Synthesis and Anticonvulsant Activity of Some 2/3-Benzoylaminopropionanilide Derivatives

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Key words
- propanamide
- anilide
- lacosamide
- α/β-alanine
- anticonvulsant activity

Abstract

In this study, the synthesis and anticonvulsant properties of sixteen 2/3-benzoylaminopropionanilide derivatives were described. Molecular design of the compounds has been based on the modification of lacosamide which is a functionalized amino acid with a novel anticonvulsant activity. The structural confirmation of the title compounds was achieved by spectral and analytical data. The anticonvulsant activity profile of synthesized compounds was determined by maximal electroshock (MES) and subcutaneous metrazole (scMet) seizure tests, whereas their neurotoxicity was examined using rotarod test. All these tests were performed in accordance with the procedures of the Antiepileptic Drug Development (ADD) program. The majority of the compounds were effective in the MES or scMet screening tests. None of the compounds showed neurotoxicity according to the rotarod test at studied doses. Most active compounds in the series were **3**, **12** and **13**, which bearing 2-methyl, 2-ethyl and 2-isopropyl substituent on the *N*-phenyl ring, respectively.

Introduction

Epilepsy is an important chronic neurological disorder characterized by recurrent unprovoked seizures. Drug therapy with current antiepileptic drugs has important disadvantages and limitations. Approximately 70–80% of epilepsy patients are currently controlled by this drugs, the rest are resistance to conventionally available medical therapies [1,2]. Moreover, the current drug therapy is associated with adverse side effects such as ataxia, gastrointestinal disturbance, gingival hyperplasia, hirsutism, megaloblastic anemia and hepatotoxicity. Therefore, one of the main challenges in drug development is to discover new more effective antiepileptic molecules with less side effects [2–6].

The search of new antiepilept ic molecules is mainly based on 2 different approaches. Those are mechanism based design and structured based design. Many of the newer antiepileptic drugs as well as those currently in clinical development were designed through structural modifications of the pre-existing compounds [7–9].

In recent studies, functionalized amino acids (FAA) and α-amino acids (AAA) are 2 classes of antiepileptic molecules with pronounced anticonvulsant activity. Lacosamide (LCM) {(*R*)-2-acetamido-*N*-benzyl-3-methoxypropionamide} developed as functionalized amino acid, was approved in Europe and in the USA for antiepileptic therapy (**Fig. 1**). According to the literature survey, lacosamide represent a new mechanism of action with a favorable safety profile [10–22].

Anilide is the other pharmacophore moiety known to produce potent anticonvulsant drugs [23–25]. The structure-activity studies on anilide derivatives indicated the importance of substitution pattern on *N*-phenyl ring [26,27].

On the basis of these encouraging results, using lacosamide and anilide as structural leads, we aimed to synthesize some 2/3-benzoylaminopropionanilide derivatives as potential anticonvulsant compounds and then evaluate their anticonvulsant activities against MES and scMet screening tests.

Materials and Methods

Chemistry

Melting points were determined on an Electrothermal IA 9100 (Electrothermal, Essex, U.K.) melting point apparatus and are uncorrected. The IR spectra of compounds were recorded as potassium bromide pellets on a Jasco FT/IR-400

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(Jasco, Tokyo, Japan) and Perkin Elmer FT-IR Spectrometer 100 (Perkin Elmer Inc., Massachusetts, USA). The ^{1}H NMR spectra were recorded on a Varian As 400 Mercury Plus NMR (Varian Inc., Palo Alto, CA, USA) spectrophotometer using a $CDCl_3$ and DMSO-d_6 as solvent. Chemical shifts were reported in parts per million (δ) j values were given in Hz. Mass spectra (API-ES) were measured on an AGILENT 1100 MSD (Agilent Technologies, Palo Alto, CA, USA). Elemental analyses (C, H and N) were performed by Leco CHNS-932 (Leco-932, St. Joseph, MI, USA). The analytical results for the elements were within ±0.4% of the theoretical values.

Synthesis of 2/3-aminopropionanilide hydrochlorides (1a–14a)

The intermediates were prepared according to the method reported in the literature [28,29]. For this purpose, α/β-alanine (11.2 mmol) was dissolved in a mixture dioxane (20 ml) and 0.5 M NaOH (20 ml). After cooling with an ice-bath, di-tert-butyl dicarbonate ($(Boc)_2O$) (12.3 mmol) was added dropwise to this solution. The solution was stirred at room temperature overnight and the solvent was evaporated under reduced pressure. 5% $KHSO_4$ (150 ml) solution was added to the residue and extracted with dichloromethane (50 ml) twice, dried over anhydrous Na_2SO_4 and evaporated under reduced pressure. The residue was purified by crystallization with ethyl acetate to give N-Boc-α/β-alanine. The solution of N-Boc-α/β-alanine (5 mmol) in THF (20 ml) was cooled to 0–5 °C, and N-methylmorpholine (NMM) (5 mmol) was added under the nitrogen atmosphere. After stirring for 5 min, isobutyl chloroformate (IBCF) (5 mmol) was added in one portion. And then, aniline or substituted anilines were added dropwise to this solution for 10 min. The reaction mixture was stirred at room temperature for 1 h. The precipitated N-methylmorpholine hydrochloride was filtered off and the filtrate was evaporated under reduced pressure. The crude product was purified by crystallization ethanol-water (1:1) to yield N-Boc-α/β-alaninanilide. The solution of N-Boc-α/β-alaninanilide (10.6 mmol) in dichloromethane (50 ml) was cooled to 0–5 °C and was saturated with hydrogen chloride gas. The mixture was stirred for 2 h, and the solvent was removed under reduced pressure to yield an oil which solidified upon trituration with cold diethyl ether. The crude product was crystallized from ethanol.

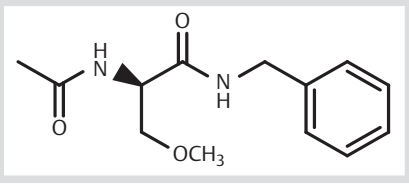

Fig. 1 Lacosamide.

Synthesis of 3-aminopropionanilide hydrochlorides (15a and 16a)

The intermediates **15a** and **16a** were prepared according to the method reported in the literature [30–32]. For this purpose, appropriately substituted aniline (66 mmol) was dissolved in 25 ml glacial acetic acid, and 3-chloropropionyl chloride (74 mmol) was added dropwise to this solution while cooling in ice-bath. The reaction mixture was stirred in the ice-bath for 15 min and at room temperature for 45 min. The mixture was poured into saturated sodium acetate solution. The precipitate was filtered, washed with cold water and purified by crystallization ethanol-water (1:1). 3-Chloropropionanilide acquired by this method (80 mmol) and phthalimide potassium salt (120 mmol) were refluxed in DMF (15 ml). By monitoring with TLC, the reaction was terminated. The reaction mixture was poured into cold water. The precipitate was filtered and washed with water. After drying, the precipitate was purified by crystallization from ethanol. The phthalimide intermediate prepared by this method (1.2 mmol) was suspended in absolute ethanol (99%) (20 ml) and hydrazine hydrate (80% aqueous) (0.2 ml) was added to this solution. The reaction mixture was refluxed for 3 h. After the mixture was cooled under 50 °C, 1.2 M HCl (5 ml) solution was added to the reaction medium and refluxed for 1 h. The resulting crude product was filtered and crystallized from ethanol.

Synthesis of 2/3-benzoylaminopropionanilides (1–16)

Compounds 1a–16a (2 mmol) were dissolved in 50% NaOH (15 ml), and benzoyl chloride (2 mmol) in chloroform (15 ml) was added to this solution. The reaction mixture was stirred at room temperature for 4 h. The organic phase was washed with 5% HCl (15 ml), 5% $NaHCO_3$ (15 ml) and water (20 ml) respectively and then evaporated under reduced pressure. The precipi-

Table 1 Yields, melting points, formulas, IR and API-ES data of compounds 1–16.

Comp.	Yield (%)	M.p. (°C)	Formula	IR (cm^{-1})	API-ES m/z (% intensity)
1	31	179	$C_{16}H_{16}N_2O_2$	3303, 3250, 1685, 1635, 1588, 1552	176 (100), 269 (M+H, 47)
2	58	111	$C_{16}H_{15}ClN_2O_2$	3484, 3235, 1643, 1581, 1550	176 (100), 305 (M+2, 9), 303 (M+H, 28)
3	90	131	$C_{17}H_{18}N_2O_2$	3473, 3239, 1644, 1625, 1588, 1552	176 (100), 283 (M+H, 84)
4	75	150	$C_{17}H_{18}N_2O_3$	3303, 3058, 1691, 1643, 1588, 1531	299 (M+H, 100)
5	70	170	$C_{18}H_{20}N_2O_2$	3288, 3250, 1656, 1631, 1581, 1538	176 (100), 297 (M+H, 32)
6	86	136	$C_{19}H_{22}N_2O_2$	3299, 3235, 1661, 1633, 1577, 1538	176 (100), 311 (M+H, 43)
7	75	178	$C_{18}H_{20}N_2O_2$	3347, 2977, 1689, 1656, 1591, 1535	176 (100), 259 (M+H, 59)
8	13	175[a]	$C_{16}H_{16}N_2O_2$	3306, 3056, 1670, 1632, 1598, 1578	269 (M+H, 100)
9	73	142	$C_{16}H_{15}ClN_2O_2$	3353, 3284, 1664, 1633, 1579, 1531	176 (100), 305 (M+2, 33), 303 (M+H, 87)
10	93	172	$C_{17}H_{18}N_2O_2$	3353, 3237, 1668, 1635, 1538	283 (M+H, 100)
11	80	132	$C_{17}H_{18}N_2O_3$	3328, 3284, 1664, 1620, 1540	299 (M+H, 100)
12	73	166	$C_{18}H_{20}N_2O_2$	3335, 3239, 1671, 1631, 1570, 1542	297 (M+H, 100)
13	92	169	$C_{19}H_{22}N_2O_2$	3344, 3235, 1664, 1629, 1591, 1542	311 (M+H, 100)
14	69	220	$C_{18}H_{20}N_2O_2$	3285, 3223, 1659, 1620, 1574, 1543	297 (M+H, 100)
15	93	232	$C_{16}H_{14}Cl_2N_2O_2$	3359, 3261, 1670, 1639, 1541, 1521	176 (100), 337 (M^+, 45)
16	12	249	$C_{16}H_{15}N_3O_4$	3378, 3247, 1670, 1651, 1576, 1545	242 (100), 314 (M+H, 18)

[a] 191°C, Ref. [34]

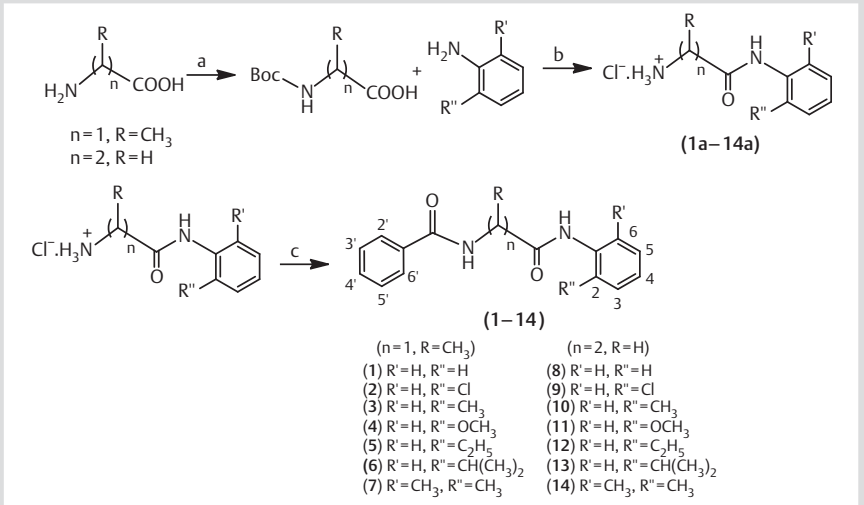

Fig. 2 Synthesis of the compounds 1–14.

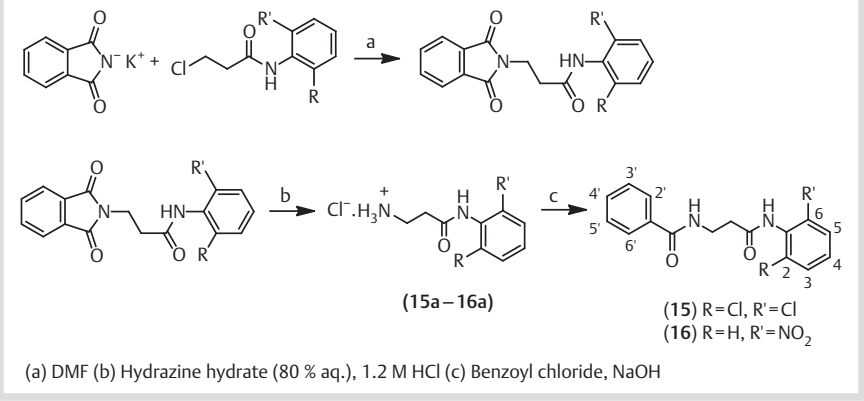

Fig. 3 Synthesis of the compounds 15 and 16.

tate was crystallized from ethanol-water (1:1) to furnish title compounds. Yields and melting points are given in **Table 1**.

Anticonvulsant Activity Screening

The compounds were tested for their anticonvulsant activity against maximal electroshock (MES) and subcutaneous Metrazole (scMet)-induced seizure treshold tests. The acute neurological toxicity was determined in the rotarod test. All these tests were performed in male mice according to the phase-1 tests of the Antiepiletic Drug Development (ADD) program which were developed by National Institute of Health (NIH), National Institute of Neurological Disorders and Stroke (NINDS) [38]. Stimulator (Grass S88, Astro-Med. Inc. Grass Instrument Division, W. Warwick, RI, USA), constant current unit (Grass CCU1A, Grass Medical Instrument, Ouincy, MA, USA) and corneal electrodes were used for the evaluation of anticonvulsant activity against MES test. All synthesized compounds were suspended in 30% aqueous of PEG 400 and administered to the mice intraperitoneally in a volume of 0.01 mL/g at body weight. 12 Swiss Albino male mice (20±2 g) were used for each compound (mice were obtained from The Hacettepe University Animal Farm) according to the ADD-NINDS program. The animal were kept under standard conditions at an ambient temperature of 25±2 °C and allowed free access to food except at the time they were brought out of the cage. All the experimental protocols were carried out with the permission of Hacettepe University, "Laboratory Animals Ethic Committee" decision (01.09.2010 date 2010/46-11 number). Control animals received 30% aqueous PEG 400. Metrazole was administered subcutaneously (sc) on the back of the neck. The rotarod toxicity test was performed on 1-inch-diameter knurled wooden rod, rotating at 6 rpm (the rotarod used in phase-1 test was made by Hacettepe University Technical Department).

Maximal Electroshock (MES)-induced seizure test

MES seizures were elicited with a 60-cycle alternating current of 50 mA intensity (5–7 times more than that required to elicite minimal seizures) delivered for 0.2 s via corneal electrodes. A drop of 0.9% saline was instilled into the eye prior to application of the electrodes in order to prevent the death of the animal. Abolition of the hind limb tonic extension component of the seizure was defined as protection.

Subcutaneous Metrazole (scMet)-induced seizure test

85 mg/kg of Metrazole (produces seizures in more than 95% of mice) was administered as a 0.5% solution sc into the posterior midline. The animal was observed for 30 min to decide whether the failure of the treshold seizure (a single episode of clonic spasms of at least 5 s duration) could be defined as protection.

Neurotoxicity

The rotarod test was used to evaluate neurotoxicity. The animal was placed on a 1-inch-diameter knurled wooden rod rotating at 6 rpm. Normal mice remain on a rod rotating at this speed indefinitely. Neurological toxicity was defined as the failure of the animal to remain on the rod for 1 min.

Table 2 ^{1}H NMR data and elemental analysis of compounds 1–16.

Compound	^{1}H NMR and Elemental analysis (C, H, N, %)
1	^{1}H NMR (DMSO-d_6): δ 1.43 (3H, d, J = 7.2 Hz, CH(CH_3)), 4.59–4.63 (1H, m, CH(CH_3)), 7.04 (1H, td, J = 1.2; 7.0 Hz, H-4), 7.30 (2H, td, J = 1.6; 7.4 Hz, H-3 and H-5), 7.47 (2H, td, J = 1.3;7.4 Hz, H-3′ and H-5′), 7.52–7.56 (1H, m, H-4′), 7.60–7.62 (2H, m, H-2 and H-6), 7.90–7.93 (2H, m, H-2′ and H-6′), 8.59 (1H, d, J = 7.6 Hz, NH-CH(CH_3)), 10.01 (1H, bs, NH-phenyl) ppm. Anal.calcd. for $C_{16}H_{16}N_2O_2$. 0.2H_2O: C, 70.67; H, 6.08; N,10.30. Found: C, 70.82; H, 6.11; N, 10.26.
2	^{1}H NMR (DMSO-d_6): δ 1.47 (3H, d, J = 7.2 Hz, CH(CH_3)), 4.71–4.75 (1H, m, CH(CH_3)), 7.17 (1H, td, J = 1.6; 8.0 Hz, H-4), 7.33 (1H, td, J = 1.0; 8.0 Hz, H-5), 7.46–7.53 (3H, m, H-3′, H-5′ and H-3), 7.54–7.57 (1H, m, H-6), 7.82 (1H, dd, J = 1.6; 8.4 Hz, H-4′), 7.92 (2H, dd, J = 1.6; 8.8 Hz, H-2′ and H-6′), 8.73 (1H, d, J = 7.2 Hz, NH-CH(CH_3)), 9.5 (1H, bs, NH-phenyl) ppm. Anal.calcd. for $C_{16}H_{15}ClN_2O_2$. 1.25H_2O: C, 59.08; H, 5.42; N, 8.61. Found: C, 59.14; H, 5.36; N, 8.74.
3	^{1}H NMR (DMSO-d_6): δ 1.47 (3H, d, J = 7.2 Hz, CH(CH_3)), 2.19 (3H, s, Ar-CH_3), 4.66–4.69 (1H, m, CH(CH_3)), 7.07 (1H, t, J = 7.2 Hz, H-4), 7.14–7.21 (2H, m, H-3 and H-5), 7.40 (1H, d, J = 8.0 Hz, H-6), 7.47 (2H, td, J = 1.6; 7.4 Hz, H-3′ and H-5′), 7.52–7.56 (1H, m, H-4′), 7.92 (2H, dd, J = 1.4; 8.6 Hz, H-2′ and H-6′), 8.61 (1H, d, J = 6.8 Hz, NH-CH(CH_3)), 9.35 (1H, bs, NH-phenyl) ppm. Anal.calcd. for $C_{17}H_{18}N_2O_2$ .1H_2O: C, 67.98; H, 6.71; N, 9.33. Found: C, 67.91; H, 7.01; N, 9.31.
4	^{1}H NMR (DMSO-d_6): δ 1.42 (3H, d, J = 7.2 Hz, CH(CH_3)), 3.76 (3H, s, OCH_3), 4.68–4.71 (1H, m, CH(CH_3)), 6.89 (1H, td, J = 2.0; 7.4 Hz, H-3), 7.02 (2H, td, J = 1.8; 8.4 Hz, H-4 and H-5), 7.48 (2H, t, J = 7.4 Hz, H-3′ and H-5′), 7.53–7.56 (1H, m, H-4′), 7.89 (2H, dd, J = 1.2; 7.2 Hz, H-2′ and H-6′), 8.03 (1H, d, J = 7.2 Hz, H-6), 8.74 (1H, d, J = 6.8 Hz, NH-CH(CH_3)), 9.1 (1H, bs, NH-phenyl) ppm. Anal.calcd. for $C_{17}H_{18}N_2O_3$: C, 68.44; H, 6.08; N, 9.39. Found: C, 68.13; H, 6.08; N, 9.15.
5	^{1}H NMR ($CDCl_3$): δ 1.09 (3H, t, J = 7.4 Hz, CH_2CH_3), 1.47 (3H, d, J = 7.2 Hz, CH(CH_3)), 2.55–2.61 (2H, m, CH_2CH_3), 4.67–4.70 (1H, m, CH(CH_3), 7.12–7.17 (2H, m, H-4 and H-5), 7.21–7.23 (1H, m, H-3), 7.39 (1H, d, J = 7.6 Hz, H-6), 7.47 (2H, t, J = 7.4 Hz, H-3 and H-5′), 7.52–7.54 (1H, m, H-4′), 7.92 (2H, dd, J = 1.6; 8.0 Hz, H-2′ and H-6′), 8.62 (1H, d, J = 7.6 Hz, NH-CHCH_3), 9.32 (1H, bs, NH-phenyl) ppm. Anal.calcd. for $C_{18}H_{20}N_2O_2$: C, 72.95; H, 6.80; N, 9.45. Found: C, 72.84; H, 6.60; N, 9.44.
6	^{1}H NMR (DMSO-d_6): δ 1.12 (6H, d, J = 6.8 Hz, CH(CH_3)$_2$), 1.47 (3H, d, J = 7.2 Hz, CH(CH_3)), 3.13–3.19 (1H, m, CH(CH_3)$_2$), 4.68 (1H, m, CH(CH_3)), 7.13–7.20 (2H, m, H-4 and H-5), 7.29 (2H, td, J = 2.4; 8, H-3 and H-6), 7.47 (2H, t, J = 7.4, H-3′ and H-5′), 7.54 (1H, t, J = 7.2 Hz, H-4′), 7.93 (2H, d, J = 7.6 Hz, H-2′ and H-6′), 8.6 (1H, d, J = 7.2 Hz, NH-CH(CH_3)), 9.37 (1H, bs, NH-phenyl) ppm. Anal.calcd. for $C_{19}H_{22}N_2O_2$: C, 73.52; H, 7.14; N, 9.03. Found: C, 73.38; H, 7.42; N, 8.90.
7	^{1}H NMR (DMSO-d_6): δ 1.48 (3H, d, J = 7.2 Hz, CH(CH_3)), 2.12 (6H, s, 2xAr-CH_3), 4.59–4.62 (1H, m, CH(CH_3)), 7.03–7.04 (3H, m, H-3, H-4 and H-5), 7.45 (2H, t, J = 7.4 Hz, H-3′ and H-5′), 7.50–7.51(1H, m, H-4′), 7.90 (2H, dd, J = 1.6; 7.6 Hz, H-2′ and H-6′), 8.54 (1H, d, J = 6.4 Hz, NH-CH(CH_3)), 9.29 (1H, bs, NH-phenyl) ppm. Anal.calcd. for $C_{18}H_{20}N_2O_2$. 1H_2O: C, 68.48; H, 7.24; N, 8.93. Found: C, 68.77; H, 7.05; N, 8.91.
8	^{1}H NMR (DMSO-d_6): δ 2.62 (2H, t, J = 7.2 Hz, CH_2-CO), 3.55 (2H, q, J = 6.3 Hz, NH-CH_2), 7.02 (1H, t, J = 7.4 Hz, H-4), 7.28 (2H, t, J = 6.0 Hz, H-3 and H-5), 7.45 (2H, tt, J = 1.3; 7.4 Hz, H-2 and H-6), 7.49–7.51 (1H, m, H-4′), 7.57 (2H, d, J = 7.4 Hz, H-3′ and H-5′), 7.83 (2H, td, J = 1.6; 7.0 Hz, H-2′ and H-6′), 8.56 (1H, t, J = 8.0 Hz, NH-CH_2), 9.93 (1H, bs, NH-phenyl) ppm. Anal.calcd. for $C_{16}H_{16}N_2O_2$. 0.1H_2O: C, 71.08; H, 5.99; N, 10.36. Found: C, 71.45; H, 6.34; N, 9.91.
9	^{1}H NMR (DMSO-d_6): δ 2.63 (2H, t, J = 7.0 Hz, CH_2-CO), 3.56 (2H, q, J = 6.5 Hz, NH-CH_2), 7.17 (1H, td, J = 1.6; 7.8 Hz, H-4), 7.31 (1H, td, J = 1.6; 7.6 Hz, H-5), 7.43–7.48 (3H, m, H-3′, H-5′ and H-3), 7.50–7.54 (1H, m, H-4′), 7.69 (1H, d, J = 7.8 Hz, H-6), 7.84 (2H, dd, J = 1.2; 8.4 Hz, H-2′ and H-6′), 8.53 (1H, t, J = 5.0 Hz, NH-CH_2), 9.53 (1H, bs, NH-phenyl) ppm. Anal.calcd. for $C_{16}H_{15}ClN_2O_2$.0.6C_2H_6O: C, 62.40; H, 5.63; N, 8.47. Found: C, 62.04; H, 5.71; N, 8.87.
10	^{1}H NMR (DMSO-d_6): 2.14 (3H, s, Ar-CH_3), 2.62 (2H, t, J = 7.0 Hz, CH_2-CO), 3.54 (2H, q, J = 6.5 Hz, NH-CH_2), 7.05 (1H, t, J = 7.2 Hz, H-4), 7.11–7.17 (2H, m, H-3 and H-5), 7.36 (1H, d, J = 7.8 Hz, H-6), 7.46–7.42 (2H, m, H-3′ and H-5′), 7.48–7.52 (1H, m, H-4′), 7.82 (2H, dd, J = 1.6; 7.8 Hz, H-2′ and H-6′), 8.53 (1H, t, J = 11.4 Hz, NH-CH_2), 9.28 (1H, bs, NH-phenyl) ppm. Anal.calcd. for $C_{17}H_{18}N_2O_2$: C, 72.32; H, 6.43; N, 9.92. Found: C, 72.19; H, 6.11; N, 9.89.
11	^{1}H NMR (DMSO-d_6): δ 2.66 (2H, t, J = 6.6 Hz, CH_2-CO), 3.52 (2H, q, J = 5.8 Hz, NH-CH_2), 3.75 (3H, s, Ar-OCH_3), 6.87 (1H, td, J = 1.2; 7.9 Hz, H-5), 6.98–7.06 (2H, m, H-3 and H-4), 7.42–7.45 (2H, m, H-3′ and H-5′), 7.48–7.52 (1H, m, H-4′), 7.82 (2H, dd, J = 1.2; 8.0 Hz, H-2′ and H-6′), 7.91 (1H, d, J = 8.2 Hz, H-6), 8.48 (1H, t, J = 14 Hz, NH-CH_2), 9.07 (1H, bs, NH-phenyl) ppm. Anal.calcd. for $C_{17}H_{18}N_2O_3$: C, 68.44; H, 6.08; N, 9.39. Found: C, 68.23; H, 6.00; N, 9.42.
12	^{1}H NMR (DMSO-d_6): δ 1.18 (3H, t, J = 7.6 Hz, CH_2CH_3), 2.56 (2H, q, J = 7.6 Hz, CH_2CH_3), 2.77 (2H,t, J = 5.6 Hz, CH_2-CO), 3.84 (2H, q, J = 5.8 Hz, NH-CH_2), 7.15–7.26(5H, m, H-3, H-4, H-5; NH- phenyl and NH-CH_2), 7.38–7.43 (2H, m, H-3′ and H-5′), 7.50–7.46 (1H, m, H-6), 7.68 (1H, d, J = 7.8 Hz, H-4′), 7.77 (2H, dt, J = 1.6; 6.7 Hz, H-2′ and H-6′) ppm. Anal.calcd. for $C_{18}H_{20}N_2O_2$: C, 72.95; H, 6.80; N, 9.45. Found: C, 72.73; H, 6.76; N, 9.42.
13	^{1}H NMR (DMSO-d_6): 1.06 (6H, d, J = 7.0 Hz, 2xCH_3), 2.64 (2H, t, J = 6.6 Hz, CH_2-CO), 3.07–3.13 (1H, m, CH(CH_3)$_2$), 3.56 (2H, q, J = 6.5 Hz, NH-CH_2), 7.19–7.11 (2H, m, H-4 and H-5), 7.23 (1H, d, J = 7.4 Hz, H-3), 7.27 (1H, d, J = 7.4 Hz, H-6), 7.45 (2H, t, J = 7.1 Hz, H-3′and H-5′), 7.52 (1H, t, J = 7.4 Hz, H-4′), 7.85 (2H, d, J = 7.0 Hz, H-2′ and H-6′), 8.54 (1H, t, J = 6.0 Hz, NH-CH_2), 9.35 (1H, bs, NH-phenyl) ppm. Anal.calcd. for $C_{19}H_{22}N_2O_2$. 0.1H_2O: C, 73.03; H, 7.11; N, 8.96. Found: C, 72.81; H, 7.30; N, 8.86.
14	^{1}H NMR (DMSO-d_6): δ 2.10 (6H, s, 2xAr-CH_3), 2.64 (2H, t, J = 7.0 Hz, CH_2-CO), 3.57 (2H, q, J = 6.5 Hz, NH-CH_2), 7.03–7.04 (3H, m, H-3, H-4 and H-5), 7.44 (1H, t, J = 1.6 Hz, H-3′or H-5′), 7.46 (1H, dd, J = 1.6; 6.6 Hz, H-3′ or H-5′), 7.50–7.52 (1H, m, H-4′), 7.85 (2H, td, J = 1.6; 2.3 Hz, H-2′ and H-6′), 8.55 (1H, t, J = 6 Hz, NH-CH_2), 9.26 (1H, bs, NH-phenyl) ppm. Anal.calcd. for $C_{18}H_{20}N_2O_2$: C, 72.95; H, 6.80; N, 9.45. Found: C, 72.56; H, 6.39; N, 9.46.
15	^{1}H NMR (DMSO-d_6): 2.65 (2H, t, J = 7.0 Hz, CH_2-CO), 3.53 (2H, q, J = 6.8 Hz, NH-CH_2), 7.31 (1H, t, J = 8.2 Hz, H-4), 7.42–7.45 (3H, m, H-3′,H-4′ and H-5′), 7.48–7.50 (2H, m, H-3 and H-5), 7.82 (2H, d, J = 7.0 Hz, H-2′ and H-6′), 8.51 (1H, t, J = 14 Hz, NH-CH_2), 9.86 (1H, bs, NH- phenyl) ppm. Anal.calcd. for $C_{16}H_{14}Cl_2N_2O_2$: C, 56.99; H, 4.18; N, 8.31. Found: C, 56.86; H, 4.38; N, 8.42.
16	^{1}H NMR (DMSO-d_6): δ 2.65 (2H, t, J = 7.2 Hz, CH_2-CO), 3.53 (2H, q, J = 6.4 Hz, NH-CH_2), 7.31 (1H, t, J = 7.9 Hz, H-4), 7.41–7.45 (3H, m, H-3′, H-4′and H-5′), 7.48–7.50 (3H, m, H-3, H-5 and H-6), 7.82 (2H, d, J = 7.0 Hz, H-2′ and H-6′), 8.49 (1H, t, J = 9.9 Hz, NH-CH_2), 9.86 (1H, bs, NH-phenyl) ppm. Anal.calcd. for $C_{16}H_{15}N_3O_4$: C, 61.34; H, 4.83; N, 13.41 Found: C, 57.70; H, 4.54; N, 8.47.

Table 3 Anticonvulsant and neurotoxicity screening data of title compounds.

Compound	MES[a]						scMet[b]						Toxicity[c]					
	0.5 h (mg/kg)			4 h (mg/kg)			0.5 h (mg/kg)			4 h (mg/kg)			0.5 h (mg/kg)			4 h (mg/kg)		
	30	100	300	30	100	300	30	100	300	30	100	300	30	100	300	30	100	300
1	0/1	0/1	0/1	0/1	0/1	**1/1**	0/1	0/1	**1/1**	0/1	0/1	0/1	0/4	0/4	0/4	0/2	0/2	0/2
2	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/4	0/4	0/4	0/2	0/2	0/2
3	0/1	0/1	0/1	**1/1**	**1/1**	**1/1**	0/1	0/1	0/1	0/1	0/1	0/1	0/4	0/4	0/4	0/2	0/2	0/2
4	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/4	0/4	0/4	0/2	0/2	0/2
5	0/1	0/1	0/1	0/1	0/1	0/1	0/1	**1/1**	**1/1**	0/1	0/1	0/1	0/4	0/4	0/4	0/2	0/2	0/2
6	0/1	0/1	0/1	0/1	0/1	**1/1**	0/1	0/1	0/1	0/1	0/1	0/1	0/4	0/4	0/4	0/2	0/2	0/2
7	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	**1/1**	0/1	0/1	0/1	0/4	0/4	0/4	0/2	0/2	0/2
8	0/1	0/1	**1/1**	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/4	0/4	0/4	0/2	0/2	0/2
9	0/1	0/1	0/1	0/1	0/1	**1/1**	0/1	0/1	0/1	0/1	0/1	0/1	0/4	0/4	0/4	0/2	0/2	0/2
10	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/4	0/4	0/4	0/2	0/2	0/2
11	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	**1/1**	0/1	0/1	0/1	0/4	0/4	0/4	0/2	0/2	0/2
12	0/1	0/1	0/1	0/1	0/1	0/1	**1/1**	**1/1**	**1/1**	0/1	0/1	0/1	0/4	0/4	0/4	0/2	0/2	0/2
13	0/1	0/1	0/1	0/1	0/1	0/1	**1/1**	**1/1**	**1/1**	0/1	0/1	0/1	0/4	0/4	0/4	0/2	0/2	0/2
14	0/1	0/1	0/1	0/1	0/1	0/1	0/1	**1/1**	**1/1**	0/1	0/1	0/1	0/4	0/4	0/4	0/2	0/2	0/2
15	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	**1/1**	0/1	0/1	0/1	0/4	0/4	0/4	0/2	0/2	0/2
16	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/4	0/4	0/4	0/2	0/2	0/2

[a] maximal electroshock test; [b] subcutaneous metrazole test; [c] rotarod test, **0/1**: No activity at dose level; **1/1**: noticeable activity at dose level; **0/4**: number of animals exhibited toxicity/number of animals tested for 05 h; **0/2**: number of animals exhibiting toxicity/number of animals tested for 4 h

Results and Discussion

Chemistry

In this study, sixteen 2/3-benzoylaminopropionanilide derivatives have been synthesized to evaluate anticonvulsant activity profile. The synthesis of the title compounds was realized in 2 steps. For this purpose, in the first step, 2/3-aminopropionanilide hydrochlorides were prepared according to the reported procedures [28–32]. In the second step, those intermediates were reacted with benzoyl chloride to furnish title compounds [33]. The synthetic pathways are given in **Fig. 2, 3**. The structures of the compounds were confirmed by spectral (IR, ^{1}H NMR, and API-ES Mass) and elemental analyses (**Table 1, 2**). The yields and melting points are summarized in **Table 1**. According to the literature survey, compunds **1** and **8** are reported derivatives [34,35] and **2–6, 9–14** are listed substances in the literature with the CAS registry numbers 72029-13-1, 1268331-32-3, 1266377-87-0, 1266366-95-3, 1266374-66-6, 1266376-33-3, 108679-33-0, 909513-33-3, 909513-87-7, 909512-98-7, 178566-26-8, 909512-08-9 and 909512-89-6, respectively, but corresponding scientific reference data are not available for those compounds.

The purity levels of compounds were determined by elemental analyses (C, H, N) and results were within 0.4% of the calculated values except for compound **16** (**Table 2**).

In the IR spectra, the presence of C=O stretching bands were the confirmative signals for the constructed functional groups in the title compounds. For instance, amide C=O stretching bands of anilide and benzamide groups were observed between 1691–1643 and 1620–1651 cm^{-1}, respectively. N-H strecthing bands of the secondary amide structure of the title compounds were observed between 3 484 and 3 056 cm^{-1} (**Table 1**), [36,37].

^{1}H NMR spectra of the title compounds were consistent with expected resonance signals in term of chemical shifts and integrations. The chemical shifts and splitting patterns of *N*-phenyl protons in each compound differed depending on the nature of the substituents (**Table 2**).

The structure of the title compounds was further verified by API-ES Mass spectra where the m/z values of molecular ion peaks were in complete agreement with the calculated molecular weight for individual compounds (**Table 1**).

Anticonvulsant Activity Screening

The preclinical discovery and development of new chemical agents for the treatment of epilepsy are based mainly on the use of animal models. At the present time, there are 3 in-vivo screening methods used routinely, namely maximal electroshock (MES) seizure, the subcutaneous metrazole (scMet), and the kindled model. The MES and scMet screening tests are claimed to predict the potential protection activity against generalized tonic-clonic seizures and generalized absence seizures, respectively and those tests recognized as the "gold standards" in the early stages of anticonvulsant testing protocole according to the Antiepileptic Drug Development (ADD) Program of National Institute of Health (NIH). Therefore, the anticonvulsant activities of the title compounds were evaluated against MES and scMet tests by the standard procedures of utilized by the NINDS Anticonvulsant Screening Program (ASP) [38].

The title compounds were tested after intraperitoneal (i.p.) injection to mice at doses of 30, 100 and 300 mg/kg. An observation was carried out at 2 different time intervals (0.5 h and 4 h). The rotarod test was used to demonstrate the possible neurotoxicity. None of the compounds showed neurotoxicity at studied doses. Preliminary screening results are presented in **Table 3**. The anticonvulsant activities of the title compounds are reported for the first time in this study.

The results indicated that majority of compounds were effective in MES or scMet tests. In the whole series only compound 1 was active in both tests, whereas compounds 2, 4, 10 and 16 were found inactive in both models.

According to the MES test results, compounds 1, 3, 6, 8 and 9 were active in the MES test indicating the ability to prevent seizure spread. Compound 8 showed protection at 300 mg/kg after 0.5 h, whereas compounds 1, 6 and 9 were active at same dose after 4 h. Compound 3 was the most active compound of the series against the MES test at the dose of 30 mg/kg at time periods 4 h. The delayed anticonvulsant activity of compounds 1, 3,

6 and 9 at 4 h could be associated with their absorbtion and distribution characteristics and long duration of action.

Compounds 1, 5, 7, 11, 12, 13, 14 and 15 were found to be active after 0.5 h in the scMet test indicating the ability to elevate seizure threshold. Compounds 12 and 13 were most active compounds in the scMet test. Compounds 1, 7, 11 and 15 showed activity at a dose of 300 mg/kg while compounds 5 and 14 exhibited activity at doses of 100 and 300 mg/kg. All of active compounds in scMet screening test displayed rapid onset and shorter duration of action.

Under the set of the compounds studied, the most active compound in the both series against MES test was aroused from the 2-benzoylaminopropionanilide structure, whereas the most active ones in scMet test were originated from the 3-benzoylaminopropionanilide (see compound 3 and 12, 13 respectively in **Table 3**). According to the contribution of the substituent on *N*-phenyl ring in both series, mono alkyl substitutions seem to yield superior compounds.

Conclusion

A series of 2/3-benzoylaminopropionanilide derivatives were designed, synthesized and their anticonvulsant activities were evaluated. As a result, 2 general trends may be summarized for both series. First, majority of the compounds synthesized had greater activity at the 0.5 h than 4 h and secondly, they showed greater activity in the scMet test rather than MES test.

As observed through biological data analysis, under the set of *ortho* mono substituent studied on *N*-phenyl ring, compounds with methyl (**3**), ethyl (**12**) and isopropyl (**13**) substitutions were found to be more potent in the MES or scMet tests. Those compounds emerged as lead compounds for future investigations.

Conflict of Interest

The authors report no conflicts of interest.

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