Original Article

Design, Synthesis and Molecular Docking Studies of (S)-1-(2-(substituted benzylamino)-3-methylbutanoyl)pyrrolidin-2-one analogues as GABA Mediated Anticonvulsant Agents

Obaid Afzal[1], Gopal Prasad Agrawal[2], Ghadah Rashed Ghazi Alotaibi[3], Raid Saleh Ali Shatat[4], Habibullah Khalilullah[5], Zabih Ullah[6], Mohammad Rashid[7], Ahmed F Elkirdasy[8], Benson Mathai K[9,*]

[1]Department of Pharmaceutical Chemistry, College of Pharmacy, Prince Sattam Bin Abdulaziz University, Al-Kharj, SAUDI ARABIA.
[2]Institute of Pharmaceutical Research, GLA University, Mathura-Delhi Road, Chaumuhan, Mathura, Uttar Pradesh, INDIA.
[3]Clinical Pharmacist, Al Nahdi Medical Company, SAUDI ARABIA.
[4]Riyadh ELM University, Riyadh, SAUDI ARABIA.
[5]Department of Pharmaceutical Chemistry and Pharmacognosy, Unaizah College of Pharmacy, Qassim University, Qassim, SAUDI ARABIA.
[6]Department of Pharmaceutical Sciences, College of Dentistry and Pharmacy, Buraydah Colleges, Qassim, SAUDI ARABIA.
[7]Department of Pharmaceutical Chemistry and Pharmacognosy, College of Dentistry and Pharmacy, Buraydah Colleges, Qassim, SAUDI ARABIA.
[8]Department of Biochemistry and Chemistry of Nutrition, Faculty of Veterinary Medicine, University of Sadat City, Sadat, EGYPT.
[9]Department of Pharmacology, Dr. Joseph Mar Thoma Institute of Pharmaceutical Sciences and Research, Kattanam, Alappuzha, Kerala, INDIA.

ABSTRACT

Background: Epilepsy is a deep rooted, partially curable brain illness that affects all individuals irrespective of ages and genders. Despite the discovery of various innovative antiepileptic drugs (AEDs), selectivity and toxicity issues remain. **Materials and Methods:** A series of (S)-1-(2-(substituted benzylamino)-3-methylbutanoyl)pyrrolidin-2-one analogues (5a-r) were synthesized and evaluated for their anticonvulsant activity. The analogues were screened by two gold standard methods, i.e., electroshock (MES) and chemoshock (scPTZ) seizure tests. In addition, the rotarod method was used to test motor impairment in all synthetic analogues for acute neurotoxicity. The paper also reports ADME prediction of all the 18 synthesized compounds, with each parameter discussed in detail. Furthermore, the GABA-A target protein was used in molecular docking experiments. **Results:** In the MES model, compounds 5e, 5h, 5k, 5l, 5o, and 5r were identified to be the most effective, while compounds 5j, 5k, 5l, 5o, and 5r were the most active against the scPTZ model. The majority of the synthesized analogues passed the neurotoxicity test, according to the findings. ADME prediction of the compounds exhibited good agreement with the *in vivo* outcomes. The results of molecular docking showed important interactions with TYR 62, ASN 85, ARG 114, ARG 129, and MET 115 at the active site of GABA-A and the results showed good agreement with *in vivo* results. **Conclusion:** The findings of the study indicated some of the compounds possessed excellent anticonvulsant activity without noticeable neurotoxicity. These compounds can be investigated further for the formation of newer/novel anticonvulsant agents.

Keywords: Pyrrolidine, Anticonvulsant activity, Molecular Docking, GABA-A, Toxicity, ADME prediction.

Submission Date: 28-03-2022;
Revision Date: 04-07-2022;
Accepted Date: 29-08-2022.

DOI: 10.5530/001954641726

Correspondence:
Dr. Benson Mathai K,
Department of Pharmacology, Dr. Joseph Mar Thoma Institute of Pharmaceutical Sciences and Research, Kattanam, Alappuzha-690503, Kerala, INDIA.
Email id: bensonpharma@gmail.com

INTRODUCTION

Epilepsy is a deep rooted, partially curable brain illness that affects all individuals irrespective of ages and genders. It is defined by repeated voluntary convulsions of upper brain origin, and more often with loss of responsiveness.[1] Any momentary aberrant behavior, such as loss of consciousness, a stare, temporary perplexed, a strange

www.ijper.org

mood, an emotional outburst, a strange sensation, or uncontrollable movements, can be symptoms of epilepsy.

According to the most recent WHO Figures, around 50 million individuals worldwide suffer from epilepsy, with nearly 80 percent of these persons living in low or middle-income countries. According to studies, up to 70% of persons with epilepsy may enjoy seizure-free lives if properly diagnosed and treated.[2]

Despite the development of several novel antiepileptic medicines (AEDs), there is still a problem with selectivity and toxicity. As a result, there is a continuous need for newer and safer anticonvulsant medications. The complexities of epilepsy's mechanism (e.g., enhancement of GABA-mediated inhibition or the other effect on the GABA system, modulation of voltage-dependent Na^+ and/or Ca^{2+} channels, inhibition of synaptic excitation mediated by ionotropic glutamate receptors or modulation of synaptic release) and lack of understanding restrict the development of novel anticonvulsant medicines. Nitrogen heterocycles are a significant family of natural and synthetic compounds, many of which exhibit beneficial pharmacological properties. Nitrogen-containing heterocycles are found in pyrrole, indole, pyrrolidine, oxadiazole, triazole, thiadiazole, triazines, pyrimidines, pyridines, and quinazolines. The presence of nitrogen-containing heterocycles, mainly lactam or imide, with linked phenyl or alkyl groups has been widely reported as a major core element of anti-seizure drugs in the past.[3,4]

Socała *et al.* 2019, discovered KA-11 (pyrrolidine derivative), a strong anticonvulsant that displayed its activity by blocking voltage-gated sodium streams in pyramidal neurons of the frontal part of the rat's brain.[5] The first-generation anticonvulsant, such as ethosuximide has this template, which can be discovered in its structure.[6-8] Also, many previous studies report a series of anticonvulsant compounds with a hybrid structure based on pyrrolidine scaffold viz., brivaracetam, piracetam, seletracetam and isomers levetiracetam and etiracetam all have the same fundamental skeleton and are FDA approved drugs.[9,10] (Figure 1).

In the early phases of testing, several pyrrolidine analogues were found to be beneficial in the subcutaneous pentylenetetrazole (*sc*PTZ) and maximum electroshock (MES) screening that is broadly considered as the "gold standards". Structure-activity relationships (SAR) studies have revealed that pyrrolidine-2,5-diones and pyrrolidine-2-ones with significantly electronegative fluoro, chloro, or trifluoromethyl replacements at the nitrogen atom of imide group have powerful anticonvulsant activity.[11,12]

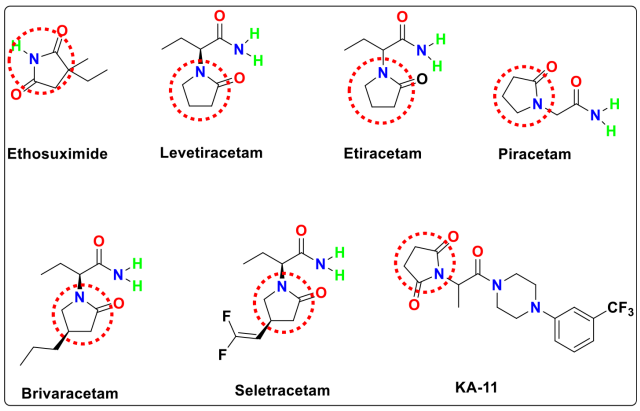


Figure 1: FDA approved anticonvulsant drugs.

The current study is an attempt to identify chemicals that are active in the standard MES or scPTZ tests, based on the previous findings. A library of 18 chemicals was synthesized with the pyrrolidine-2-one system as a key scaffold. The molecular docking analysis was carried out on all the synthesized compounds to determine their affinity to bind with Gamma-aminobutyric acid receptors that are ligand-gated ion-channel (GABA-A) receptors to qualitatively rationalize their anticonvulsant actions. The information acquired by molecular docking was compared to that gleaned from pharmacological screening.

MATERIALS AND METHODS

Chemistry

General

Merck Chemicals provided all of the reagents and chemicals utilized in the investigation. The melting points of derivatives were determined using the Hicon melting point instrument with open capillary tubes and were presented uncorrected.

Thin-layer chromatography (TLC) plates (silica gel G) were used to evaluate the purity of synthesized analogues using the mobile phase of ethyl acetate: hexane (4:6) v/v. The detecting reagents used were iodine vapors and UV light. FTIR spectrophotometer (Shimadzu) was used to obtain the FTIR Spectra. The value of υ_{max} (wavenumber) was recorded in cm^{-1}. Bruker model DRX-400MHz NMR was used to perform the NMR analysis (^1H at 400 MHz and ^{13}C at 100 MHz) of synthesized compounds using $CDCl_3$ solvent. Chemical shifts or resonant frequencies (δ) are measured in ppm using tetrmethylsilane (TMS) as an internal standard, while J values (coupling constants) are measured in hertz (Hz). Applied Biosystems Mass spectrometer (Applied Biosystems; API-3000) was used to obtain the mass of the synthesized compound and the value was

reported in dalton. Perkin-Elmer (240C analyzer) was used to perform the elemental analysis (C, H and N) of synthesized derivatives.

Synthesis of methyl (2S)-2-(Substituted-benzylamino) -3-methylbutanoate (3a-3r)

The mixture was stirred for 30 min at room temperature after adding L-Valine methyl ester HCl (0.0012 mol; 2) to a substituted benzaldehyde solution (0.001 mol; 1a-1r) in methanol (15 mL). Iodine (0.0004 mol) was added to the resulting mixture while it was being stirred at ambient temperature until all of the iodine was dissolved. Slowly adding sodium borohydride to this combination (0.0014 mol) and stirring at room temperature for the remainder of the reaction time was necessary to complete the reaction. Compounds (3a-3r) were obtained by the process of filtration of solid precipitate followed by washing with water and recrystallization using ethanol. TLC plates were used to examine the reaction progress and purity using mobile phase comprising of formic acid: ethyl acetate: toluene (1:4:5) *v/v*.

Synthesis of (2S)-2-(substituted-benzylamino)-3-methylbutanoic acid (4a-4r)

1 mL of 20 percent NaOH was added to a solution of compound 3a (0.001 mol) in 20 mL alcohol, and the reaction blend was refluxed and stirred until the reaction was completed. TLC was used to observe the reaction's progression from start to finish. Once the reaction was completed, the solution was cooled at RT and dilute HCl was used to neutralize this solution followed by 1hr stirring. When the precipitate came out of the filter, it was rinsed with water, dried, and then re-crystalized with ethanol. TLC was used to evaluate the progress of reaction and purity of compounds using mobile phase comprising of acetone: benzene (2:8) *v/v*.

Synthesis of target compounds ((S)-1-(2-(substituted benzylamino)-3-methylbutanoyl)pyrrolidin-2-one analogues)

To a solution of (2S)-2-(substituted-benzylamino)-3-methylbutanoic acid (5a, 0.001 mol), EDC·HCl (0.0015 mol), 2-pyrrolidone (0.001 mol), HOBT (0.0015 mol: in 20 mL DMF), and Et3N (0.001 mol) were slowly added at an ambient temperature condition. The mixture was stirred for 12-15 hr. After the reaction was concluded, 60 mL water was mixed to the reaction mixture, and then in the presence of ethyl acetate the compound was recovered. The organic layer obtained was washed consecutively with an aqueous citric acid solution (10%) followed by water, a sodium bicarbonate solution (saturated), water and brine (saturated) and then anhydrous sodium sulfate for drying. The distillation of solvent was carried out under reduced pressure, to obtain the residue that was washed, dried, and recrystallized with ethyl acetate.

1-[(2S)-2-(benzylamino)-3-methylbutanoyl]pyrrolidin-2-one (5a)

IR (KBr) (cm^{-1}): 3378 (NH), 1707 (C=O), 1683 (C=O), 1249 (C-N); ^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 1.68 (m, 6H, 2xCH_3), 2.09 (2H, m, CH_2), 2.35 (2H, m, CH_2), 2.52 (m, 1H, CH), 3.10 (m, 1H, CH), 4.01 (2H, m, CH_2), 4.17 (bs, 1H, NH, D_2O-exchangeble), 4.46 (s, 2H, CH_2), 6.94 (d, 2H, Ar-H, J = 7.3 Hz), 7.18 (m, 3H, Ar-H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ(ppm): 19.6, , 22.1, 31.8, 34.5, 41.0, 52.6, 65.4, 119.5, 125.6, 178.6, 126.7, 138.3, 174.5; ESI MS (m/z): 275 [M+H]; Anal. Calculated for $C_{16}H_{22}N_2O_2$: C, 70.04; H, 8.08; N, 10.21; Found: C, 70.39; H, 8.13; N, 10.28%.

1-{(2S)-3-methyl-2-[(4-methylbenzyl)amino]butanoyl} pyrrolidin-2-one (5b)

IR (KBr) (cm^{-1}): 3390 (NH), 1710 (C=O), 1680 (C=O), 1255 (C-N); ^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 1.70 (m, 6H, 2xCH_3), 2.06 (2H, m, CH_2), 2.22 (s, 3H, CH_3), 2.37 (2H, m, CH_2), 2.52 (m, 1H, CH), 3.12 (m, 1H, CH), 4.04 (2H, m, CH_2), 4.2 (bs, 1H, NH, D_2O-exchangeble), 4.41 (s, 2H, CH_2), 6.97 (d, 2H, Ar-H, J = 7.5 Hz), 7.05 (d, 2H, Ar-H, J = 7.4); ^{13}C-NMR (100 MHz, $CDCl_3$) δ(ppm): 19.2, 22.7, 23.0, 31.4, 33.8, 41.3, 51.8, 65.9, 120.6, 125.9, 135.6, 137.8, 174.8, 179.0; ESI MS (m/z): 289 [M+H]; Anal. Calculated for $C_{17}H_{24}N_2O_2$: C, 70.80; H, 8.39; N, 9.71; Found: C, 70.58; H, 8.42; N, 9.77%.

1-{(2S)-2-[(4-methoxybenzyl)amino]-3-methylbutanoyl} pyrrolidin-2-one (5c)

IR (KBr) (cm^{-1}): 3405 (NH), 1700 (C=O), 1690 (C=O), 1253 (C-N); ^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 1.69 (m, 6H, 2xCH_3), 2.10 (2H, m, CH_2), 2.33 (2H, m, CH_2), 2.52 (m, 1H, CH), 3.06 (m, 1H, CH), 3.38 (s, 3H, OCH_3), 4.10 (2H, m, CH_2), 4.35 (bs, 1H, NH, D_2O-exchangeble), 4.46 (s, 2H, CH_2), 6.99 (d, 2H, Ar-H, J = 7.4 Hz), 7.08 (d, 2H, Ar-H, J = 7.3); ^{13}C-NMR (100 MHz, $CDCl_3$) δ (ppm): 19.3, 21.7, 30.9, 32.8, 41.6, 51.3, 56.2, 69.2, 117.5, 131.7, 134.2, 154.6, 174.1, 179.3; ESI MS (m/z): 305 [M+1]; Anal. Calculated for $C_{17}H_{24}N_2O_3$: C, 67.08; H, 7.95; N, 9.20; Found: C, 67.33; H, 7.91; N, 9.28%.

1-{(2S)-2-[(4-hydroxybenzyl)amino]-3-methylbutanoyl} pyrrolidin-2-one (5d)

IR (KBr) (cm^{-1}): 3407 (NH), 1711 (C=O), 1694 (C=O), 1240 (C-N); ^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 1.76 (m, 6H, 2xCH_3), 2.05 (2H, m, CH_2), 2.38 (2H, m, CH_2),

2.58 (m, 1H, CH), 3.04 (m, 1H, CH), 3.38 (s, 1H, OH), 4.06 (2H, m, CH_2), 4.28 (bs, 1H, NH, D_2O-exchangeble), 4.41 (s, 2H, CH_2), 6.95 (d, 2H, Ar-H, J = 7.3Hz), 7.14 (d, 2H, Ar-H, J = 7.4); ^{13}C-NMR (100 MHz, $CDCl_3$) δ(ppm): 19.6, 21.8, 30.3, 32.1, 41.2, 52.1, 69.4, 117.5, 131.3, 133.6, 154.8, 174.4, 178.7; ESI MS (m/z): 291 [M+1]; Anal. Calculated for $C_{16}H_{22}N_2O_3$: C, 66.18; H, 7.64; N, 9.65; Found: C, 66.47; H, 7.67; N, 9.72%.

1-{(2S)-3-methyl-2-[(4-nitrobenzyl)amino]butanoyl} pyrrolidin-2-one (5e)

IR (KBr) (cm^{-1}): 3388 (NH), 1705 (C=O), 1690 (C=O), 1258 (C-N); ^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 1.70 (m, 6H, 2xCH_3), 2.10 (2H, m, CH_2), 2.30 (2H, m, CH_2), 2.61 (m, 1H, CH), 3.08 (m, 1H, CH), 4.03 (2H, m, CH_2, 4.21 (bs, 1H, NH, D_2O-exchangeble), 4.39 (s, 2H, CH_2), 6.98 (d, 2H, Ar-H, J = 7.3Hz), 7.28 (d, 2H, Ar-H, J = 7.2); ^{13}C-NMR (100 MHz, $CDCl_3$) δ(ppm): 19.5, 21.2, 30.6, 32.4, 42.1, 52.2, 70.0, 121.7, 129.8, 136.9, 149.1, 175.14, 178.4; ESI MS (m/z): 320 [M+1]; Anal. Calculated for $C_{16}H_{21}N_3O_4$: C, 60.17; H, 6.63; N,13.16; Found: C, 60.42; H, 6.68; N,13.23%.

1-{(2S)-2-[(4-chlorobenzyl)amino]-3-methylbutanoyl} pyrrolidin-2-one (5f)

IR (KBr) (cm^{-1}): 3409 (NH), 1710 (C=O), 1697 (C=O), 1259 (C-N); ^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 1.63 (m, 6H, 2xCH_3), 2.06 (2H, m, CH_2), 2.31 (2H, m, CH_2), 2.58 (m, 1H, CH), 3.11 (m, 1H, CH), 4.08 (2H, m, CH_2), 4.20 (bs, 1H, NH, D_2O-exchangeble), 4.43 (s, 2H, CH_2), 6.94 (d, 2H, Ar-H, J = 7.1Hz), 7.24 (d, 2H, Ar-H, J = 7.23); ^{13}C-NMR (100 MHz, $CDCl_3$) δ(ppm): 20.0, 21.4, 31.2, 33.0, 42.2, 52.1, 69.8, 126.3, 129.7, 131.7, 137.3, 175.3, 178.8; ESI MS (m/z): 309 [M+1]; Anal. Calculated for $C_{16}H_{22}N_2O_2$: C, 62.23; H, 6.85; N, 9.07; Found: C, 62.53; H, 6.88; N, 9.01%.

1-{(2S)-2-[(4-fluorobenzyl)amino]-3-methylbutanoyl} pyrrolidin-2-one (5g)

IR (KBr) (cm^{-1}): 3387 (NH), 1712 (C=O), 1688 (C=O), 1260 (C-N); ^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 1.69 (m, 6H, 2xCH_3), 2.02 (2H, m, CH_2), 2.22 (2H, m, CH_2), 2.53 (m, 1H, CH), 3.19 (m, 1H, CH), 4.04 (2H, m, CH_2), 4.26 (bs, 1H, NH, D_2O-exchangeble), 4.49 (s, 2H, CH_2), 6.97 (d, 2H, Ar-H, J = 7.1Hz), 7.25 (d, 2H, Ar-H, J = 7.3); ^{13}C-NMR (100 MHz, $CDCl_3$) δ(ppm): 19.7, 21.1, 31.6, 33.4, 41.8, 52.7, 69.3, 117.1, 129.7, 134.3, 158.8, 175.2, 178.5; ESI MS (m/z): 293 [M+1]; Anal. Calculated for $C_{16}H_{21}FN_2O_2$: C, 65.73; H, 7.24; N, 9.58; Found: C, 65.49; H, 7.29; N, 9.64%.

1-{(2S)-2-[(4-bromobenzyl)amino]-3-methylbutanoyl} pyrrolidin-2-one (5h)

IR (KBr) (cm^{-1}): 3380 (NH), 1713 (C=O), 1680 (C=O), 1244 (C-N); ^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 1.71 (m, 6H, 2xCH_3), 2.07 (2H, m, CH_2), 2.21 (2H, m, CH_2), 2.59 (m, 1H, CH), 3.15 (m, 1H, CH), 4.09 (2H, m, CH_2), 4.21 (bs, 1H, NH, D_2O-exchangeble), 4.40 (s, 2H, CH_2), 6.96 (d, 2H, Ar-H, J = 7.3 Hz), 7.19 (d, 2H, Ar-H, J = 7.3); ^{13}C-NMR (100 MHz, $CDCl_3$) δ(ppm): 19.7, 20.9, 31.8, 33.7, 42.1, 52.5, 69.6, 119.7, 129. 2, 132.6, 138.3, 175.7, 178.2; ESI MS (m/z): 353 [M+1]; Anal. Calculated for $C_{16}H_{21}BrN_2O_2$: C, 54.40; H, 5.99; N, 7.93; Found: C, 54.67; H, 5.95; N, 7.99%.

1-{(2S)-2-[(3-chlorobenzyl)amino]-3-methylbutanoyl} pyrrolidin-2-one (5i)

IR (KBr) (cm^{-1}): 3383 (NH), 1715 (C=O), 1689 (C=O), 1258 (C-N); ^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 1.66 (m, 6H, 2xCH_3), 2.11 (2H, m, CH_2), 2.33 (2H, m, CH_2), 2.50 (m, 1H, CH), 3.13 (m, 1H, CH), 4.07 (2H, m, CH_2), 4.22 (bs, 1H, NH, D_2O-exchangeble), 4.49 (s, 2H, CH_2), 6.91 (m, 1H, Ar-H), 7.03 (s,1H, Ar-H), 7.13 (m, 2H, Ar-H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ(ppm): 20.1, 21.3, 31.7, 33.4, 40.9, 52.0, 69.2, 125.7, 126.8, 129.4, 133.9, 138.8, 175.7, 179.1; ESI MS (m/z): 309 [M+1]; Anal. Calculated for $C_{16}H_{21}ClN_2O_2$: C, 62.23, H, 6.85, N, 9.07; Found: C, 62.52, H, 6.89, N, 9.16%.

1-{(2S)-3-methyl-2-[(3-methylbenzyl)amino]butanoyl} pyrrolidin-2-one (5j)

IR (KBr) (cm^{-1}): 3390 (NH), 1702 (C=O), 1688 (C=O), 1262 (C-N); ^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 1.66 (m, 6H, 2xCH_3), 2.03 (2H, m, CH_2), 2.27 (s, 3H, CH_3), 2.35 (2H, m, CH_2), 2.49 (m, 1H, CH), 3.12 (m, 1H, CH), 4.11 (2H, m, CH_2), 4.27 (bs, 1H, NH, D_2O-exchangeble), 4.48 (s, 2H, CH_2), 6.93 (s, 1H, Ar-H), 7.05 (m, 3H, Ar-H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ(ppm): 19.8, 22.4, 23.2, 31.8, 33.3, 41.7, 51.5, 68.4, 119.7, 122.6, 123.9, 135.2, 136.8, 174.6, 179.40; ESI MS (m/z): 289 [M+1]; Anal. Calculated for $C_{17}H_{24}N_2O_2$: C, 70.80; H, 8.39; N, 9.71; Found: C, 70.98; H, 8.33; N, 9.80%.

1-{(2S)-3-methyl-2-[(3-nitrobenzyl)amino]butanoyl} pyrrolidin-2-one (5k)

IR (KBr) (cm^{-1}): 3422 (NH), 1714 (C=O), 1681 (C=O), 1254 (C-N); ^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 1.70 (m, 6H, 2xCH_3), 2.14 (2H, m, CH_2), 2.31 (2H, m, CH_2), 2.56 (m, 1H, CH), 3.15 (m, 1H, CH), 4.10 (2H, m, CH_2), 4.26 (bs, 1H, NH, D_2O-exchangeble), 4.44 (s, 2H, CH_2), 6.99 (s, 1H, Ar-H), 7.09 (m, 3H, Ar-H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ(ppm): 17.5, 19.1, 31.3, 40.5, 50.8, 68.2,

122.6, 124.5, 129.8, 134.9, 137.5, 147.8, 174.2, 175.9; ESI MS (m/z): 320 [M+1]; Anal. Calculated for $C_{16}H_{21}N_3O_4$ C, 60.17; H, 6.63; N, 13.16; Found: C, 60.42; H, 6.69; N, 13.22%.

1-{(2S)-2-[(3-methoxy-4-methylbenzyl)amino]-3-methylbutanoyl}pyrrolidin-2-one (5l)

IR (KBr) (cm^{-1}): 3392 (NH), 1717 (C=O), 1689 (C=O), 1245 (C-N); ^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 1.72 (m, 6H, 2xCH_3), 2.17 (2H, m, CH_2), 2.29 (m, 5H, CH_3 & CH_2), 2.58 (m, 1H, CH), 3.12 (m, 1H, CH), 3.43 (m, 3H, OCH_3), 4.11 (2H, m, CH_2), 4.24 (bs, 1H, NH, D_2O-exchangeble), 4.48 (s, 2H, CH_2), 6.91 (s, 1H, Ar-H), 7.09 (m, 2H, Ar-H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ (ppm): 17.9, 19.6, 21.4, 31.9, 32.3, 41.3, 51.0, 53.7, 68.6, 122.7, 126.2, 131.0, 133.8, 157.8, 174.8, 178.7; ESI MS (m/z): 319 [M+1]; Anal. Calculated for $C_{16}H_{22}N_2O_2$: C,67.90; H, 8.23; N, 8.80; Found: C,67.67; H, 8.27; N, 8.88%.

1-[(2S)-2-{[4-(dimethylamino)benzyl]amino}-3-methylbutanoyl]pyrrolidin-2-one (5m)

IR (KBr) (cm^{-1}): 3386 (NH), 1709 (C=O), 1693 (C=O), 1258 (C-N); ^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 1.64 (m, 6H, 2xCH_3), 2.13 (2H, m, CH_2), 2.29 (2H, m, CH_2), 2.62 (m, 4H, CH, & N-$(CH_3)_2$), 3.09 (m, 1H, CH), 4.08 (2H, m, CH_2), 4.32 (bs, 1H, NH, D_2O-exchangeble), 4.44 (s, 2H, CH_2), 6.94 (d, 2H, Ar-H, J = 7.4 Hz), 7.11 (d, 2H, Ar-H, J = 7.3); ^{13}C-NMR (100 MHz, $CDCl_3$) δ(ppm): 19.3, 20.4, 31.3, 32.8, 40.8, 42.6, 52.6, 69.1, 116.1, 116.9, 130.0, 131.4, 151.3, 175.2, 178.3; ESI MS (m/z): 318 [M+1]; Anal. Calculated $C_{18}H_{27}N_3O_2$: C, 68.11; H, 8.57; N, 13.24; Found: C, 68.38; H, 8.62; N, 13.32%.

1-{(2S)-2-[(3-fluorobenzyl)amino]-3-methylbutanoyl}pyrrolidin-2-one (5n)

IR (KBr) (cm^{-1}): 3379 (NH), 1711 (C=O), 1687 (C=O), 1260 (C-N); ^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 1.71 (m, 6H, 2xCH_3), 2.12 (2H, m, CH_2), 2.33 (2H, m, CH_2), 2.53 (m, 1H, CH), 3.14 (m, 1H, CH), 4.10 (2H, m, CH_2), 4.31 (bs, 1H, NH, D_2O-exchangeble), 4.52 (s, 2H, CH_2), 6.96 (m, 1H, Ar-H), 7.07 (s,1H, Ar-H), 7.17 (m, 2H, Ar-H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ(ppm): 17.2, 18.8, 31.8, 40.3, 51.5, 68.4, 113.8, 119.0, 123.5, 127.0, 138.0, 162.7, 174.5, 175.7; ESI MS (m/z): 293 [M+1]; Anal. Calculated $C_{16}H_{21}FN_2O_2$: C 65.73; H, 7.24; N, 9.58; Found: C 65.96; H, 7.28; N, 9.64%.

1-{(2S)-2-[(3-chloro-4-methylbenzyl)amino]-3-methylbutanoyl}pyrrolidin-2-one (5o)

IR (KBr) (cm^{-1}): 3390 (NH), 1715 (C=O), 1680 (C=O), 12455 (C-N); ^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 1.69 (m, 6H, 2xCH_3), 2.26 (m, 5H, CH_3 & CH_2), 2.37 (2H, m, CH_2), 2.48 (m, 1H, CH), 3.17 (m, 1H, CH), 4.11 (2H, m, CH_2), 4.28 (bs, 1H, NH, D_2O-exchangeble), 4.49 (s, 2H, CH_2), 6.96 (m, 1H, Ar-H), 7.02 (s,1H, Ar-H), 7.16 (m, 2H, Ar-H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ(ppm): 19.4, 21.1, 31.6, 33.5, 41.4, 52.4, 69.1, 126.8, 130.1, 132.6, 135.2, 174.9, 177.8; ESI MS (m/z): 323 [M+1]; Anal. Calculated for $C_{17}H_{23}ClN_2O_2$: C, 63.25; H, 7.18; N, 8.68; Found: C, 63.53; H, 7.13; N, 8.59%.

1-{(2S)-2-[(3-chloro-4-methoxybenzyl)amino]-3-methylbutanoyl}pyrrolidin-2-one (5p)

IR (KBr) (cm^{-1}): 3423 (NH), 1705 (C=O), 1692 (C=O), 1253 (C-N); ^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 1.63 (m, 6H, 2xCH_3), 2.18 (m, 2H, CH_2), 2.34 (2H, m, CH_2), 2.46 (m, 1H, CH), 3.29 (m, 4H, CH & OCH3), 4.15 (2H, m, CH_2), 4.231 (bs, 1H, NH, D_2O-exchangeble), 4.51 (s, 2H, CH_2), 6.95 (m, 1H, Ar-H), 7.01 (s,1H, Ar-H), 7.20 (m, 2H, Ar-H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ(ppm): 19.7, 21.2, 30.8, 32.9, 41.1, 52.7, 54.0, 69.3, 116.5, 127.7, 131.8, 150.2, 174.3, 178.5; ESI MS (m/z): 339 [M+1]; Anal. Calculated for $C_{17}H_{23}ClN_2O_3$: C, 60.26; H, 6.84; N, 8.27; Found: C, 60.55; H, 6.81; N, 8.33%.

1-{(2S)-2-[(3-chloro-4-hydroxybenzyl)amino]-3-methylbutanoyl}pyrrolidin-2-one (5q)

IR (KBr) (cm^{-1}): 3390 (NH), 1709 (C=O), 1687 (C=O), 1245 (C-N); ^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 1.67 (m, 6H, 2xCH_3), 2.11 (m, 2H, CH_2), 2.29 (2H, m, CH_2), 2.44 (m, 1H, CH), 3.16 (m, 1H, CH), 4.09 (2H, m, CH_2), 4.23 (bs, 1H, NH, D_2O-exchangeble), 4.48 (s, 2H, CH_2), 6.95 (m, 1H, Ar-H), 7.03 (s,1H, Ar-H), 7.16 (m, 2H, Ar-H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ(ppm): 20.2, 21.6, 31.2, 32.8, 41.6, 52.8, 68.9, 116.7, 125.8, 128.3, 131.5, 149.3, 175.0, 178.2; ESI MS (m/z): 325 [M+1]; Anal. Calculated for $C_{16}H_{21}ClN_2O_3$: C, 59.17; H, 6.52, N, 8.62; Found: C, 59.46; H, 6.55, N, 8.69%.

1-[(2S)-3-methyl-2-{[4-(trifluoromethyl)benzyl]amino}butanoyl]pyrrolidin-2-one (5r)

IR (KBr) (cm^{-1}): 3437 (NH), 1711 (C=O), 1689 (C=O), 1256 (C-N); ^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm):): 1.729 (m, 6H, 2xCH_3), 2.08 (2H, m, CH_2), 2.26 (2H, m, CH_2), 2.57 (m, 1H, CH), 3.17 (m, 1H, CH), 4.09 (2H, m, CH_2), 4.29 (bs, 1H, NH, D_2O-exchangeble), 4.452 (s, 2H, CH_2), 7.02 (d, 2H, Ar-H, J = 7.2 Hz), 7.25 (d, 2H, Ar-H, J = 7.1); ^{13}C-NMR (100 MHz, $CDCl_3$) δ(ppm): 20.0, 21.16, 31.6, 32.6, 41.2, 52.8, 69.3, 123.5, 128.8, 130.2, 139.8, 175.3, 178.7; ESI MS (m/z): 343 [M+1]; Anal. Calculated for $C_{17}H_{21}F_3N_2O_2$: C, 59.64; H, 6.18; N, 8.18; Found: C, 59.87; H, 6.21; N, 8.09%.

Pharmacology

Anticonvulsant Screening

All animal handling procedures, sample administration, and disposal were done in accordance with IACUC

standards at the Faculty of Veterinary Medicine, University of Sadat City, Egypt, under compliance number VUSC-002-1-22.

Male albino mice ranging 25-30g were employed as experimental animals in this study. In order to test the compounds for both MES and *sc*PTZ, they were mixed with PEG 200. A group of six animals per cage were kept in a standard laboratory environment at ambient temperature with free availability of water and food.

Maximal Electroshock Seizure (MES) Test

To investigate the anticonvulsant effects at specific time intervals (0.5 h and 4h), test substances were given as an intraperitoneal (*i.p.*) injection at doses of 30, 100, and 300 mg/kg. Using ear clip electrodes, mice were given 60Hz, 50 mA electrical shocks for 0.2 sec, resulting in maximal electroshock convulsions. Protection is characterized by the lack of the seizure in 50% or more of the animals treated with the drug.[13]

Subcutaneous Pentylenetetrazole (*sc*PTZ) Seizure Test

The *sc*PTZ is a test that can be used to find substances that raise the seizure threshold. The *sc*PTZ test employs 85 mg/kg of the pentylenetetrazole dose. This causes clonic convulsions lasting at least five seconds in 97 percent (CD97) of the animals investigated. The test chemicals were delivered intraperitoneally to each animal at doses of 30, 100, and 300 mg/kg.

The convulsant (PTZ) was given subcutaneously at the estimated testing time (0.5 h and 4.0 h) and the animals were monitored for 30 min. The lack of clonic seizures in 50% or more of the animals over the desired time determined the capacity of synthesised analogues to remove the impact of pentylenetetrazole on the seizure cutoff.[14]

Neurotoxicity Study

Rotarod Test

The rotarod test was used to keep track of mice's minimal motor damage. During the experiment, the mice were trained to stay on a rotating rod with a diameter of 3.2 cm having a rotational speed of 10 revolutions per minute (RPM). The animal's failure to keep balance on the rotating rod for at least one minute is used to determine neurotoxicity. The dose at which half of the animals qualified to balance themselves on the rotating rod and the half dropped off was determined.[15,16]

Ethanol Potentiation Test

Mice were administered the test compounds. Except for control animals, test animals received a dosage of 2.5 g kg^{-1} ethanol one hour later. There will be no lateral position due to ethanol in control animals. The animals from each group that were in the lateral posture after being fed ethanol was counted.[17]

Molecular Docking Studies and ADME Prediction

The ADME characteristics of synthetic compounds were examined using pre-ADMET software. A number of indices, including plasma protein binding (PPB), log P, skin permeability (SP), blood-brain barrier penetration (BBB), buffer solubility (BS), and human intestinal absorption (HIA), were determined in this study.

AutoDock Vina software was used to perform molecular docking to get the probable orientation and confirmation for the ligand at the binding site. Chem draw software (Cambridge Soft) was used to draw the structures of synthesized analogues of pyrrolidine (5a-r). The Chem3D Ultra 8.0 programme was used to turn the 2D structures into 3D structures.

Crystal structure of GABA-A receptor, also known as GABA(A)R-beta3 homopentamer (code 4COF), was obtained by using PDB (Protein Data Bank). The protein preparation wizard in UCSF Chimera 1.15 was used to create the protein. The protein was imported into PyRx software, which generated a PDBQT file containing the protein's structure with hydrogens present in all polar residues. Lamarckian Genetic Algorithm (LGA) approach was used to determine ligand-receptors docking.

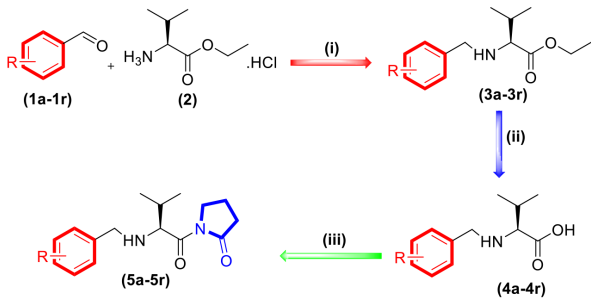
Following the completion of the docking search, the best conformation with the lowest docked energy was selected as the best candidate for further investigation. Ten AutoDock Vina runs were done for each ligand structure, with the best pose being collected and preserved for each run. The protein-ligand conformation interactions were explored in Discovery Studio 3.5. The docking score, hydrogen bonds, and pi-pi interactions were utilised to calculate the compound's binding affinities at the receptor's active site.

RESULTS AND DISCUSSION

Chemistry

The work reported here explains the synthesis and characterization of (S)-1-(2-(substituted benzylamino)-3-methylbutanoyl)pyrrolidin-2-one analogues. Simultaneously, anticonvulsant activity and molecular docking experiments were performed on these newly synthesised compounds. The preparation processes, reagents, and ambient conditions for the production of a total of 18 target compounds were specified in Scheme 1. Various physicochemical parameters were investigated and reported in Table 1. These

new analogues were characterized with the help of NMR and IR spectroscopical data using crystalized compounds isolated with a suitable solvent. Elemental analyses of new products were also performed. IR data depicted N–H, C=O and C=N stretching bands in the region of 3437-3378, 1717-1715 and 1260-1240 cm^{-1}, respectively. The ^1H NMR spectral data of compounds were obtained in $CDCl_3$/ DMSO-*d6* solvent using TMS as an internal standard. These newly synthesized products showed Ar-H in the region of δ 6.91-7.28 ppm with varied EDG and EWG substitutions, a broad singlet in the region δ 4.20 - 4.35 ppm was observed for N-H. The pyrrolidinone carbonyl carbon showed the peak in the region δ 174.1-175.3 ppm and CH_2 protons were obtained at region δ 2.01-2.40 ppm as multiplets. Further, the dimethyl protons were seen as multiplets in the δ 1.63-1.75 ppm region. Also, the benzyl CH_2 appeared as a singlet in the δ 4.23-4.50 ppm. Observed values for Carbon, Hydrogen and Nitrogen in elemental analysis were within ±0.4 percent of theoretical values.



R=H, OCH_3, CH_3, F, Cl, NO_2, OH, Br, N $(CH_3)_2$

Scheme 1: Production of 2-(arylamino)-3-methylbutanoyl] pyrrolidin-2-one analogues. Chemicals and conditions: (i) Methanol, agitation; 30 min, Iodine, Sodium borohydride, stirring; room temperature, water, recrystallization with ethanol; (ii) NaOH, EtOH; reflux, neutralization by dilute HCl, water, recrystallization with ethanol; (iii) EDC·HCl, HOBT, DMF, triethylamine, stirring;12-15 hrs, water, brine, sodium sulphate, recrystallization with ethyl acetate.

Pharmacology Evaluation

Various pharmacological investigations were undertaken in accordance with the NIH's standard approach for anticonvulsant drug development (ADD) programme. Neurotoxicity studies of the product molecules were also performed. The analysis protocols comprised subcutaneous pentylenetetrazole (*sc*PTZ), maximal electroshock (MES) and neurotoxicity (Tox). The synthesized analogues were directed as an intraperitoneal (*i.p.*) injection with a dose level of 30, 100 and 300 mg/kg and the anticonvulsant action was calculated after 0.5 hr and 4 hr intervals of administration. Ethanol potentiation and rotarod test were used to measure neurotoxicity. Results of anticonvulsant and neurotoxicity data were tabulated in Table 2.

Table 1: Physicochemical variables of the synthesized analogues (5a-r).

Compound	Substitution (R)	Molecular Formulae	Molecular weight	% Yield	Melting point (°C)	R_f^*	Color (State)
5a	H	$C_{16}H_{22}N_2O_2$	274.3640	47	145	0.51	Yellow (Solid)
5b	p-CH_3	$C_{17}H_{24}N_2O_2$	288.3910	52	171	0.45	Off white (Crystal)
5c	p-OCH_3	$C_{17}H_{24}N_2O_3$	304.3900	41	162	0.39	White (Solid)
5d	p-OH	$C_{16}H_{22}N_2O_3$	290.3630	55	186	0.49	White (Powder)
5e	p-NO_2	$C_{16}H_{21}N_3O_4$	319.3610	50	208	0.59	White (Crystal)
5f	p-Cl	$C_{16}H_{21}ClN_2O_2$	308.8060	47	166	0.41	White (Solid)
5g	p-F	$C_{16}H_{21}FN_2O_2$	292.3544	40	158	0.44	Cream (Solid)
5h	p-Br	$C_{16}H_{21}BrN_2O_2$	353.2600	55	148	0.55	Off white (Crystal)
5i	m-Cl	$C_{16}H_{21}ClN_2O_2$	308.8060	43	173	0.46	Yellow (Solid)
5j	m-CH_3	$C_{17}H_{24}N_2O_2$	288.3910	46	155	0.63	Off white (Crystal)
5k	m-NO_2	$C_{16}H_{21}N_3O_4$	319.3610	46	196	0.64	Tan white (Crystal)
5l	3-OCH_3,4-CH_3	$C_{18}H_{26}N_2O_3$	318.4170	53	184	0.57	Cream (Solid)
5m	p-$N(CH_3)_2$	$C_{18}H_{27}N_3O_2$	317.4330	46	182	0.67	White (Crystal)
5n	m-F	$C_{16}H_{21}FN_2O_2$	292.3544	42	164	0.61	Pale yellow (Solid)
5o	3-Cl, 4-CH_3	$C_{17}H_{23}ClN_2O_2$	322.8330	48	186	0.69	Yellow (Powder)
5p	3-Cl, 4-OCH_3	$C_{17}H_{23}ClN_2O_3$	338.8320	45	197	0.73	Yellow (Solid)
5q	3-Cl, 4-OH	$C_{16}H_{21}ClN_2O_3$	324.8050	51	212	0.48	Cream (Solid)
5r	p-CF_3	$C_{16}H_{21}F_3N_2O_2$	342.3622	42	190	0.58	Pale yellow (Solid)

* Solvent system: Toluene: Ethyl acetate: Formic acid (TEF, 5:4:1)

Table 2: Neurotoxicity and anticonvulsant screen data of analogues (5a-r).

Compound Number	Intraperitoneal/subcutaneous injection in mice[a]						
	MES screening		scPTZ screening		Neurotoxicity screening		Ethanol
	0.5 hr	4.0 hr	0.5 hr	4.0 hr	0.5 hr	4.0 hr	Potentiation
5a	-	300	-	-	X	X	X
5b	300	-	300	-	X	X	X
5c	-	-	X	X	X	X	X
5d	-	300	-	-	X	X	X
5e	100	300	300	-	-	-	-
5f	-	300	300	-	X	X	X
5g	300	-	X	X	X	X	X
5h	30	300	300	-	-	-	-
5i	-	-	X	X	X	X	X
5j	300	-	100	-	-	-	-
5k	30	300	100	300	-	+	-
5l	30	100	100	300	-	-	-
5m	-	-	X	X	X	X	X
5n	300	--	-	-	X	X	X
5o	100	100	100	-	+	+	+
5p	300	-	-	-	X	X	X
5q	300	-	-	-	X	X	X
5r	30	100	100	300	-	-	-
Phenytoin	30	30	-	-	100	100	-
Carbamazepine	30	100	100	300	100	300	-

[a]Doses of 30, 100 and 300 mg/kg of the compound were injected, then the protection action and neurotoxicity studies were analyzed after 0.5 and 4hr intervals. The given data described the amount of minimum dose that was required to lead protection or neurotoxicity in either 50% or higher number of the animals. The symbol dash (—) showed that those compounds were inactive towards neurotoxicity or anticonvulsant activity. X— indicates not analyzed. [b]Ethanol potentiation test: (+), half or more animals showed positive results; (–), half or more animals depicted negative results. X— denotes not tested.

In Maximal Electroshock (MES) model, molecules 5h, 5k, 5l and 5r depicted electroshock protection at 0.5 hr which signified that they were having a quick onset of action. Molecules 5e and 5o showed positive results at 100 mg/kg at 0.5 hr. At the interval of 4.0 hr, molecules 5l, 5o and 5r showed good activity at a dose of 100 mg/kg which indicated that the analogues possess a long time of action at a moderate dose. The standard drugs used were phenytoin and carbamazepine.

In the scPTZ model, compounds 5j, 5k, 5l, 5o and 5r were observed to show activity at 100 mg/kg at 0.5 hr. Compound 5k, 5l and 5r were protective at 300 mg/kg at 4.0 hr. Most of the compounds showed negative results during neurotoxicity evaluation. Only compound 5o was found to be neurotoxic at 0.5 and 4 hr and 5k were found neurotoxic at 4 hr.

Moreover, compounds 5l and 5r were observed to be highly potent from all the synthesized compounds and also acted as selective GABA facilitators. In the case of MES and scPTZ analysis, compounds either showed inactivity or did not possess any significant property.

The SAR studies revealed that compounds 5l and 5r were most potent amongst the series. The disubstituted compound 5l having EDG (-OCH_3) at meta position and (-CH_3) at the para position was observed to be highly active. The other compound 5r also found to be active in MES and scPTZ screening, having a powerful EWG (-CF_3) at the para position. Other active compounds (5e, 5h, 5k, and 5o) suggested that the molecules substituted with strong EWG at meta or para positions possessed significant anticonvulsant activity.

ADME Prediction

ADME prediction was performed on the generated compounds, and the findings are summarised in Table 3. Analyzing BBB penetration revealed whether the target molecule could cross the BBB. The resulting values further served in decreasing the adverse effects and toxicity and also may improve the efficacy of drugs that possessed pharmacological action of the brain. Almost all analyzed targets presented positive values, supporting their potential to easily cross the BBB. The

Table 3: Prediction of the ADME of synthesized derivatives.

Compds.	Substitution (R)	LogP	[a]BBB	[b]BS (mg/l)	CYP-inhibition	[c]HIA	[d]PPB	[e]SP
5a	H	2.000100	0.070029	4438.47	2D6 weak inhibitor	95.663326	51.952000	-3.23102
5b	p-CH_3	2.486300	0.160443	3189.83	2D6 weak inhibitor	95.701136	59.708721	-3.17241
5c	p-OCH_3	1.983700	0.0237141	4279.65	2D6 weak inhibitor	96.077157	51.525775	-3.47775
5d	p-OH	1.732700	0.0919382	5387.02	2D6 weak inhibitor	92.325521	52.346291	-3.50838
5e	p-NO_2	1.894500	0.131241	899.497	2D6 weak inhibitor	87.836075	41.786914	-3.29512
5f	p-Cl	2.664500	0.12346	2715.29	2D6 weak inhibitor	95.789296	74.337909	-3.31111
5g	p-F	2.205600	0.0611122	8527.99	2D6 weak inhibitor	95.666521	61.210023	-3.55678
5h	p-Br	2.748500	0.141878	1286.74	2D6 weak inhibitor	95.952512	81.903092	-3.1823
5i	m-Cl	2.664500	0.174952	1620.04	2D6 weak inhibitor	95.789296	72.285339	-3.31338
5j	m-CH_3	2.486300	0.135021	1903.17	2D6 weak inhibitor	95.701136	59.236636	-3.1819
5k	m-NO_2	1.894500	0.00894785	536.673	2D6 weak inhibitor	87.836075	52.087732	-3.2943
5l	3-OCH_3,4-CH_3	2.719500	1.58839	1180.8	2D6 weak inhibitor	88.798688	68.571927	-3.48546
5m	p-$N(CH_3)_2$	2.162300	0.0445739	1702.15	2D6 weak inhibitor	95.882777	52.021030	-3.38995
5n	m-F	2.205600	0.101433	5088.11	2D6 weak inhibitor	95.666520	63.953767	-3.56159
5o	3-Cl, 4-CH_3	3.150700	0.346792	648.797	2D6 weak inhibitor	95.825707	76.273092	-3.2481
5p	3-Cl, 4-OCH_3	2.648100	0.0479278	1544.88	2D6 weak inhibitor	96.169360	67.677842	-3.53493
5q	3-Cl, 4-OH	2.397100	0.242633	3929.02	2D6 weak inhibitor	93.544097	73.573799	-3.55604
5r	p-CF_3	2.942400	0.231226	1545.73	2D6 weak inhibitor	95.706629	60.781361	-2.2046

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

value of 5l is 1.58839, indicating that it is the most active. HIA is the process through which the orally administered drugs were absorbed from GIT into the bloodstream. The synthesized compounds reported good results in the range 80–100% depicting them to be well-absorbed compounds and, also, these compounds can be assimilated through the human intestine. PPB has an effect on how long a molecule stays in the body and can also influence how effective a medicine is. The extent to which a medication binds to plasma proteins significantly affects its pharmacological and pharmacokinetic properties. As indicated, the % bound value of less than 90 was considered as low and the % bound value of more than 90 was considered as high.

As presented, molecules 5h, 5o, 5f, 5i and 5q displayed good affinity for a plasmatic protein with a value of more than 70%. Also, the ability to bind protein plasma highly affects the process of distribution of the drug. One of the important parameters is the SP rate for the transdermal delivery of drugs. The diffusion of the drug into the intercellular lipid matrix is recognized as an important factor determining the absorption of the drug by the skin. All the analyzed compounds showed negative results for SP check, ranging from -3.56159 (compounds 5n) to -2.2046 (compound 5r), proving that these cannot be directed via transdermal routes. The permeability potential of the compound to reach the target tissue in the body is indicated by the Log *P* values. The tested analogues were lipophilic because the Log $P > 0$ (or $P > 1$). Compound 5o is most lipophilic with Log *P* 3.150700.

Molecular docking

To find the possible binding pattern and energies of the synthesized analogues (**5a-r**) the molecular docking studies were carried out which helped to outline the promising leads. The potential molecules analyzed against the GABA-A receptor presented a diverse docking score in the range of -5.4 to -6.3. The docking pattern of molecules 5a-r depicted that there were two important amino acids ARG 129 and MET 115 which acted as a pathway for the opening of ligand to the GABA-A receptor. The image of the top-ranked docking score of compounds 5e, 5j, 5k, 5l, 5o, and 5r is depicted in Figure 2. The docking score of pyrrolidine derivatives (5a-r) with $GABA_A$ (4 COF) is given in Table 4. The residues of amino acids such as TYR 62, ASN 85, ARG 114, ARG 129, MET 115, ASP 84, THR 110, TRP 443 and THR 110 generate the active site of the receptor (GABA-A). The phenyl ring substitution in analogues was responsible for important interactions of pi-alkyl with ARG 129, pi-sulphur interactions with MET 115, pi-pi T-shaped interactions with TYR 62 and pi-pi stacked interactions with TRP443 in the binding region of receptor (GABA-A). The amino residues

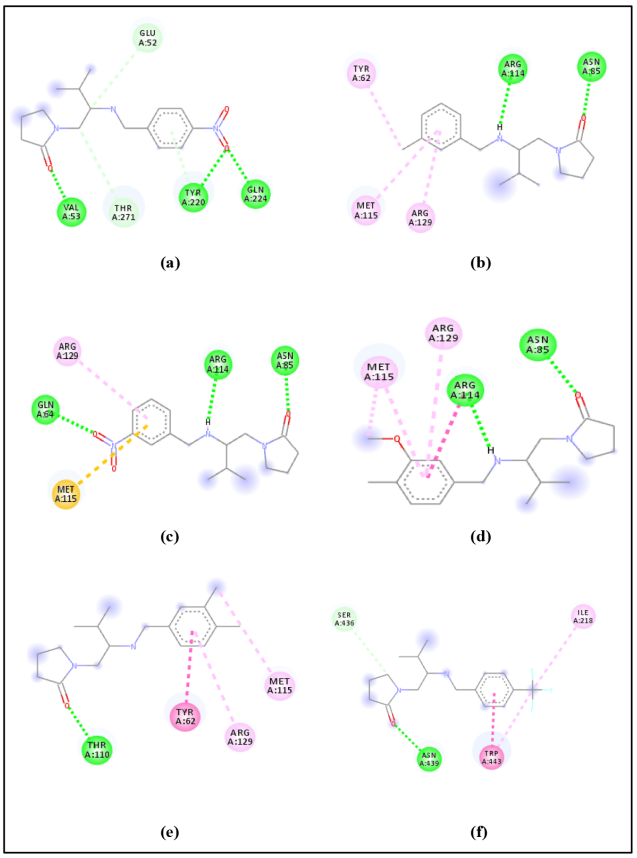


Figure 2: 2D Ligand interaction of (a) compound 5e (b) compound 5j (c) compound 5k (d) compound 5l (e) compound 5o (f) compound 5r using LigPlot.

Table 4: 4COF Docking score of pyrrolidine derivatives (5a-r) with GABA-A.

Compound	Docking score (kcal/mol)	Compound	Docking score (kcal/mol)	Compound	Docking score (kcal/mol)
5a	-5.4	5g	-5.7	5m	-5.5
5b	-5.7	5h	-5.6	5n	-5.5
5c	-5.8	5i	-5.4	5o	-6.0
5d	-5.6	5j	-6.1	5p	-5.7
5e	-6.2	5k	-6.4	5q	-5.6
5f	-5.6	5l	-6.3	5r	-6.3

ARG 114 and ASN 85 are responsible for conventional hydrogen bond formation with the –NH group.

The *in-silico* studies data presented that the substitution of EWG on aryl ring can significantly increase the GABA concentration and therefore anticonvulsant activity. The results co-relate with the results of the animal model. This result of *in silico* and biological activity also indicates that the presence of a Cl group in the aryl ring may boost the anticonvulsant activity of compound 5° by regulating the physicochemical properties and pharmacokinetic parameters of compound 5° to increase bioavailability and metabolic stability, as well as the binding affinity to receptors. In general, the presence of halogens such as chlorine (Cl) in the compounds contributes to the hydrophobic interactions between the molecules and the target protein.

CONCLUSION

New (S)-1-(2-(substituted benzylamino)-3-methylbutanoyl) pyrrolidin-2-one analogues (5a-r) were synthesised and their anticonvulsant activity was assessed utilising the MES and scPTZ models in this study. Compounds 5l and 5r were shown to be highly potent among the series against both models with a quick onset of action and therefore, they are supposed to be the most active and selective GABA facilitators. All compounds of the series passed the neurotoxicity test except compound 5o which was found to be neurotoxic. The paper also includes ADME prediction and molecular docking of all analogues. All ADME parameters are discussed in detail in the manuscript. The molecular docking was done using Autodock Vina using GABA-A as the target protein. The important interactions found from the results are TYR 62, ASN 85, ARG 114, ARG 129 and MET 115 at the active site of GABA-A. The significant pi-pi, pi-alkyl, pi-sulfur interactions are discussed in detail. The results of docking co-relates with the results of the animal model. This result of *in-silico* and biological activity indicates that substitution of the EWG on the aryl ring at the meta or para position can significantly increase the GABA concentration and, consequently, the anticonvulsant effect. The series of synthesized analogues can be considered as promising candidates for further exploration.

ACKNOWLEDGEMENT

The work is self-funded; however, all authors acknowledge their respective institutions for receiving various kinds of support to carry out this research work.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

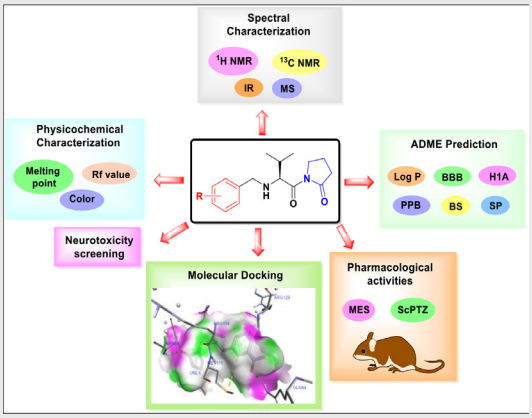
ADD: Anticonvulsant drug development; **AEDs:** antiepileptic drugs; **Anal:** Analytical; **aq:** aqueous; **Ar:** aromatic; **BBB:** Blood-brain barrier; **BS:** Buffer solubility; **EDG:** Electron donating group; **EWG:**

Electron withdrawing group; **ESI:** Electrospray ionization; **FDA:** Food and Drugs Administration; **FTIR:** Fourier transform infrared; **GABA-A:** Gamma-aminobutyric acid; **HIA:** Human Intestinal Absorption; **i.p.:** Intraperitoneal; **IACUC:** Institutional Animal Care and Use Committee; **KBr:** Potassium bromide; **LCMS/MS:** Liquid chromatography-mass spectrometry; **m.p.:** melting point; **MS:** Mass spectrometry; **MES:** Maximal electroshock seizures; **NIH:** National institutes of health; **NMR:** Nuclear magnetic resonance; **PDB:** protein data bank; **PEG:** Polyethylene glycol; **PPB:** Plasma Protein Binding; **RPM:** Revolutions per minute; **Rf:** retention factor; **RT:** Room Temperature; **SP:** Skin Permeability; **scPTZ:** Subcutaneous pentylenetetrazole; **TLC:** Thin layer chromatography; **UCSF:** University of California, San Francisco.

REFERENCES

1. Figueiredo KA, Medeiros SC, Neves JKO, Da Silva JA, Da Rocha Tomé A, Carvalho ALM *et al*. *In vivo* evaluation of anticonvulsant and antioxidant effects of phenobarbital microemulsion for transdermal administration in pilocarpine seizure rat model. Pharmacol Biochem Behav. 2015;131:6-12. doi: 10.1016/j.pbb.2015.01.008, PMID 25620677.
2. Kwon CS, Wagner RG, Carpio A, Jetté N, Newton CR, Thurman DJ. The worldwide epilepsy treatment gap: A systematic review and recommendations for revised definitions - A report from the ILAE Epidemiology Commission. Epilepsia. 2022;63(3):551-64. doi: 10.1111/epi.17112, PMID 35001365.
3. Kaushik D, Khan SA, Chawla G, Kumar S. N'-[(5-chloro-3-methyl-1-phenyl-1H-pyrazol-4-yl)methylene] 2/4-substituted hydrazides: Synthesis and anticonvulsant activity. Eur J Med Chem. 2010;45(9):3943-9. doi: 10.1016/j.ejmech.2010.05.049, PMID 20573423.
4. Siddiqui N, Ahsan W. Triazole incorporated thiazoles as a new class of anticonvulsants: Design, synthesis and *in vivo* screening. Eur J Med Chem. 2010;45(4):1536-43. doi: 10.1016/j.ejmech.2009.12.062, PMID 20116140.
5. Socała K, Mogilski S, Pieróg M, Nieoczym D, Abram M, Szulczyk B *et al*. KA-11, a novel pyrrolidine-2,5-dione derived broad-spectrum anticonvulsant: Its antiepileptogenic, antinociceptive properties and *in vitro* characterization. ACS Chem Neurosci. 2019;10(1):636-48. doi: 10.1021/acschemneuro.8b00476, PMID 30247871.
6. Kamiński K, Rzepka S, Obniska J. Synthesis and anticonvulsant activity of new 1-[2-oxo-2-(4-phenylpiperazin-1-yl) ethyl] pyrrolidine-2, 5-diones. Bioorg Med Chem Lett. 2011;21(19):5800-3. doi: 10.1016/j.bmcl.2011.07.118, PMID 21875804.
7. Byrtus H, Obniska J, Czopek A, Kamiński K. Synthesis and anticonvulsant activity of new N-Mannich bases derived from 5-cyclopropyl-5-phenyl-hydantoins. Arch Pharm. 2011;344(4):231-41. doi: 10.1002/ardp.201000241, PMID 21469172.
8. Siddiqui N, Alam MS, Sahu M, Naim MJ, Yar MS, Alam O. Design, synthesis, anticonvulsant evaluation and docking study of 2-[(6-substituted benzo [d] thiazol-2-ylcarbamoyl) methyl]-1-(4-substituted phenyl) isothioureas. Bioorg Chem. 2017;71:230-43. doi: 10.1016/j.bioorg.2017.02.009, PMID 28238402.
9. Grover G, Nath R, Bhatia R, Akhtar MJ. Synthetic and therapeutic perspectives of nitrogen containing heterocycles as anti-convulsants. Bioorg Med Chem. 2020;28(15):115585. doi: 10.1016/j.bmc.2020.115585, PMID 32631563.
10. Kerru N, Gummidi L, Maddila S, Gangu KK, Jonnalagadda SB. A review on recent advances in nitrogen-containing molecules and their biological applications. Molecules. 2020;25(8):1909. doi: 10.3390/molecules25081909, PMID 32326131.
11. Góra M, Czopek A, Rapacz A, Dziubina A, Głuch-Lutwin M, Mordyl B *et al*. Synthesis, anticonvulsant and antinociceptive activity of new hybrid compounds: derivatives of 3-(3-methylthiophen-2-yl)-pyrrolidine-2,5-dione. Int J Mol Sci. 2020;21(16):5750. doi: 10.3390/ijms21165750, PMID 32796594.
12. Emami L, Moezi L, Amiri-Zirtol L, Pirsalami F, Divar M, Solhjoo A *et al*. Anticonvulsant activity, molecular modeling and synthesis of spirooxindole-4H-pyran derivatives using a novel reusable organocatalyst. Mol Divers. 2022:1-13. doi: 10.1007/s11030-021-10372-7, PMID 35079947.
13. Krall RL, Penry JK, White BG, Kupferberg HJ, Swinyard EA. Antiepileptic drug development: II. Anticonvulsant drug screening. Epilepsia. 1978;19(4):409-28. doi: 10.1111/j.1528-1157.1978.tb04507.x, PMID 699894.
14. Clark CR, Wells MJ, Sansom RT, Norris GN, Dockens RC, Ravis WR. Anticonvulsant activity of some 4-aminobenzamides. J Med Chem. 1984;27(6):779-82. doi: 10.1021/jm00372a013, PMID 6737420.
15. Ali R, Siddiqui N. Preliminary Anticonvulsant and Toxicity Screening of Substituted Benzylidenehydrazinyl-N-(6-substituted benzo [d] thiazol-2-yl) propanamides. Scientific World Journal. 2014;2014:194652. doi: 10.1155/2014/194652, PMID 25580452.
16. Dunham NW, Miya TS, Edwards LD. The pharmacological activity of a series of basic esters of mono- and dialkylmalonic acids. J Am Pharm Assoc Am Pharm Assoc. 1957;46(1):64-6. doi: 10.1002/jps.3030460119, PMID 13502132.
17. Clerici F, Pocar D, Guido M, Loche A, Perlini V, Brufani M. Synthesis of 2-amino-5-sulfanyl-1, 3, 4-thiadiazole derivatives and evaluation of their antidepressant and anxiolytic activity. J Med Chem. 2001;44(6):931-6. doi: 10.1021/jm001027w, PMID 11300875.

PICTORIAL ABSTRACT



SUMMARY

A series of (S)-1-(2-(substituted benzylamino)-3-methylbutanoyl)pyrrolidin-2-one analogues (5a-r) were synthesized and evaluated for their anticonvulsant activity. The analogues were screened by the 'gold standard methods, i.e., maximal electroshock seizure (MES) test and subcutaneous pentylenetetrazole (scPTZ) test. Compounds 5e, 5h, 5k, 5l, 5o and 5r were found to be the most potent of the series in MES model and, compounds 5j, 5k, 5l, 5o and 5r were found to be most active in scPTZ model. Furthermore, all synthetic analogues were evaluated for acute neurotoxicity using the rotarod method where most of the synthesized analogues passed the test. The paper also reports ADME prediction of all the 18 synthesized compounds with each parameter discussed in detail. Further, molecular docking studies were performed and the results showed good agreement with *in vivo* results. These compounds can be investigated further for the formation of newer/novel anticonvulsant agents.

About Authors

Dr. Obaid Afzal is currently working as an assistant professor in the Department of Pharmaceutical Chemistry, College of Pharmacy, Prince Sattam Bin Abdulaziz University, Saudi Arabia. He has more than 10 years of research experience in design, synthesis and pharmacological evaluation of bioactive molecules related to anticancer, antiviral, neuroprotective and analgesic drug development. He has more than 80 publications in different peer reviewed journal.

Dr. Gopal Prasad Agrawal is an associate professor of pharmaceutical analysis and quality assurance and presently working in the Institute of Pharmaceutical Research, GLA University, Mathura, India having more than 15 years of experience in teaching and research. He has published many research and review papers in the field of pharmaceutical analysis, formulation, and pharmaceutics in SCI/ Scopus indexed journals. His research areas of interest include pharmaceutical analysis, ,formulation & development, and pharmaceutical quality assurance.

Dr. Benson Mathai Kochikuzhyil is a professor of Pharmacology with more than 20 years of experience in teaching, research and administration. His research interests and publications are in the field of diabetes mellitus, dyslipidemia, cancer, stress and neurological disorders. He is the life member of Association of Pharmaceutical Teachers of India (APTI).

Cite this article: Afzal O, Agrawal GP, Alotaibi GRG, Shatat RSA, Khalilullah H, Ullah Z, Rashid M, Elkirdasy AF, Mathai BK. Design, Synthesis and Molecular Docking Studies of (S)-1-(2-(substituted benzylamino)-3-methylbutanoyl) pyrrolidin-2-one analogues as GABA Mediated Anticonvulsant agents. Indian J of Pharmaceutical Education and Research. 2023;57(1):182-93.