB and proteases (trypsin) binding property as shown in Figure 3.

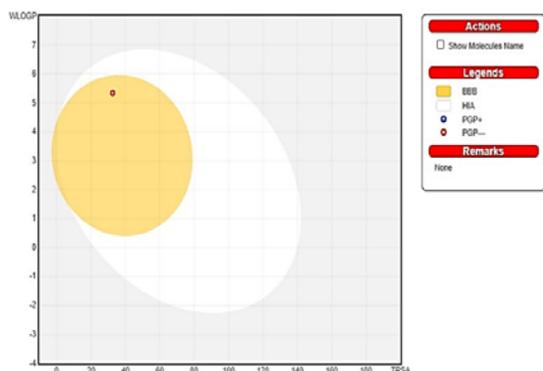


Figure 2: Boiled-egg plot for BBB prediction (Swiss ADME) ■ BBB (Blood Brain Barrier), □ HIA (Human intestinal absorption), ● PGP+ (Binding ability to P-glycoprotein), ● PGP- (Inability to bind with P-glycoprotein)

Target prediction for proteases for synthesized compounds C1 - C8 was in the range of 6.7 - 33.3 % in *H. Sapiens* while the maximum value of 33.3 % was for C1 (Figure 3).

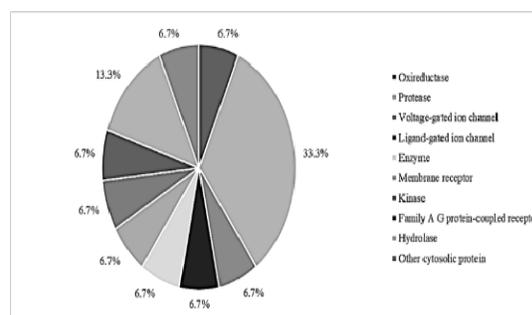


Figure 3: Target prediction for C1 in *H. sapiens*

Table 2: ADME prediction for C1

Physicochemical properties	Aqueous solubility	Pharmacokinetic parameters	Drug-likeness	Medicinal chemistry parameters
Formula	Log S (ESOL)	GI absorptibility	Lipinski	PAINS
C ₂₂ H ₁₆ N ₂ O ₂	-5.04	High	Yes; violation: MLOGP>4.15	No alert
Molecular weight	Aqueous solubility	BBB permeability	Ghose	Brenk
340.37 g/mol	3.13e-03 mg/mL	Yes	Yes	2 alerts: imine_1, stilbene
Number of atoms (heavy)	Class	P-gp substrate	Veber	Lead likeness
26	Moderate solubility	No	Yes	No; violation: XLOGP3>3.5
Number of aromatic atoms (heavy)	Log S (Ali)	CYP1A2 inhibition	Egan	Synthetic accessibility
18	-5.23	Yes	Yes	3.49
Csp3 fraction	Solubility	CYP2C19 inhibition	Muegge factor	
0	2.01e-03 mg/mL	Yes	Yes	
Number of bonds i.e., rotatable	Class	CYP2C9 inhibition	Score of bioavailability	
3	Moderate solubility	Yes	0.55	
Acceptors (Number Hydrogen bond)	Log S (SILICOS-IT)	CYP2D6 inhibition		
3	-6.28	No		
Donors (Number of Hydrogen bond)	Solubility	CYP3A4 inhibition		
1	1.77e-04 mg/mL	No		
Refractivity	Class	Log K_p (skin permeability)		
108.34	Poorly soluble	-5.25 cm/s		
TPSA				
52.90 Å ²				

Table 3: Predicted pharmacokinetic properties of carbamazepine synthesized derivatives

Compound	Pharmacokinetics								Medicinal chemistry		
	GI absorption	BBB permeation	CYP1A2 inhibition	CYP2C19 inhibition	CYP2C9 inhibition	CYP2D6 inhibition	CYP3A4 inhibition	Log Kp (Skin permeation)	Bioavailability score	Drug likelihoods	Synthetic accessibility
C1	High	Yes	Yes	Yes	Yes	No	No	-5.25	0.55	Yes	3.49
C2	High	Yes	Yes	Yes	Yes	No	No	-4.90	0.55	Yes	3.50
C3	High	Yes	Yes	Yes	Yes	No	No	-4.83	0.55	Yes	3.55
C4	High	Yes	No	Yes	Yes	No	No	-4.76	0.55	Yes	3.74
C5	High	Yes	No	Yes	Yes	No	No	-5.87	0.55	Yes	3.47
C6	High	Yes	No	Yes	Yes	No	No	-5.87	0.55	Yes	3.47
C7	High	Yes	Yes	Yes	Yes	No	No	-4.54	0.55	Yes	3.10
C8	High	Yes	Yes	Yes	Yes	No	No	-5.38	0.55	Yes	3.51

Molecular docking, anti-protease ability and structure activity relationship

Molecular docking studies were conducted for ligand (synthesized derivatives) and targeted protein (trypsin). The docking of designed derivatives with trypsin exhibited more likelihood of ligands to be bonded to the target site at trypsin due to induce fitting at binding site of the target protein molecule at THR157, ILE156 and GLY154 as shown by amino acid residues in Figure 4 [19].

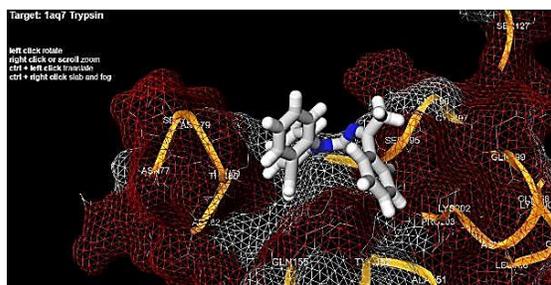


Figure 4: Docking pose for C3 with trypsin

Anti-protease activity

The total anti-protease potential at different concentrations was tested using azocasein assay [12]. The trend found was as follows; C7 > C3 > C5 > C2 > C6 > C4 > C2 > C1 as shown in Figure 5. The ability to inhibit proteases link the synthesized compounds as potential antiviral agents and therapeutic moieties that inhibit convulsive disorders such as epilepsy.

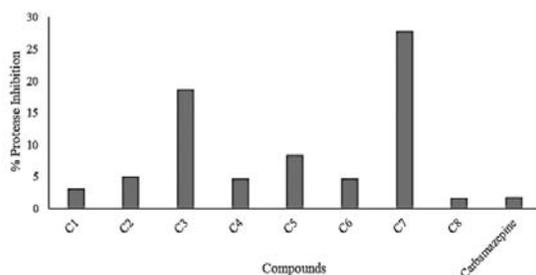


Figure 5: Antiproteases activity of synthesized derivatives (C1-C8) compared to parent drug carbamazepine

Antioxidant potential

Antioxidant potential revealed C3, C4, C5 as more actively antioxidant while C1 and C2 as least active as the radical scavenging agent as shown in Figure 6. This behavior shows that the synthesized compounds exert the least oxidative

stress, especially when the compound can cross BBB [20].

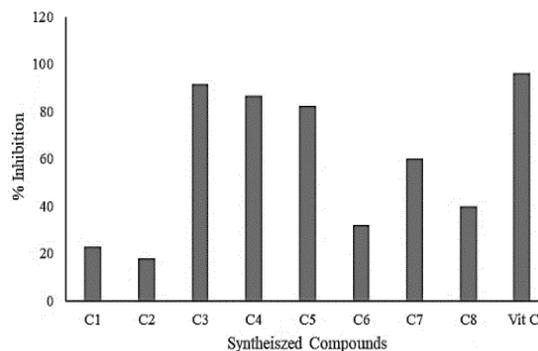


Figure 6: DPPH free radical scavenging activity of synthesized derivatives (C1-C8) compared to vitamin C

Antimicrobial activity

As shown in Figure 7, antimicrobial activity against *B. bronchiseptica* was in rank order of C5 > C2 > C8 > C1 > C6 > C4 > C3 > C7, C5 > C4 > C2 > C1 > C6 > C8 > C3 > C7 for *B. subtilis*, C2 > C6 > C4 > C8 > C1 > C3 > C5 > C7 for *E. coli*, C5 > C7 > C8 > C6 while C4, C3, C1 and C2 gave negligible results for *B. pumilis*, C2 > C8 > C4 > C5 > C1 > C6 > C3 > C7 for *M. luteus*, C5 > C4 > C2 > C6 > C8 > C1 > C7 > C3 for *S. epidermidis*, C5 > C4 > C8 > C1 > C2 > C3 > C6 > C7 for *S. aureus*. All derivatives had antimicrobial activity, while C1 derivative showed prominent activity [11].

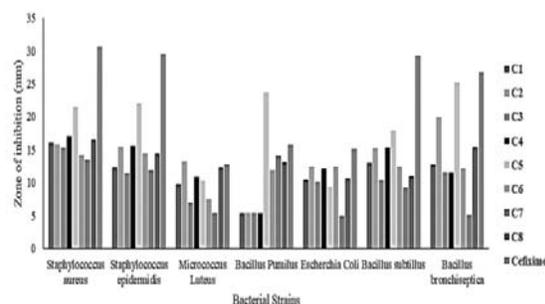


Figure 7: Antimicrobial activity of synthesized derivatives (C1-C8) compared to drug cefixime

Anticonvulsant activity

The results shown by C7 and C5 were having comparable activity with standard drug, mild to the moderate activity of C1, C2, C4, and C8, whereas C3 showed the least activity as shown in Figure 8.

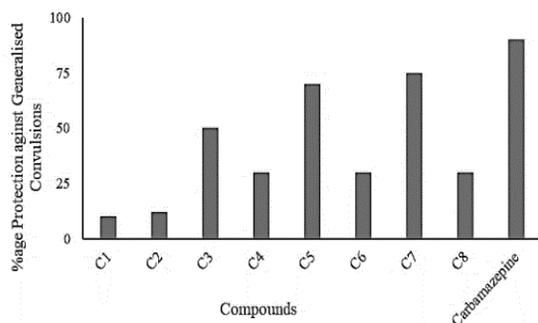


Figure 8: Anticonvulsant activity of synthesized derivatives (C1-C8) compared to parent drug – carbamazepine

Structure-activity relationship

Anti-protease activity increased by adding an alkyl group at the primary amine group of the parent molecule as C7, while the activity became lesser when an aryl group was added in the alkyl chain like C3. Anti-protease activity decreased when the secondary amine group added along with aryl group in C5. By increasing chain length at substitution site i.e., primary amine of parent molecule in the remaining synthesized derivatives, activity also reduced.

Antioxidant potential was increased with the attachment of aryl groups i.e., C3, C4, and C5. In contrast, attachment of the alkyl chain decreased the antioxidant properties in the compounds, i.e., C7 and C8, while the remaining compounds exhibited the least potential.

Antimicrobial activity of derivatives was maximum against *S. aureus*, and *B. bronchiseptica* and aryl substituted compounds were more active against *S. aureus*. Amine group increased the activity of such derivatives. Anticonvulsant activities of the synthesized derivatives showed that C3, C5, and C7 are the derivatives that have comparable anticonvulsant activities with their parent drug. They have aryl substitution in C3 and C5, and the alkene group is attached in C7.

DISCUSSION

C1 has greater activity against *Bacillus subtilis*, while it has comparable activity with cefixime (standard drug) against *Staphylococcus aureus*, *Bacillus bronchiseptica* and *Staphylococcus epidermidis*. C2 has greater activity against *Staphylococcus aureus* and *Staphylococcus epidermidis* as compared to standard drug while it has moderate activity against *Micrococcus luteus*, *Bacillus pumilus*, *Escherchia coli*, *Bacillus subtilis* and *Bacillus bronchiseptica*. C8 had

moderate activity against *Bacillus bronchiseptica* and *Staphylococcus aureus* as compared to standard drug. C9 exhibited least activity against *Micrococcus luteus* compared to the standard drug cefixime.

Almost all the derived Schiff bases of carbamazepine had moderate to maximum antibacterial activity against all the studied strains but most of the derived compounds are more active against *Staphylococcus aureus* and *Bacillus bronchiseptica*.

Among the derivatives of carbamazepine, C4 and C5 showed significant radical scavenging activity i.e., 86.69 and 82.43 %, respectively as compare to standard i.e., ascorbic acid which had 96.28 % radical scavenging activity. C2 and C6 has 17.75 and 31.89 % radical scavenging activity respectively which was not significant ($p > 0.05$).

Amongst the derivatives of carbamazepine; C5, C6 and C7 had greater or significant anti-protease activity i.e., 8.39, 4.69 and 27.79 %, respectively while carbamazepine itself has 1.74 % anti-protease activity.

C3, C5 and C7 showed considerable anticonvulsant activity with percentage protection of 50, 70 and 70 %, respectively while their parent drug - carbamazepine gave 90 % protection and their mortality rate was considerably low.

CONCLUSION

Among the newly synthesized carbamazepine derivatives, C3 demonstrates appreciable antioxidant activity while C5 exhibits prominent antibacterial activity, compared with ascorbic acid and cefixime, respectively. The protease inhibitory potential of C3 and C7 is the highest while C5 and C7 present moderate anticonvulsant activity relative to carbamazepine. Thus, these compounds are potential anticonvulsant agents with a predictable mechanism of action, unlike other conventional anticonvulsants with unknown or non-specific mechanism of action.

DECLARATIONS

Acknowledgement

The authors acknowledge the Pharmacy Department, The Islamia University Bahawalpur for providing essential equipment and resources to perform this work.

Conflict of Interest

No conflict of interest associated with this work.

Contribution of Authors

The authors declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by them.

Open Access

This is an Open Access article that uses a funding model which does not charge readers or their institutions for access and distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/4.0>) and the Budapest Open Access Initiative (<http://www.budapestopenaccessinitiative.org/read>), which permit unrestricted use, distribution, and reproduction in any medium, provided the original work is properly credited.

REFERENCES

1. Stafstrom CE, Carmant L. Seizures and epilepsy: an overview for neuroscientists. *Cold Spring Harb Perspect Med* 2015; 5(6): a022426.
2. Aliabadi A, Gholamine B, Karimi T. Synthesis and antiseizure evaluation of isoindoline-1, 3-dione derivatives in mice. *Med Chem Res* 2014; 23: 2736-2743.
3. Khajouei MR, Mohammadi-Farani A, Moradi A, Aliabadi A. Synthesis and evaluation of anticonvulsant activity of (Z)-4-(2-oxoindolin-3-ylideneamino)-N-phenylbenzamide derivatives in mice. *Res Pharm Sci* 2018; 13(3): 262.
4. Lohman R-J, O'Brien TJ, Cocks TM. Protease-activated receptor-2 regulates trypsin expression in the brain and protects against seizures and epileptogenesis. *Neurobiol Dis* 2008; 30(1): 84-93.
5. Oikonomopoulou K, Hansen KK, Saifeddine M, Vergnolle N, Tea I, Diamandis EP, Hollenberg MD. Proteinase-mediated cell signalling: targeting proteinase-activated receptors (PARs) by kallikreins and more. *Biol Chem* 2006; 387: 677-685.
6. Alshibli HM, Al-Abdullah ES, Haiba ME, Alkahtani HM, Awad GE, Mahmoud AH, Ibrahim BM, Bari A, Villingier A. Synthesis and Evaluation of New Coumarin Derivatives as Antioxidant, Antimicrobial, and Anti-Inflammatory Agents. *Molecules* 2020; 25(14): 3251.
7. Ambrósio AF, Soares-da-Silva P, Carvalho CM, Carvalho AP. Mechanisms of action of carbamazepine and its derivatives, oxcarbazepine, BIA 2-093, and BIA 2-024. *Neurochem Res* 2002; 27(Pt 1): 121-130.
8. Sinha D, Tiwari AK, Singh S, Shukla G, Mishra P, Chandra H, Mishra AK. Synthesis, characterization and biological activity of Schiff base analogues of indole-3-carboxaldehyde. *Euro J Med Chem* 2008; 43(1): 160-165.
9. Joseyphus RS, Nair MS. Antibacterial and antifungal studies on some schiff base complexes of zinc (II). *Mycobiol* 2008; 36(2): 93-98.
10. Padul MV, Patil MT, Chougale AD, Zambare VP, Patil RM, Ghule RB, Naikwade SV, Garad AS, Shaikh FL, Gadge PP et al. In vitro screening of proteinase inhibitors (trypsin, chymotrypsin and Helicoverpa gut proteinase inhibitors) in different plant tissue extracts. *Trends Biotechnol Res* 2012; 1(1): 07-14.
11. Silva RH, Andrade A, Nóbrega DF, Castro RDd, Pessôa HL, Rani N, De Sousa DP. Antimicrobial Activity of 4-Chlorocinnamic Acid Derivatives. *BioMed Res Int* 2019; 22: 4217864.
12. Brock FM, Forsberg CW, Buchanan-Smith JG. Proteolytic activity of rumen microorganisms and effects of proteinase inhibitors. *App Environ Microbiol* 1982; 44(3): 561-569.
13. Safitri A, Fatchiyah F, Sari DRT, Roosdiana A. Phytochemical screening, in vitro anti-oxidant activity, and in silico anti-diabetic activity of aqueous extracts of *Ruellia tuberosa* L. *J App Pharm Sci* 2020; 10(03): 101-108.
14. Harriss D, Atkinson G. Update—ethical standards in sport and exercise science research. *Int J Sports Med* 2011; 32(11): 819-821.
15. Opo FADM, Rahman MM, Ahammad F, Ahmed I, Bhuiyan MA, Asiri AM. Structure based pharmacophore modeling, virtual screening, molecular docking and ADMET approaches for identification of natural anti-cancer agents targeting XIAP protein. *Sci Rep* 2021; 11(1): 1-17.
16. Berman HM, Westbrook J, Feng Z, Gilliland G, Bhat TN, Weissig H, Shindyalov IN, Bourne PE. The Protein Data Bank. *Nucleic Acids Res* 2000; 28(1): 235-242.
17. Odhar HA, Ahjel SW, Albeer AAMA, Hashim AF, Rayshan AM, Humadi SS. Molecular docking and dynamics simulation of FDA approved drugs with the main protease from 2019 novel coronavirus. *Bioinformation* 2020; 16(3): 236-244.
18. Daina A, Michielin O, Zoete V. SwissADME: a free web tool to evaluate pharmacokinetics, drug-likeness and medicinal chemistry friendliness of small molecules. *Sci Rep* 2017; 7(1): 1-13.
19. Latha KNM, Babu GRM. A novel approach for identification of possible GSK-3 β inhibitors using computational virtual screening analysis of drugs. *Int J Comput Biol Drug Des* 2019; 12(4): 312-331.
20. Fási L, Meo FD, Kuo CY, Buric SS, Martins A, Kúsz N, Béni Z, Dékány M, Balogh GT, Pesic M et al. Antioxidant-inspired drug discovery: Antitumor metabolite is formed in situ from a hydroxycinnamic acid derivative upon free-radical scavenging. *J Med Chem* 2019; 62(3): 1657-1668.