Ind. J. Pharm. Edu. Res., 2024; 58(3s):s1062-s1074.
https://www.ijper.org

Original Article

Design, Synthesis, Molecular Docking, *in silico* ADME Assessment of Pyrrole-Based Heterocyclic Amino Acid Derivatives as Potential Anticonvulsant Agent

Md Shamsher Alam[1,*], Mohammed Albratty[1], Hassan Ahmed Alhazmi[1,2,3], Asim Najmi[1], Khalid Zoghebi[1], Hafiz Antar Makeen[4], Safaa Fathy Saleh[1], Ahmed Farag El Kirdasy[5], Benson Mathai Kochikuzhyil[6]

[1]Department of Pharmaceutical Chemistry and Pharmacognosy, College of Pharmacy, Jazan University, Jazan, SAUDI ARABIA.
[2]Substance Abuse and Toxicology Research Centre, Jazan University, Jazan, SAUDI ARABIA.
[3]Medical Research Center, Jazan University, Jazan, SAUDI ARABIA.
[4]Department of Clinical Pharmacy, Pharmacy Practice Research Unit, College of Pharmacy, Jazan University, Jazan, SAUDI ARABIA.
[5]Department of Biochemistry, College of Veterinary Medicine, Sadat City University, Sadat, EGYPT.
[6]Department of Pharmacology, Dr. Joseph Mar Thoma Institute of Pharmaceutical Sciences and Research, Kattanam, Alappuzha, Kerala, INDIA.

ABSTRACT

Background: Epilepsy is a neurological disorder characterized by anomalous brain activity, convulsions and odd behaviour. Ten pyrrole carbohydrate based analogues (Va-Vj) were synthesized with amide as intermediate in the current research with the goal of reducing convulsions and seizures. **Materials and Methods:** The newly developed compounds were synthesized. Numerous methods (IR, NMR, mass, elemental analysis, etc.,) were used to characterize these substances. Several models were used to test each of these molecules for anticonvulsant activity. By using the rotarod and ethanol potentiation techniques, neurotoxicity was also evaluated. The study meticulously examined each parameter and showed ADME predictions for each of the 10 congeners that were produced. In addition, studies on molecular docking employed the GABA-A target protein. **Results:** Anticonvulsant screening results identified compounds Ve, Vd, Vc and Va as the most efficacious of the series. All synthesized equivalents largely passed the neurotoxicity test. The results of molecular docking revealed significant interactions at the active site of GABA-A with Ile C:242, Asp C:424, Phe D:307, Arg C:250 Trp C:241 and Phe C:240 and the outcomes were good and in agreement with *in vivo* findings. **Conclusion:** The study's findings showed that some substances had promising anticonvulsant properties that were comparable to those of the standard drug. The highly active novel anticonvulsant analogues may therefore represent a possible lead and additional studies may result in a potential new drug candidate.

Keywords: N-glycosides, Carbohydrate, Anticonvulsant activity, Pyrrole, ADME.

Correspondence:
Dr. Md Shamsher Alam
Department of Pharmaceutical Chemistry and Pharmacognosy, College of Pharmacy, Jazan University, Jazan 45142, SAUDI ARABIA.
Email: mosalam@jazanu.edu.sa

Received: 27-04-2023;
Revised: 12-02-2024;
Accepted: 14-06-2024.

INTRODUCTION

There exists a global population of around 50 million individuals who are affected by epilepsy. The current taxonomy of seizure types, as stated by the International League Against Epilepsy, has three primary categories: generalized onset (motor or absent), localized onset (which may encompass aberrant behaviors, responses, sensations, or movements) and unknown onset.[1,2] Seizures are characterized as disturbances in neurological function that arise from abnormal neuronal signaling inside the brain. Despite the existence of a wide range of Antiepileptic Drugs (AEDs), it is commonly recognized that around one-third of individuals with epilepsy fail to achieve adequate seizure management using currently available treatments.[3-5] Furthermore, in instances of status epilepticus, a condition distinguished by unusually protracted seizures, the potential for neuronal mortality, neuronal injury and modifications to neuronal networks to result in fatality is contingent upon the specific type and duration of the convulsions.

According to recent estimates by the World Health Organization (WHO), the global population affected by epilepsy is over 50 million individuals, the majority of whom, nearly 80%, reside in nations categorized as low or middle-income. According to empirical research, individuals who receive accurate diagnosis and suitable treatment for epilepsy have the potential to achieve a seizure-free existence, as indicated by studies reporting rates as high as 70%.[6]

DOI: 10.5530/ijper.58.3s.106

Therefore, there exists a substantial clinical demand to discover suitable and efficacious interventions for the treatment of particularly drug-resistant epilepsy. There remains a prevailing concern over the selectivity and toxicity of the presently Accessible Antiepileptic Drugs (AEDs). Hence, there exists a perpetual need for enhanced anticonvulsant medications that exhibit reduced hazards. The advancement of novel anticonvulsant medications is hindered by our constrained comprehension of the intricate mechanisms that underlie epilepsy. These mechanisms include the alteration of voltage-dependent sodium and/or calcium channels, the enhancement of inhibitory effects mediated by Gamma-Aminobutyric Acid (GABA), the potential impact on the GABA system, the decrease in synaptic excitation mediated by ionotropic glutamate receptors and the modification of synaptic release. Numerous pharmacological effects of synthetic nitrogen heterocycles have been demonstrated, elevating the significance of this class of molecules. Quinazolines, pyrrole, indole, pyridine, pyrrolidine, triazole, oxadiazole, thiadiazole, triazines and pyrimidines are nitrogen-containing heterocycles. Historically, there was a prevalent belief that numerous anticonvulsant medications were composed of nitrogen-containing heterocycles, predominantly lactam or imides, with phenyl or alkyl groups attached.[7,8]

In nature, pyrrole and its derivatives are constantly present. Several therapeutically useful chemicals, such as fungicides, antibiotics, anti-inflammatory medications, cholesterol-lowering medications, anticancer medicines, anti-convulsant agents and many more, include the pyrrole subunit.[9]

Numerous biological activity have been discovered for pyrrole, an essential ring structure. A wide variety of physiologically active chemicals are integrated into pyrrole ring either as substituents or with different substitutions on the ring itself. As shown in Figure 1 below, certain medications with pyrrole moiety are now on the market, while others are undergoing clinical studies.[10]

Almost every part of living things involves carbohydrates and compounds containing carbohydrates carry out a number of crucial biological tasks. Therefore, a significant field of therapeutic research has long been in the study of compounds based on carbohydrates. Since most living things use glucose as an energy source, glucose conjugates and derivatives can be employed to treat metabolic diseases like diabetes. Since RNA and DNA are composed of D-ribose and D-deoxyribose, their derivatives are frequently utilized to insert into and stop the reproduction processes of viruses and pathogenic cells. The different glycans that are found on the surface of bacteria, viruses and eukaryotic cells are also noteworthy. These glycans are important for identification, invasion and communication, making them potential candidates for disease detection and treatment. Furthermore, a wide range of secondary metabolites based on carbohydrates are secreted by both plants and microbes as defense or signaling molecules. These metabolites can serve as potential targets for future therapeutic development.[11]

Significant advancements in the disciplines of carbohydrate chemistry, glycobiology and chemical glycobiology have been made since 2000, opening up a wide range of possibilities for the identification of drugs based on carbohydrates.

More than 15 Novel Chemical Entities (NCEs) based on carbohydrates were introduced globally after 2015; these include antiviral, antibacterial, anticancer and antidiabetic medications (Table 1).[12-14]

When it comes to medications for the nervous system that are based on carbohydrates, two drugs have been approved in the last 20 years: sugammadex, which reverses neuromuscular block and sodium oligomannate, which treats Alzheimer's Disease (AD). Green Valley produced sodium oligomannate (GV-971, 42), which is obtained from marine brown algae. Multiple targeting methods of sodium oligomannate were shown to have a significant ameliorative effect on animal models of cognitive impairment, according to ethological tests.

Merck Sharp and Dohme created Sugammadex, a selective relaxant binding agent that reverses NMB through a unique and innovative method. It is an eight-D-glucose unit modified g-cyclodextrin connected by β-1,4-glycosidic linkages.[15,16]

For a long time, researchers have been exploring the potential of heterocyclic amino acids as anticonvulsant agents (Figure 2). Our team has been dedicated to developing GABA modulators for many years and we recently identified pyrrole, a nitrogen-containing heterocyclic moiety, as a promising candidate for further study. To synthesize potential anticonvulsant agents, we converted pyrrole to intermediate amides and coupled them with glucose and lactose to form N-glycosides. This effort was based on our previous work and we hope it leads to the discovery of new candidates that effectively modulate GABA and prevent seizures.

MATERIALS AND METHODS

Chemistry

Equipment used

Equipment such as a Rota evaporator (Buchi), a Magnetic stirrer and water bath (JSGW, India), a Weighing balance (made by Mettlor, India), an UV Chamber (made by M/S Schimadzu, India), a Deep Freezer (made by M/S REMI, India) were used.

Solvents and Reagents

For analytical thin layer chromatography, pre-coated TLC plates and glass plates with silica gel G were employed. The process of detection involved the use of various spray reagents or UV lamps. For thin layer chromatography, silica gel G (160-120 mesh) was acquired from SRL. Additional solvents and reagents for the investigation were obtained from Merck, India, Rankem, India; and M/S Qualigens Fine Chemicals, Mumbai, India.

N-glycoside Preparation

This stage comprises the subsequent subdivisions: a) Acetylation of sugars b) Bromination of acetylated sugars c) Amineum acid ester synthesis d) Amidoamide preparation e) N-glycoside synthesis f) N-glycoside deacetylation g) Hydrolysis.

Synthesis of Initial Nucleus

Acetylated sugar was produced by refluxing sugar with sodium acetate and acetic anhydride. An additional step involved the bromination of this acetylated sugar using a solution composed of HBr and acetic anhydride (Ia and Ib) (Figure 3a). The presence of brominated sugar derivatives was validated via spectral analysis and thin layer chromatography. Subsequently, amino methyl esters were produced by combining amino acid with concentrated H_2SO_4 and methanol. The amides (IIa-IIe) were produced by coupling these amino acid esters with pyrrole carboxylic acid (Figure 3b).

Synthesis of Final Compounds

In the presence of HOBT, an equivalent quantity of brominated sugar reacted with the amide (IIa-IIe), producing N-glycosides (IIIa-IIIe). Following this, acetylated sugar was deprotected using methanol and sodium methoxide.[13,14] Ion exchange resin IR-120H+ was utilized to terminate the reaction, resulting in the formation of carbohydrate-coupled esters (IVa-IVe). After hydrolyzing these compounds with dilutionary HCl, the resulting compounds (Va-Ve) were obtained. Except for a few, all products and intermediates were obtained with a satisfactory yield. As shown in Figure 4, final compounds were verified by means of TLC, IR and NMR spectroscopy.

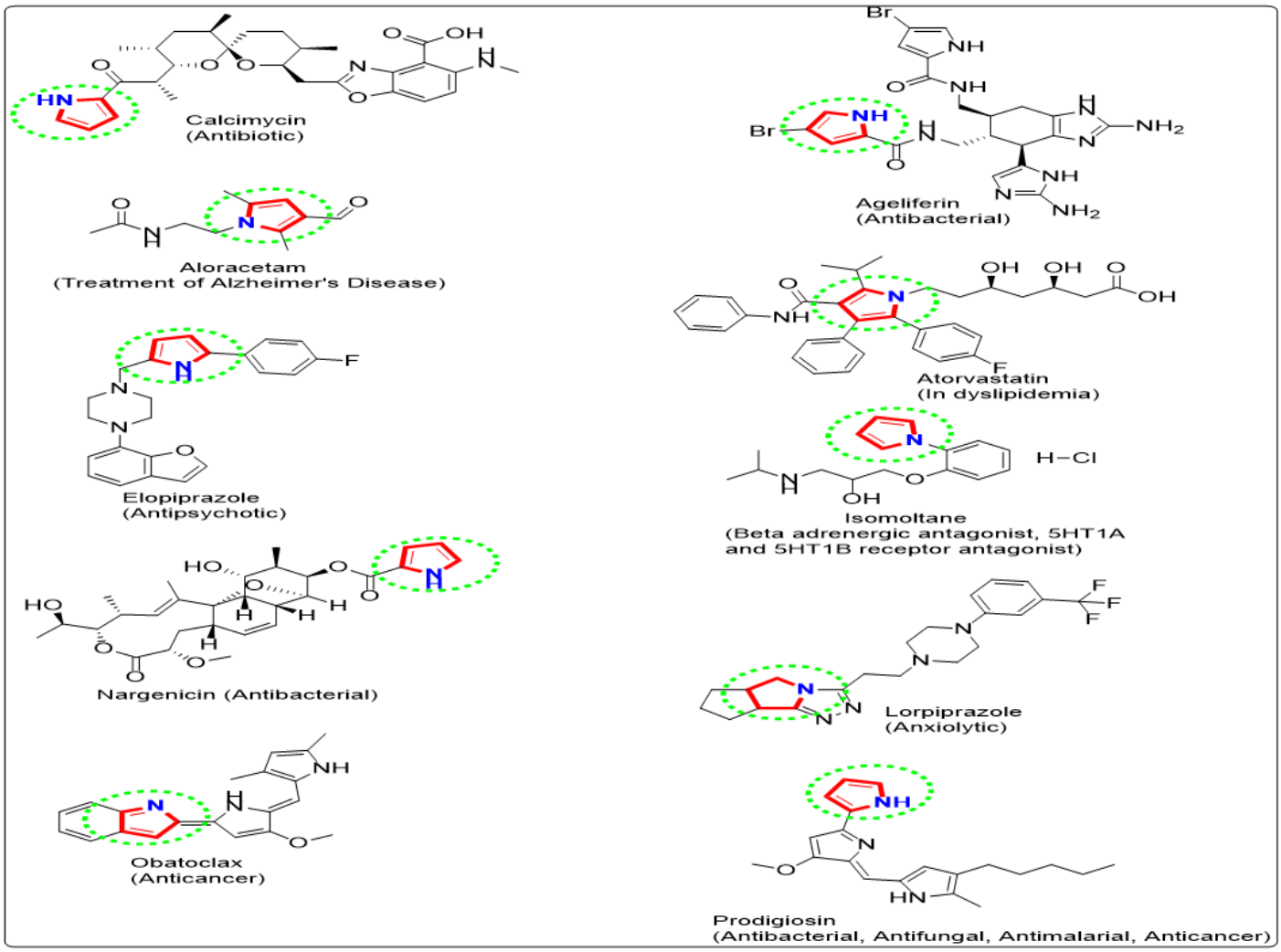

Figure 1: Drugs containing pyrrole moiety available in market.

Pharmacology

Anticonvulsant action assessment

Every step of the animal handling process, including sample administration and disposal, was followed as per IACUC regulations, Faculty of veterinary-medicine, University of Sadat-City, with Ethical approval number VUSC-036-1-23.

Table 1: Carbohydrate based drugs launched after 2015.

Name of the Drug	Year	Indications
Molnupiravir	2021	Antiviral
Azvudine	2021	Antiviral
Maribavir	2021	Antiviral
Remedesevir	2020	Antiviral
Carrimycin	2019	Antibacterial
Plazomicin	2018	Antibacterial
Forodesine	2017	Anticancer
Midostaurin	2017	Anticancer
Sotaglifozin	2019	Antidiabetic
Remogliflozin etabonate	2019	Antidiabetic
Ertugliflozin	2017	Antidiabetic
Sodium oligomannate	2019	Alzheimer's disease
Lactilol	2020	Chronic idiopathic constipation
Migalastat	2016	Fabry disease
Uridine triacetate	2015	Hereditary orotic aciduria
S. Pneumoniae vaccines	2000-2021	*S. Pneumoniae* prevention

The experimental animals used were male albino mouse weighing 25-30 g. For MES and scPTZ screening, test compounds were suspended in PEG 200. In a typical laboratory setting, six animals per cage were kept at room temperature with unfettered access to food and water.

Maximal Electroshock Seizure (MES) Test

Test compounds at a dose level of 30, 100 and 300 mg/kg were administered as an intraperitoneal (i.p.) injection to measure the anticonvulsant effect at a different time interval (0.5 hr and 4 hr). The most severe electroshock seizures in mice were induced utilising ear clip electrodes and 0.2 sec of 60 Hz, 50 mA electrical shocks. Protection is defined as the absence of the tonic extensor component of seizures in 50% or more of the animals.[17]

Subcutaneous Pentylenetetrazole (*sc*PTZ) Seizure Test

To determine which chemicals raise the threshold for seizures, the scPTZ is utilized. Pentylenetetrazole is used in the scPTZ test at a dose of 85 mg/kg. In 97% (CD97) of the tested animals, this results in clonic convulsions lasting at least five seconds. Each animal received intraperitoneal doses of 30, 100 and 300 mg/kg of the test chemicals.

PTZ was given subcutaneously at 0.5 hr and 4.0 hr and the animals were watched for 30 min. The lack of clonic seizures in 50% or more of the animals over the specified time period served as a measure of the ability of synthesized analogues to counteract pentylenetetrazole's influence on the seizure threshold.[18]

Neurotoxicity study

Rotarod test

Utilizing the rotarod test, the motor function of rodents was evaluated. Mice were instructed to maintain their position on a revolving rod measuring 3.2 cm in diameter and rotating at 10 Revolutions Per Minute (RPM) throughout the experiment. In

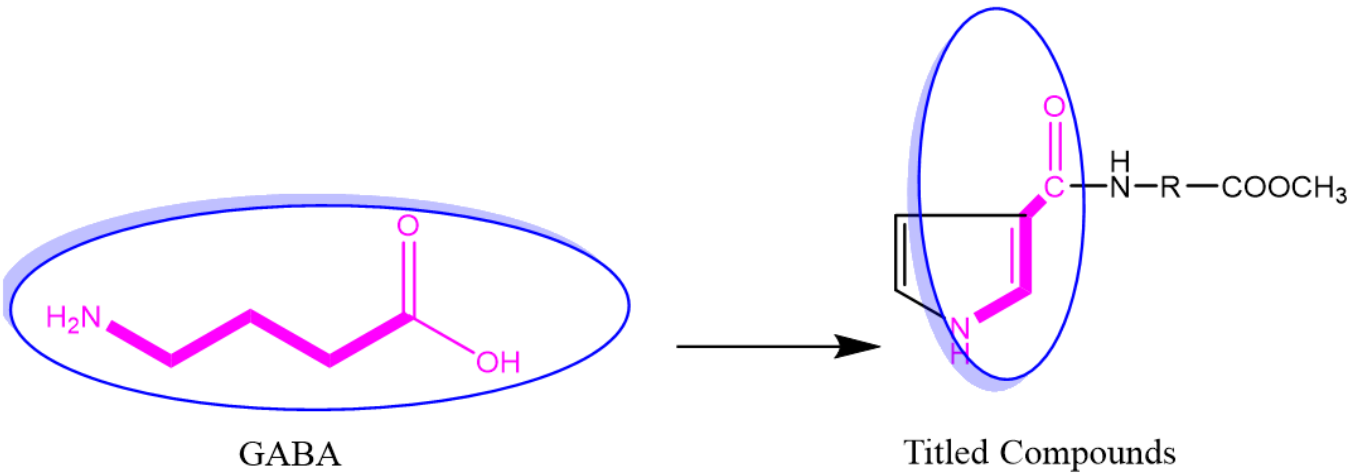

Figure 2: Structurally GAB-similar heterocyclic aminoacids as potential GABA-modulators.

all three experiments, the degree of neurotoxicity is determined by the animal's inability to maintain equilibrium on the rotating rod for a minimum of 1 min. The dosage at which 50% of the animals exhibited balance impairment while on the rotating rod was determined to be.[19,20]

Ethanol Potentiation Test

Test compounds were given to mice. 1 hr later, the dose of 2.5 g kg^{-1} of ethanol was administered to test animals except for control animals. In control animals, there won't be any lateral positioning brought on by ethanol. After being given ethanol, the number of animals in each group that were in the lateral posture was counted.

Molecular docking studies and ADME prediction

To evaluate the ADME properties of compounds that were synthesized, pre-ADMET software was utilized. Human Intestinal Absorption (HIA), log P, Plasma Protein Binding (PPB), Blood-Brain Barrier penetration (BBB) and Skin Permeability (SP) were among the attributes that were evaluated.

The AutoDock Vina software was utilized to conduct molecular docking in order to ascertain the likely orientation and validate the presence of the ligand at the site of binding. The laboratories utilized Chem Draw software (Cambridge Soft) to illustrate the structures of the synthesized pyrrolidine analogues (Va-j). The Chem3D Ultra 8.0 software was employed to transform the 2D structures into 3D.

The Protein Data Bank (PDB) contained the X-ray crystal structure of the GABA-A receptor, denoted as the GABA(A) R-beta3 homopentamer (code 4COF). The protein preparation was accomplished using UCSF Chimera 1.15 with the assistance of the protein preparation wizard. The protein was inputted into the PyRx program, which generated a PDBQT file containing the hydrogen atoms present in each of the polar residues of the protein. The Lamarckian Genetic Algorithm (LGA) methodology was employed for the computation.

After the completion docking search, the best conformation with the lowest docked energy was chosen. For each ligand structure, ten AutoDock Vina runs were performed and the best pose was captured and saved for each run. Discovery studio 3.5 investigated

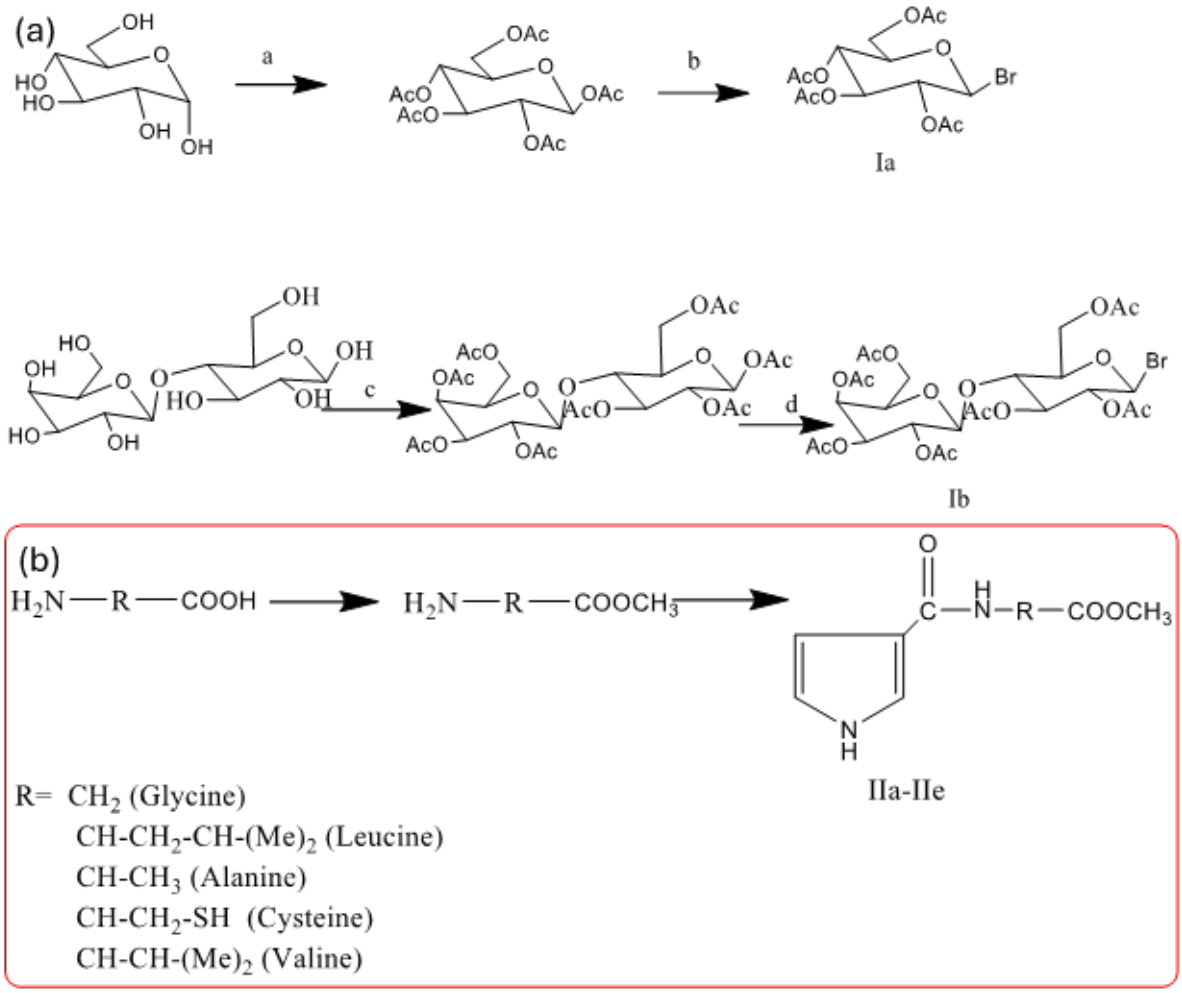

Figure 3: (a) Scheme for the synthesis of the initial nucleus; (b) Scheme for the synthesis of the pyrrole intermediates.

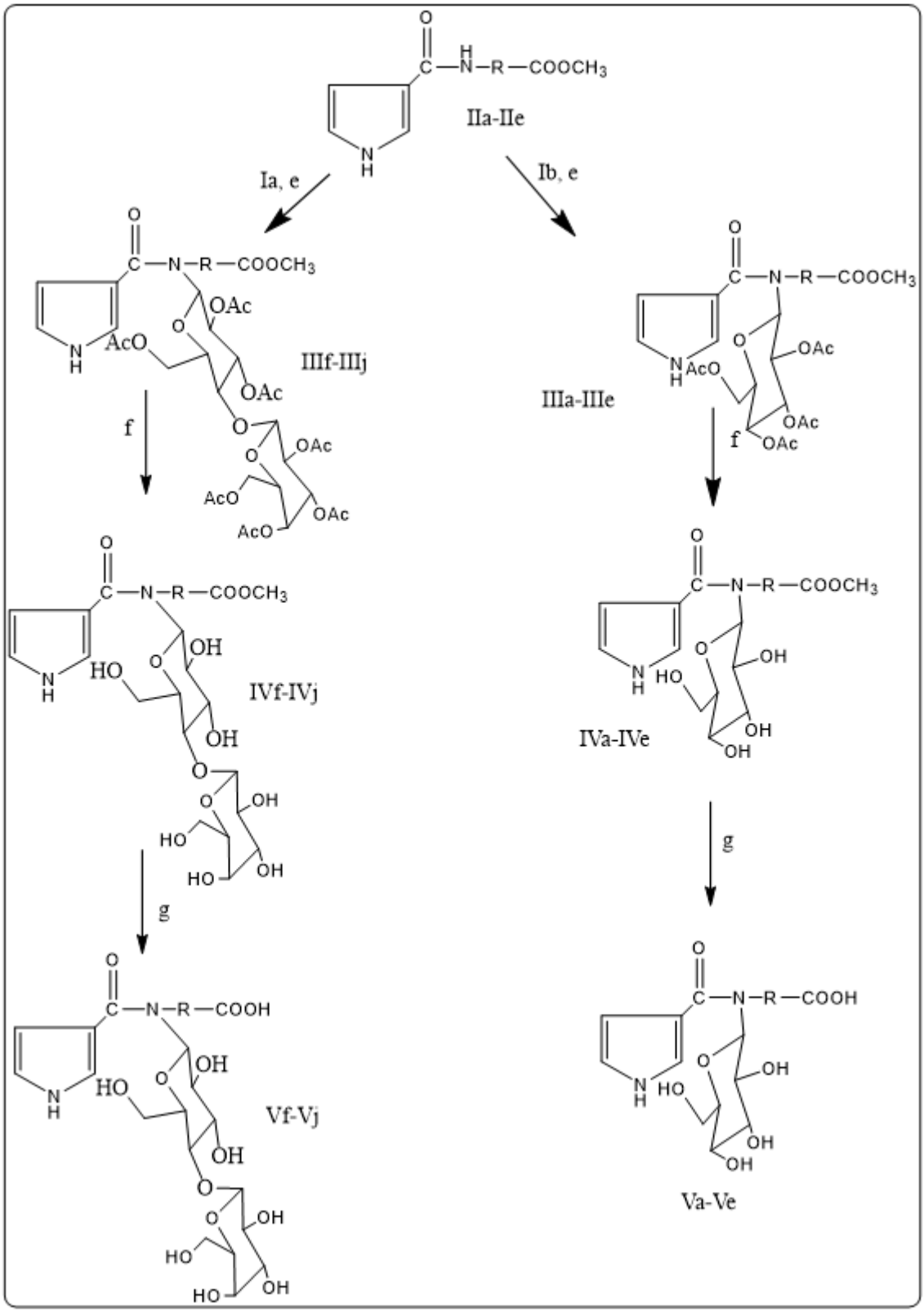

Figure 4: Scheme for synthesis of the final compounds.

protein-ligand conformation interactions. The affinities of the molecule at the receptor's active site were determined using the docking score, pi-pi interactions and hydrogen bonds.

RESULTS AND DISCUSSION

Chemistry

In this paper, the synthesis and characterization carbohydrate-based analogues are described as shown in Figure 4. These compounds have been designed keeping in mind the structure of the indigenous neurotransmitter Gamma Amino Butyric Acid (GABA) which plays a vital role in controlling seizure generation and spread.

The work reported here explains the synthesis and characterization pyrrole-carbohydrate analogues. Simultaneously, these newly synthesized compounds were evaluated for anticonvulsant activities and molecular docking studies were also performed. A total 10 number of substrates were synthesized according to the synthetic procedure described in Figure 4. Table 2 presents the results of an investigation into various physicochemical properties. Utilizing compounds that had been crystallized and isolated with an appropriate solvent, these novel analogues were characterized with the use of NMR and IR spectroscopical data. New product elemental analyses were also carried out. N-H, C=O (acid and amide), C=N, C-O, C-N and N-H stretching bands were visible in IR measurements.

Table 2: Physicochemical properties of prepared analogs (Va-Vj).

Compd	Structure	IUPAC Name	Melting point range	Yield	Molecular weight
Va	2	N-(1H-pyrrole-3-carbonyl)-N-3,4,5-trihydroxy-6-(hydroxymethyl)tetrahydro-2H-pyran-2-yl)glycine.	182-185°C	80%	330.10
Vb	2	N-(1H-pyrrole-3-carbonyl)-N-3,4,5-trihydroxy-6-(hydroxymethyl)tetrahydro-2H-pyran-2-yl)cysteine.	175-178°C	56%	376.09
Vc	2	N-(1H-pyrrole-3-carbonyl)-N-3,4,5-trihydroxy-6-(hydroxymethyl)tetrahydro-2H-pyran-2-yl)valine.	195-198°C	70%	372.37
Vd	2	N-(1H-pyrrole-3-carbonyl)-N-3,4,5-trihydroxy-6-(hydroxymethyl)tetrahydro-2H-pyran-2-yl)alanine.	187-191°C	71%	344.12
Ve	2	N-(1H-pyrrole-3-carbonyl)-N-3,4,5-trihydroxy-6-(hydroxymethyl)tetrahydro-2H-pyran-2-yl)leucine.	184-187°C	59%	386.16
Vf	2	N-3,4-dihydroxy-6-(hydroxymethyl)-5-(3,4,5-trihydroxy-6-(hydroxymethyl) tetrahydro-2H-pyran-2-yl)oxy) tetrahydro-2H-pyran-2-yl)-N-(1H-pyrrole-3-carbonyl)glycine.	211-213°C	30%	492.15
Vg	2	N-3,4-dihydroxy-6-(hydroxymethyl)-5-(3,4,5-trihydroxy-6-(hydroxymethyl) tetrahydro-2H-pyran-2-yl)oxy) tetrahydro-2H-pyran-2-yl)-N-(1H-pyrrole-3-carbonyl)cysteine.	207-211°C	21%	538.52
Vh	2	N-3,4-dihydroxy-6-(hydroxymethyl)-5-(3,4,5-trihydroxy-6-(hydroxymethyl) tetrahydro-2H-pyran-2-yl)oxy) tetrahydro-2H-pyran-2-yl)-N-(1H-pyrrole-3-carbonyl)valine.	237-241°C	29%	534.51
Vi	2	N-3,4-dihydroxy-6-(hydroxymethyl)-5-(3,4,5-trihydroxy-6-(hydroxymethyl) tetrahydro-2H-pyran-2-yl)oxy) tetrahydro-2H-pyran-2-yl)-N-(1H-pyrrole-3-carbonyl)alanine.	228-231°C	32%	506.46
Vj	2	N-3,4-dihydroxy-6-(hydroxymethyl)-5-(3,4,5-trihydroxy-6-(hydroxymethyl) tetrahydro-2H-pyran-2-yl)oxy) tetrahydro-2H-pyran-2-yl)-N-(1H-pyrrole-3-carbonyl)leucine.	224-228°C	49%	548.54

Table 3: Data on analogues (1-10)'s neurotoxicity and anticonvulsant screening.

Compound Number	Mice receiving an intraperitoneal/subcutaneous injection[a]						
	MES assessment		scPTZ assessment		Neurotoxicity assessment		Ethanol
	0.5 hr	4.0 hr	0.5 hr	4.0 hr	0.5 hr	4.0 hr	Potentiation
Va	100	300	100	300	X	X	X
Vb	300	300	300	300	-	-	-
Vc	100	100	100	300	X	X	X
Vd	30	100	100	300	-	-	-
Ve	30	100	100	100	-	-	-
Vf	100	300	300	300	-	-	-
Vg	300	300	300	300	-	-	X
Vh	100	300	300	300	+	+	-
Vi	300	300	300	300	-	-	X
Vj	300	300	300	300	+	+	-
Phenytoin	30	30	-	-	100	100	-
Valproic acid	30	100	100	300	100	300	-

Using TMS as an internal standard, the ^{1}H NMR spectrum data of the compounds were collected in $CDCl_3$/DMSO-d6 solvent. These newly developed products revealed pyrrole-H in the range of 7.2-8.8 ppm with glucose and lactose attachments, a singlet for COOH in the range of 12.0-13.0 ppm and a singlet for N-H in the range of 9.1-9.6 ppm, a singlet for OH is observed in the range 2.1-2.5. Various signals for sugar moieties are also observed in proton NMR.

Reagents: (a) Methanol, sodium acetate and acetic-anhydride (b) Dichloromethane, HBr-AcOH and acetic anhydride (c) Concentrated sulphuric acid, Ethanol and aq. ammonia solution. (d) Dichloromethane, HOBT, nicotinic acid, Et_3N.

Reagents: (e) Dichloromethane, DMF, Et_3N (f) anhydrous Methanol, NaOMe, DCM, ion exchange resin (IR-120H$^+$) (g) dil. HCl.

The determination of the compound structures was achieved by analyzing and interpreting spectral data. KBr pellet/Neat infrared transmissions, expressed as vmax in cm^{-1}, were measured using a PerkinElmer spectrophotometer. The proton NMR spectra were acquired using deutectic solvents and a PerkinElmer NMR spectrometer operating at 300 MHz. The internal standard utilized for the spectra was Me_4Si. The values of Hz were used to record the coupling constants (J) and ppm (δ) for chemical shifts. The TLC solvent system consisted of a 6:4 ratio of ethyl acetate to petroleum ether. The TLC, FT-IR and proton NMR data for the final compounds is mentioned below.

Va: (R_f 0.67): 3639-3604 (OH), 1727 (C=O acid), 1683 (C=N), 1634 (C=O amide), 1275(C-O), 1236 (CN), 3325 (NH), ^{1}H-NMR (300 MHz, CDCl3) δ (ppm): 7.91-8.88 (m, 3H, -CH), 5.7-4.38(m, 5H, -CH), 3.8 (s, 2H, -CH2), 3.6(d, 2H, -CH2), 2.1 (s, 4H, -OH), 9.3 (s, 1H, -NH), 12.2 (s, 1H, -COOH).

Vb: (R_f 0.64): 3632-3597 (OH), 1732 (C=O acid), 1684 (C=N), 1634 (C=O amide), 1213 (C-O), 1168 (CN), 3327 (NH), 1H-NMR (300 MHz, CDCl3) δ (ppm): 8-8.8 (m, 3H, -CH), 6-4.37 (m, 5H, -CH), 4.78 (d, 2H, -CH2), 3.7 (s, 2H, -CH2), 1.4 (s, 1H, -SH), 2.1 (s, 4H, -OH), 9.1 (s, 1H, -NH), 12.0 (s, 1H, -COOH).

Vc: (R_f 0.29): 3604-3576 (OH), 1729 (C=O acid), 1691 (C=N), 1636 (C=O amide), 1051(C-O), 1341 (CN), 3350 (NH), 1H-NMR (300 MHz, CDCl3) δ (ppm): 8.5-7.3 (m, 3H, -CH), 5.5-4.5(m, 5H, -CH), 4.27(d, 1H, -CH); 2.75 (m, 1H, -CH), 0.96 (d, 6H, -CH_3), 2.3 (s, 4H, -OH), 3.7 (s, 2H, -CH_2), 9.2 (s, 1H, -NH), 12.8 (s, 1H, -COOH).

Vd: (R_f 0.26): 3593- 3561 (OH), 1732 (C=O acid), 1668 (C=N), 1636 (C=O amide), 1095(C-O), 1363 (CN), 3405 (NH), 1H-NMR (300 MHz, CDCl3) δ (ppm): 8.5-7.8 (m, 3H, -CH), 6.3-4.12 (m, 5H, -CH), 3.8 (s, 2H, -CH2), 1.42(d, 3H, -CH3), 4.57 (q, 1H, -CH), 2.3(s, 4H, -OH), 9.4 (s, 1H, -NH), 12.5 (s, 1H, -COOH).

Ve: (R_f 0.69): 3628-3589 (OH), 1749 (C=O acid), 1671 (C=N), 1629 (C=O amide), 1238 (C-O), 1324 (CN), 3427 (NH), 1H-NMR (300 MHz, CDCl3) δ (ppm): 8.3-7.2 (m, 3H, CH); 5.4-4.4(m, 4H, -CH), 3.9 (s, 2H, -CH2), 1.75 (dd, 2H, -CH2), 1.49 (m, 1H, -CH), 2.2 (s, 4H, -OH); 0.98 (d, 6H, -CH3), 4.09 (t, 1H, -CH), 9.3 (s, 1H, -NH), 12.4 (s, 1H, -COOH).

Vf: (R_f 0.45): 3638-3587 (OH), 1731 (C=O acid), 1680 (C=N), 1654 (C=O amide), 1126 (C-O), 1076 (CN), 3330 (NH), 1H-NMR (300 MHz, CDCl3) δ (ppm): 8.1-7.4 (m, 3H, -CH), 5.5 (d, 1H, H-1, j8.1Hz), 5 (d, 1H, H-7), 4.89-4.3(m, 8H, -CH), 3.9(s, 2H, CH2), 3.7(d, 4H, -CH2), 3.4(s, 3H, -CH3), 2.3 (s, 7H, -OH), 9.4 (s, 1H, -NH), 12.7 (s, 1H, -COOH).

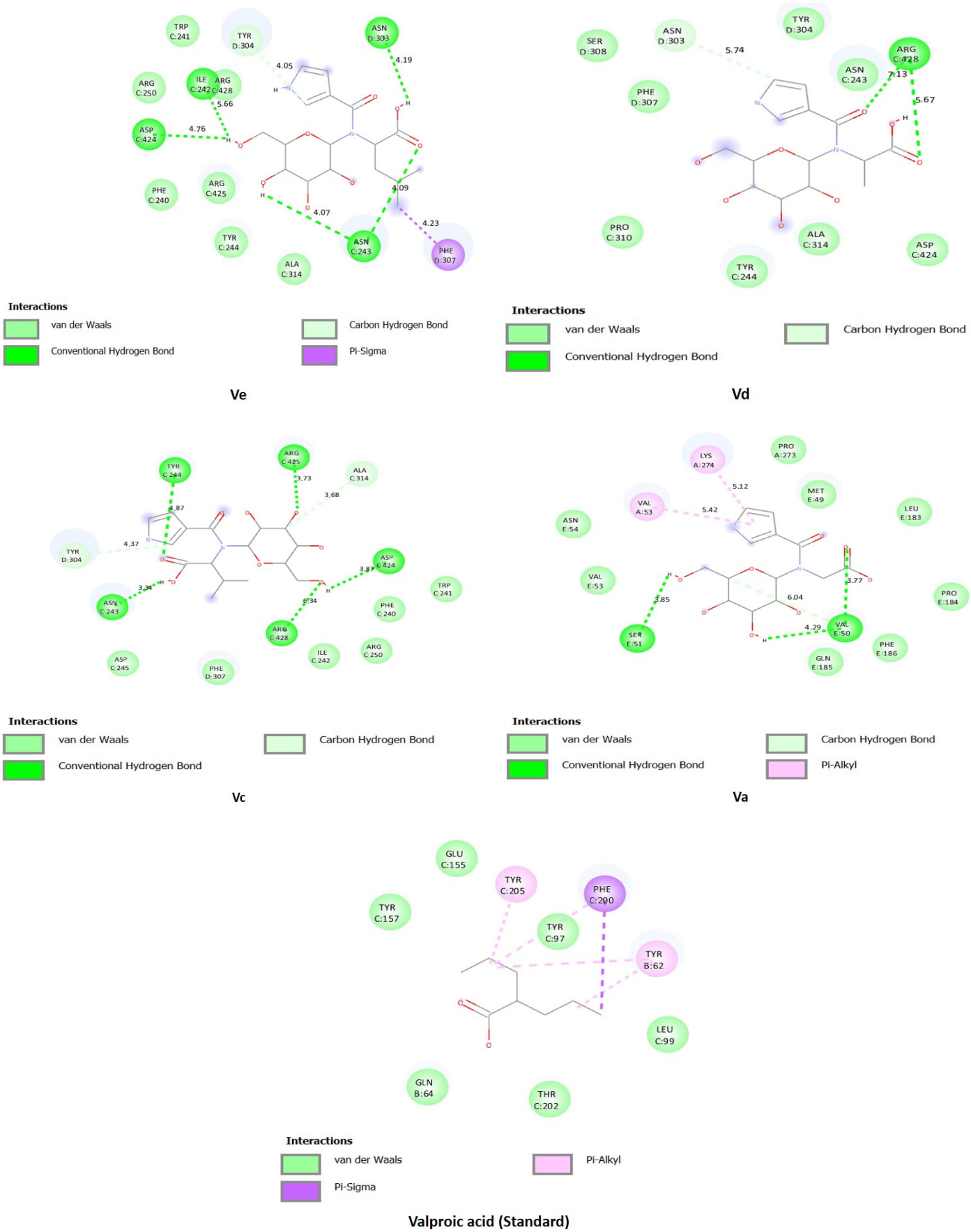

Figure 5: 2D Ligand interaction of most active compounds.

Table 4: ADME prediction and results of Docking of final analogues (Va-j) with the GABA-A receptor, 4CO.

Compd	LogP	[a]BBB	[b]BS (mg/l)	CYP-inhibition	[c]HIA	[d]PPB	[e]SP	Ligand	Binding Affinity
Va	-1.899990	0.0491962	759487	2D6 non inhibitor	11.623660	14.722877	-5.00993	4cof__uff_E=665.32	-7.0
Vb	-1.368690	0.003234	7.15513e+006	2D6 non inhibitor	8.868602	24.254852	-4.81553	4cof__uff_E=699.94	-6
Vc	-0.743060	0.0492941	179630	2D6 non inhibitor	15.626759	33.724435	-4.80932	4cof__uff_E=770.93	-7.1
Vd	-1.533480	0.0466093	420575	2D6 non inhibitor	12.807258	21.798133	-4.95178	4cof__uff_E=627.50	-7.2
Ve	-0.356750	0.0532067	97754.8	2D6 non inhibitor	17.312305	36.588190	-4.73896	4cof__uff_E=709.20	-7.5
Vf	-3.649980	0.0279247	592625	2D6 non inhibitor	1.396705	10.245790	-5.09369	4cof__uff_E=12526.49	-6.9
Vg	-3.118680	0.0297884*	5.35802e+006	2D6 non inhibitor	0.992857	13.586356	-4.84958	4cof__uff_E=12663.98	-6.8
Vh	-2.493050	0.028434	134949	2D6 non inhibitor	1.896129	23.994238	-4.9096	4cof__uff_E=12594.37	-6.9
Vi	-3.283470	0.0280494	323771	2D6 non inhibitor	1.546727	13.388004	-5.04419	4cof__uff_E=12643.86	-6.7
Vj	-2.106740	0.0286698	72630.8	2D6 non inhibitor	2.101461	26.969895	-4.84028	4cof__uff_E=1095.05	-5.7
Valproic acid (standard)								4cof__uff_E=36.29	-5.7

[a]Blood brain barrier, [b]Buffer solubility, [c]Human Intestinal Absorption, [d]Plasma Protein Binding, [e]Skin Permeability.

Vg: (R_f 0.42): 3615-3571 (OH), 1732 (C=O acid), 1669 (C=N), 1653 (C=O amide), 1094 (C-O), 1261 (CN), 3425 (NH), 1H-NMR (300 MHz, CDCl3) δ (ppm): 8.3-7.2 (m, 3H, m; -CH); 5.7 (d, 1H, H-1, j7.76), 5.1(d, 1H, H-7), 4.89-4.3(m, 8H, -CH), 3.9(s, 2H, CH2), 3.7(d, 4H,-CH2), 3.4(s, 3H, -CH3), 2.3 (s, 7H, -OH), 9.1(s, 1H, -NH), 12.9 (s, 1H, -COOH).

Vh: (R_f 0.39): 3579-3528 (OH), 1718 (C=O acid), 1636 (C=N), 1671 (C=O amide), 1123 (C-O), 1363 (CN), 3305 (NH), 1H-NMR (300 MHz, CDCl3) δ (ppm): 8.5-7.7 (m, 3H, -CH), 6.1(d, 1H, H-1, j8.17 Hz), 5.4 (d, 1H, H-7), 4.81-4.2 (m, 8H, -CH), 3.7 (t, 1H, -CH), 3.5 (d, 4H, -CH2), 3.3 (q, 2H, -CH2), 2.1(s, 7H, -OH), 1.9 (m, 1H, -CH), 1.6 (dd, 2H, -CH2), 1.4(t, 3H, -CH3), 0.9 (d, 6H, -CH3), 9.4 (s, 1H, -NH), 13.0 (s, 1H, -COOH).

Vi: (R_f 0.36): 3627-3597 (OH), 1759 (C=O acid), 1677 (C=N), 1649 (C=O amide), 1125 (C-O), 1190 (CN), 3370 (NH), 1H-NMR (300 MHz, CDCl3) δ (ppm): 8.8-7.7 (m, 3H, -CH), 6.2 (d, 1H, H-1, j8.42Hz), 5.3 (d, 1H, H-7), 4.75-4.21 (m, 8H, CH); 4.1 (d, 4H, -CH2), 3.8 (t, 1H, -CH), 3.5 (s, 3H, -CH3), 2.3 (s, 7H, -OH), 1.9 (m, 1H, -CH), 1.7 (dd, 2H, -CH2), 0.8(d, 6H, -CH3), 9.1 (s, 1H, -NH), 12.2 (s, 1H, -COOH).

Vj: (R_f 0.36): 3595-3532 (OH), 1734 (C=O acid), 1664 (C=N), 1662 (C=O amide), 1115 (C-O), 1099 (CN), 3360 (NH), 1H-NMR (300 MHz, CDCl3) δ (ppm): 8.6-7.2 (m, 3H, -CH), 5.9 (d, 1H, H-1, j7.1Hz), 5.2 (d, 1H, H-7), 4.89-4.33 (m, 8H, -CH), 3.9 (d, 4H, -CH2), 3.7 (q, 1H, -CH), 3.4 (q, 2H, -CH2), 2.4 (s, 7H, -OH), 1.5 (t, 3H, -CH3), 1.2 (d, 3H, -CH3), 9.6 (s, 1H, -NH), 12.8 (s, 1H, -COOH).

Pharmacological Assessment

Numerous pharmacological studies were carried out in line with the Anticonvulsant Drug Development (ADD) program's standard operating procedure. Studies on the compound's neurotoxicity were also carried out. The analysis techniques comprised scPTZ, MES and neurotoxicity (Tox). The anticonvulsant activity of the synthesized analogues was calculated at intervals of 0.5 and 4 hr after injections given intraperitoneally (i.p.) at doses of 30, 100 and 300 mg/kg. Neurotoxicity was evaluated using the rotarod test and ethanol potentiation. Table 3 contains the findings of the anticonvulsant and neurotoxicity data.

All molecules in the Maximal Electroshock (MES) model showed electroshock protection after 0.5 hr with Vd and Ve at a dose of 30 mg/kg, indicating a rapid beginning of action. At 100 mg/kg for 0.5 hr, molecules Va, Vc, Vf and Vh produced favorable outcomes. Molecules Vc, Vd and Ve demonstrated good activity at a dose of 100 mg/kg at the interval of 4.0 hr, indicating that the analogues

have a prolonged half-life at a moderate dose. Phenytoin and valproic acid were the usual medications utilized.

Compounds Va, Vc, Vd and Ve were found to exhibit action at 100 mg/kg at 0.5 hr in the scPTZ model. At 100 mg/kg after 4.0 hr, compounds Ve were protective. When the neurotoxicity of the compounds was tested, the majority produced negative results. Only compound Vh and Vj were discovered to be neurotoxic at 0.5 and 4 hr, respectively.

Of all the synthetic compounds, compounds Vd and Ve were shown to be the most powerful and to function as specific GABA mediators. The compounds in the MES and scPTZ studies either showed no action or had no distinguishing characteristics.

According to the SAR investigations, molecules having glucose moiety as N-glycoside were found to be most potent. Compound Ve was found to be most potent thus indicating compounds with leucine and alanine as most potent. It is also seen that substitution at position 3 of pyrrole results in active analogues.

[a]30 mg/kg, 100 mg/kg and 300 mg/kg of the drug were administered. After 0.5 and 4 hr, the protective effect and neurotoxicity were studied. The results showed how much of a minimum dose of lead was needed to protect 50% or more of the animals or cause neurotoxicity in at least 50% of them. The dash (-) meant that neither neurotoxicity nor anticonvulsant action was present in these compounds. X-means that it was not analyzed.

[b]Ethanol potentiation test: (+) means that at least half of the animals had positive results; (-) means that at least half of the animals had negative results. X-stands for "not tested."

ADME Prediction

On the designed compounds, ADME prediction was performed and the outcomes are presented shown in Table 4. It was feasible to determine whether a certain substance might pass through the BBB by examining BBB penetration. The values that were found also helped to reduce side effects and toxins and they may have made drugs that had a psychological effect on the brain work better. Almost all of the targets that were looked at had positive values, which shows that they could easily cross the BBB. Ve has the highest value, 0.0532067, that implies it is the most active. The process through which oral medications entered the circulation from the GIT is known as HIA. The results for the synthesized compounds were less than 50%, which shows that they are not well-absorbed compounds through oral route. PPB can alter a drug's effectiveness as well as how long a substance stays in the body. The amount of binding to plasma proteins has a big effect on how a drug works and how it moves through the body. A percent bound value below 90 was regarded as low and a value above 90 was seen as high.

As demonstrated, all synthetic chemicals exhibited strong affinity for a plasmatic protein with a value less than 90%. Also, the way the drug is spread out depends a lot on how well it binds to proteins in plasma. For drugs that are given through the skin, the SP rate is one of the most important factors. It is known that the way the drug moves into the lipid tissue between cells is an important part of how the skin absorbs the drug. All the compounds that were tested failed the SP check, showing that they can't be directed through the skin. The Log P numbers show how likely it is that the compound will be able to get to the target tissue in the body. Because Log P<0 (or p<1), the analogues that were tried were hydrophilic due to sugar moieties attached to them. ADME prediction of synthesized derivatives are shown in Table 4.

Molecular docking

Molecular docking studies were done to find out how the synthesized analogues (Va-j) might bind and how much energy they would need to do so. This helped to find the potential leads. The docking scores of the possible compounds that were tested against the GABA-A receptor ranged from -7.5 to -5.7. Figure 5 shows the docking images of all the compounds Va-Vj that were synthesized. Table 4 shows the results of docking in form of scores of derivatives (Va-j) with GABA-A (4 COF).

From the docking studies, Ve was found to be most active with a score of -7.5. The residue Ile C:242, Asp C:424, Asn C:243 and Asn D:303 were found to be involved in conventional hydrogen bonding with hydroxy and carboxyl group of compound at a distance of 5.66, 4.76, 4.07 and 4.19 Å respectively. Pi-Sigma was formed with Phe D:307 residue at a distance of 4.23 Å. Carbon hydrogen bond of N-H of pyrrole was formed was formed with Tyr D: 304. Van der Waals forces were seen with Arg C:250, Arg C:428, Trp C:241, Phe C:240, Arg C:425, Tyr C:244 and Ala C:314.

For compound Vd, hydrogen bond was formed with Arg C:428. Conventional hydrogen bond of N-H of pyrrole was formed with residue Asn D:303 at a distance 5.74 Å, Vander Waals forces were seen with Ser D:308, Tyr D:304, Phe D:307, Asn C: 243, Asp C:424, Ala C:314, Tyr C: 244 and Pro C:310.

In compound Vc, conventional hydrogen bonding is seen with residues Tyr C:244, Arg C:425, Asp C:424 and Arg C:428 at a distance 4.87, 3.73.6.34 and 3.87 Å. Carbon hydrogen bond was seen Tyr D:304 and Ala C:314 at a distance 4.37 and 3.68 Å. Vander Waals bonds are seen with Asp C:245, Phe D:307, Ile C:242, Arg C:250, Phe C:24 and Trp C:241.

For compound Va, conventional hydrogen bonds are formed with Val E:50, Ser E:51. Pi-Alkyl bonds are formed with Lys A:274 and Val A:53. Vander Waals forces are seen with Pro A:273, Met E:49, Leu E:183, Pro E:184, Phe E:186, Gln E:185, Val E:53 and Asn E:54.

The study of pattern of docking in synthesized molecules with maximum docking score represented that conventional hydrogen bonding, carbon hydrogen bond, Pi-alkyl bond and Vander Waals forces are the major binding source for the synthesized compound the receptor.

The most active compounds Ve, Vd, Vc and Va have glucose as sugar moiety as a substitutent.

The important amino acid residues involved are found to be Ile C:242, Asp C:424, Phe D:307, Arg C:250 Trp C:241 and Phe C:240 which served as a conduit for the ligand's access to the GABA-A receptor.

According to evidence from *in silico* experiments, adding small sugar moiety with pyrrole at position 3 can considerably boost GABA levels and, consequently, anticonvulsant action. The results correspond to those from the animal model. The results of docking score were compared with standard drug valproic acid and the results indicate the synthesized compounds to be more active than the standard.

CONCLUSION

In the current study, pyrrole-carbohydrate analogues (Va-j) were synthesized and evaluated for their anticonvulsant activity using MES and scPTZ model. The series of fused derivatives were found to be active for anticonvulsant activity. Compounds Ve revealed to be extremely effective throughout the series against both types, with a rapid start to the attack. As a result, it is hypothesized that these four compounds namely, Ve, Vd, Vc and Va are those GABA facilitators who are most active and selective. The neurotoxicity test was passed successfully by every compound in the series except for compound Vh and Vj, that was discovered to be neurotoxic. In addition, the article includes molecular interactions and ADME predictions for all analogues. In the manuscript, every ADME parameter is covered in depth. In order to carry out molecular docking, Autodock Vina was utilized and the protein GABA-A served as the target. At the active site of GABA-A, Ile C:242, Asp C:424, Phe D:307, Arg C:250 Trp C:241 and Phe C:240 are involved in significant interactions. conventional hydrogen bonding, carbon hydrogen bond, Pi-alkyl bond and Vander Waals forces are thoroughly examined. The docking results are similar to those of the animal model.

According to *in silico* and biological activity studies, combining pyrrole with carbohydrate through N-glycosidic linkage at position 2 can significantly raise GABA concentration and, have an anticonvulsant effect as a result. The group of synthesized analogues can be viewed as intriguing potential research subjects.

ACKNOWLEDGEMENT

The authors gratefully acknowledge the funding of the Deanship of Graduate Studies and Scientific Research, Jazan University, Saudi Arabia, through project number: (RG24-M018).

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

FUNDING

This research was funded by the Deanship of Graduate Studies and Scientific Research, Jazan University, Saudi Arabia, through project number: (RG24-M018).

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Every step of the animal handling process, including sample administration and disposal, was followed as per IACUC regulations, Faculty of veterinary-medicine, University of Sadat-City, with Ethical approval number VUSC-036-1-23.

ABBREVIATIONS

AEDs: Antiepileptic drugs; **GABA:** Gamma-aminobutyric acid; **NCEs:** Novel chemical entities; **AD:** Alzheimer's disease; **scPTZ:** Subcutaneous Pentylenetetrazole; **HIA:** Human intestinal absorption; **PPB:** Plasma protein binding; **BBB:** Blood-brain barrier penetration; **SP:** Skin permeability; **PDB:** Protein Data Bank; **LGA:** Genetic Algorithm; **ADD:** Anticonvulsant Drug Development; **MES:** Maximal Electroshock.

SUMMARY

Epilepsy is a medical condition where the brain behaves abnormally, leading to convulsions and unusual behavior. In current study, ten carbohydrate-based compounds were synthesized to reduce seizures. Different methods were used to characterize these derivatives. Several models were used to test each derivative for anticonvulsant activity. The study also evaluated the neurotoxicity. The results showed that compounds Ve, Vd, Vc and Va were the most effective in reducing seizures. All synthesized equivalents passed the neurotoxicity test. The study identified the active site of GABA-A protein using molecular docking. The findings of the study suggest that some of these compounds have good anticonvulsant properties. These novel anticonvulsant analogues could be used as a starting point for further studies to develop a potential new drug candidate.

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Cite this article: Alam MS, Albratty M, Alhazmi HA, Najmi A, Zoghebi K, Makeen HA, *et al*. Design, Synthesis, Molecular Docking, *in silico* ADME Assessment of Pyrrole-Based Heterocyclic Amino Acid Derivatives as Potential Anticonvulsant Agent. Indian J of Pharmaceutical Education and Research. 2024;58(3s):s1062-s1074.