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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Synthesis and anticonvulsant activity of some *N*-(benzoyl)glycinanilide derivatives

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Abstract Glycine is a major inhibitory neurotransmitter and recent studies have shown that certain lipophilic glycine derivatives demonstrate anticonvulsant activity in animal epilepsy models. On the other hand, anilide is another fruitful structure for designing potential anticonvulsant agents. Ameltolide, ralitoline and some phthalimide derivatives are the examples of anilide analogs with potent anticonvulsant activity. In this study, two key structural pharmacophores were combined and a series of *N*-benzoylglycinanilide derivatives were designed. Their anticonvulsant activities evaluated against maximal electroshock (MES) and subcutaneous metrazole seizure tests, whereas their neurotoxicity was examined by rotarod test. The preliminary screening results indicated that majority of the compounds were effective in the MES test. None of the compounds showed neurotoxicity according to the rotarod test at studied doses. The most active compound in the series is *N*-(2-((4-methoxyphenyl)amino)-2-oxoethyl)benzamide (compound **8**) which bearing 4-methoxy substituent on the *N*-phenyl ring.

Keywords Anilide · Glycine · Glycinamide · Anticonvulsant activity · Amide synthesis

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Introduction

Epilepsy is a chronic neurologic disorder characterized by recurrent unprovoked seizures which results from a temporary electrical disturbance of the brain due to an imbalance between excitatory and inhibitory neurotransmitters. About 50 million people world wide have epilepsy. Currently, the main treatment for epileptic disorder is the long term and consistent administration of anticonvulsant drugs. Although over 30 AEDs are available, approximately 30 % of epileptic patients are not seizure free. In many cases, the clinical use of AEDs is restricted by their side effects. Therefore, a substantial need remains to discover novel chemical entities for the development of new more effective and safer AEDs (McNamara, 2001; Löscher and Schmidt, 2006; Lin and Kabada, 1997; Malawska, 2005).

Many of the newer antiepileptic drugs were designed through structural modifications of available therapeutics and were developed with a specific aim to modify neurotransmitter function such as enhancement of inhibitory mediated neurotransmission or inhibition of excitatory-mediated neurotransmission (Malawska, 2005; Czabinski *et al.*, 2005). It is also known that the imbalance between inhibitory and excitatory processes leads to epileptic seizures in the brain. Therefore, attenuation of excitation and enhancement of inhibition in the brain are the prevalent goals in designing new anticonvulsant agents. GABAergic and glutamatergic systems and their receptor sites can be given as examples of those targets (Löscher, 1998; Porter, 1993; Fraser, 1996; Kohl and Dannhardt, 2001).

Glycine is one of the major inhibitory neurotransmitter which plays an important role in the control of neuronal activity in the central nervous system (CNS). Simultaneously, glycine acts as a co-agonist of the excitatory

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neurotransmitter glutamate by interacting with the strychnine-insensitive glycine binding site of the *N*-methyl-D-aspartate receptor complex. A deficiency in brain glycine levels has been shown to cause convulsions or epilepsy. Under normal physiologic conditions, glycine does not easily cross the blood–brain barrier (BBB) due to zwitterionic characteristic and the absence of an active transport mechanism. Thus, intracerebroventricular or systemic administrations of large doses of glycine are necessary for significant increases in brain glycine levels. Since the entry of glycine into the brain is negligible, several lipophilic glycine derivatives that can cross the BBB have been developed. These derivatives are milacemide, remacemide, phthaloyl glycinamide, valproyl glycinamide, and *N*-benzyloxycarbonyl glycine and its ester and amide derivatives. Some of the derivatives mentioned above have potential to become new antiepileptic and CNS drugs. Milacemide and remacemide have been undergoing clinical trials in patients with epilepsy (Fig. 1) (Sussan *et al.*, 1999; Scriba and Lambert, 1999; Geurts *et al.*, 1998; Hadad and Bialer, 1995; Salach *et al.*, 1994; Usifoh *et al.*, 2001; Dawidowski *et al.*, 2011).

On the other hand, anilide group has been observed as a productive pharmacophoric structure for the potent anticonvulsant molecules in recent studies (Clark *et al.*, 1985; Bailleux *et al.*, 1995; Vamecq *et al.*, 2000; Soyer *et al.*, 2003; Soyer *et al.*, 2004).

Depending on the data described above, glycine and anilide moieties were combined to design a series of *N*-benzoylglycinanilide derivatives which can be accepted as a open chain analogs of phthaloylglycinamides with known anticonvulsant activity. Accordingly, the synthesis and preliminary anticonvulsant activity evaluation of designed *N*-benzoylglycinanilide derivatives are reported.

Experimental

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 (Jasco, Tokyo, Japan) and Perkin Elmer FT-IR Spectrometer 100 (Perkin Elmer Inc., Massachusetts, USA). The NMR spectra were recorded on a Varian As 400 Mercury Plus NMR (Varian Inc., Palo Alto, CA, USA) spectrophotometer using DMSO-d_6 as solvent. Chemical shifts were reported in parts per million (δ). J was 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 theoretic values.

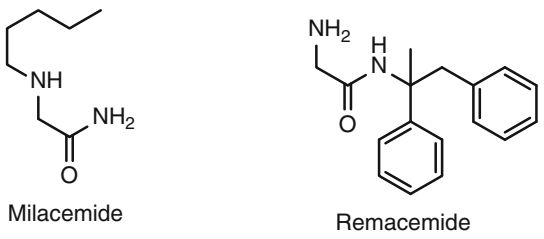


Fig. 1 Milacemide and remacemide

General procedure for the synthesis of glycinanilide intermediates (**1a–11a**)

Compounds **1a–11a** were prepared according to the method reported in the literature (Ho *et al.*, 1998). For this purpose, *N*-(*tert*-butoxycarbonyl)-glycine (*N*-Boc-glycine, 9.6 mmol) and *N*-methylmorpholine (NMM, 9.6 mmol) were dissolved in DMF (10 mL). The solution was cooled to −20 °C and isobutyl chloroformate (IBCF, 9.6 mmol) was added. After stirring for 10 min at −20 °C, aniline or substituted anilines (9.6 mmol) were added and the reaction mixture was stirred at room temperature for 4 h. Then, the precipitated NMM hydrochloride was filtered off. The solvent was evaporated under reduced pressure and the residue was partitioned between ethyl acetate (50 mL) and water (50 mL). The ethyl acetate phase was washed with 5 % $NaHCO_3$ (50 mL), dried over anhydrous Na_2SO_4 and evaporated under reduced pressure. Trifloroacetic acid (TFA, 1 mL) was added to the residue and stirred for 30 min. The solution was treated with petroleum ether–ether (1:1) and stirred for 30 min at room temperature. The resulting precipitate was filtered and dissolved in water (5 mL). The solution was basified with 10 % NaOH and extracted with dichloromethane (20 mL) twice. The dichloromethane phase was washed with water (20 mL), dried over anhydrous Na_2SO_4, filtered, and evaporated under reduced pressure. The crude product was crystallized from ethanol.

General procedure for the synthesis of title compounds (**1–11**)

Benzoic acid or substituted benzoic acids (1.3 mmol) were refluxed in 5 mL thionyl chloride for 1 h and excess thionyl chloride was removed in vacuo. The residue was dissolved in dichloromethane (20 mL) and a solution of appropriate amine (**1a–11a**) in dichloromethane (5 mL) at 0–4 °C was added dropwise. After the addition was completed, the mixture was stirred in an ice bath for 30 min, at room temperature, for a further 2 h and washed with 10 %

HCl (15 mL), 10 % NaOH (15 mL), and water (20 mL). The organic phase was dried over anhydrous Na_2SO_4 and then evaporated under reduced pressure. The precipitate was crystallized from ethanol:water (1:1) to furnish title compounds.

*N-(2-oxo-2-(phenylamino)ethyl)benzamide (**1**)*

Yield 26 %; mp 216 °C (lit.[24] 213–215 °C); IR υ_{maks} (KBr): 3274, 3139, 1683, 1639, 1540, 1492 cm^{-1}; ^1H NMR (DMSO-d_6): δ 4.05 (2H, d, $J = 5.6$ Hz, CH_2), 7.03 (1H, td, $J = 1.2$; 7.4 Hz, H-4), 7.29 (2H, td, $J = 2.0$; 8.0 Hz, H-3 and H-5), 7.45–7.49 (2H, m, H-2 and H-6), 7.52–7.56 (1H, m, H-4′), 7.58–7.60 (2H, m, H-3′ and H-5′), 7.90 (2H, dd, $J = 1.6$; 7.0 Hz, H-2′ and H-6′), 8.79 (1H, *t*, $J = 5.5$ Hz, N<u>H</u>–CH_2), 10.01 (1H, bs, N<u>H</u>–phenyl); ^{13}C NMR (DMSO-d_6): δ 43.6, 119.9, 123.9, 128.0, 129.0, 129.4, 132.1, 134.7, 139.6, 167.3, 168.5 ppm; (MS (API-ES) *m/z* (%): 255 ($M+H^+$, 70); Anal. calcd. for $C_{15}H_{14}N_2O_2$: C, 70.85; H, 5.55; N, 11.02. Found: C, 70.69; H, 5.32; N, 11.05.

*N-(2-(2-chlorophenylamino)-2-oxoethyl)benzamide (**2**)*

Yield 77 %; mp 179 °C; IR υ_{maks} (KBr): 3274, 3110, 1679, 1643, 1538, 1488 cm^{-1}; ^1H NMR (DMSO-d_6): δ 4.12 (2H, d, $J = 6.2$ Hz, CH_2), 7.16 (1H, td, $J = 1.6$; 7.7 Hz, H-4), 7.32 (1H, td, $J = 1.4$; 7.7 Hz, H-5), 7.46–7.56 (4H, m, H-3, H-3′, H-4′ and H-5′), 7.82 (1H, dd, $J = 1.6$; 8.2 Hz, H-6), 7.88 (2H, dd, $J = 1.2$; 7.0 Hz, H-2′ and H-6′), 8.90 (1H, *t*, $J = 5.9$ Hz, N<u>H</u>–CH_2), 9.49 (1H, bs, N<u>H</u>–phenyl); ^{13}C NMR (DMSO-d_6): δ 44.0, 125.7, 126.3, 126.7, 128.0, 128.2, 129.0, 130.1, 132.2, 134.5, 135.4, 167.5, 169.0 ppm; MS (API-ES) *m/z* (%): 287($M-H^-$, 100), 289($M-H+2$, 35); Anal. calcd. for $C_{15}H_{13}ClN_2O_2$: C, 62.40; H, 4.54; N, 9.70. Found: C, 62.25; H, 4.54; N, 9.76.

*N-(2-oxo-2-(o-tolylamino)ethyl)benzamide (**3**)*

Yield 22 %; mp 195 °C; IR υ_{maks} (KBr): 3268, 3050, 1673, 1641, 1542, 1490 cm^{-1}; ^1H NMR (DMSO-d_6): δ 2.19 (3H, s, CH_3), 4.02 (2H, d, $J = 5.6$ Hz, CH_2), 7.06 (1H, *t*, $J = 7.4$ Hz, H-4), 7.13–7.20 (2H, m, H-5 and H-6), 7.41–7.55 (4H, m, H-3, H-3′, H-4′ and H-5′), 7.89 (2H, dd, $J = 1.2$; 8.2 Hz, H-2′ and H-6′), 8.90 (1H, *t*, $J = 5.9$ Hz, N<u>H</u>–CH_2), 9.49 (1H, bs, N<u>H</u>–phenyl); ^{13}C NMR (DMSO-d_6): δ 18.4, 43.9, 125.4, 125.8, 126.6, 128.0, 129.0, 131.0, 132.1, 132.5, 134.7, 136.8, 167.4, 168.6 ppm; MS (API-ES) *m/z* (%):267 ($M-H^-$, 100); Anal. calcd. for $C_{16}H_{16}N_2O_2$: C, 71.62; H, 6.01; N, 910.44. Found: C, 71.50; H, 5.89; N, 10.39.

*N-(2-(2-methoxyphenylamino)-2-oxoethyl)benzamide (**4**)*

Yield 67 %; mp 106 °C; IR υ_{maks} (KBr): 3330, 3056, 1681, 1648, 1538, 1486 cm^{-1}; ^1H NMR (DMSO-d_6): δ 3.78 (3H, s, OCH_3), 4.09 (2H, d, $J = 5.5$ Hz, CH_2), 6.89 (1H, *t*, $J = 7.4$ Hz, H-4), 7.01–7.05 (2H, m, H-3 and H-5), 7.47–7.55 (3H, m, H-3′, H-4′ and H-5′), 7.90 (2H, dd, $J = 1.2$; 7.6 Hz, H-2′ and H-6′), 8.02 (1H, d, $J = 7.8$ Hz, H-6), 8.92 (1H, bs, N<u>H</u>–CH_2), 9.14 (1H, bs, N<u>H</u>–phenyl); ^{13}C NMR (DMSO-d_6): δ 44.3, 56.4, 111.8, 121.1, 124.9, 127.8, 128.0, 129.1, 132.2, 134.6, 150.0, 167.6, 168.5 ppm; MS (API-ES) *m/z* (%):285 ($M+H^+$, 73); Anal. calcd. for $C_{16}H_{16}N_2O_3$: C, 67.59; H, 5.67; N, 9.85. Found: C, 67.37; H, 5.61; N, 9.79.

*N-(2-(2-ethylphenylamino)-2-oxoethyl)benzamide (**5**)*

Yield 32 %; mp 187 °C; IR υ_{maks} (KBr): 3255, 3089, 1668, 1639, 1536, 1490 cm^{-1}; ^1H NMR ($CDCl_3$): δ 1.09 (3H, *t*, $J = 7.4$ Hz, CH_3), 2.57 (2H, q, $J = 7.4$; 15.0 Hz, C<u>H</u>$_2$$CH_3$), 4.08 (2H, d, $J = 5.1$ Hz, CH_2), 7.11–7.22 (3H, m, H-4, H-5 and H-6), 7.39–7.53 (4H, m, H-3, H-3′, H-4′ and H-5′), 7.90 (2H, dd, $J = 1.2$; 7.6 Hz, H-2′ and H-6′), 8.83 (1H, *t*, $J = 5.5$ Hz, N<u>H</u>–CH_2), 9.29 (1H, bs, N<u>H</u>–phenyl); ^{13}C NMR (DMSO-d_6): δ 14.9, 24.4, 43.9, 126.1, 126.2, 126.6, 128.0, 129.0, 129.2, 132.1, 134.7, 136.1, 138.2, 167.4, 168.9 ppm; MS (API-ES) *m/z* (%):281 ($M-H^-$, 100); Anal. calcd. for $C_{17}H_{18}N_2O_2$: C, 72.32; H, 6.43; N, 9.92. Found: C, 72.34; H, 6.31; N, 9.95.

*N-(2-(2-isopropylphenylamino)-2-oxoethyl)benzamide (**6**)*

Yield 17 %; mp 120 °C; IR υ_{maks} (KBr): 3262, 3089, 1666, 1639, 1527, 1486 cm^{-1}; ^1H NMR (DMSO-d_6): δ 1.11 (6H, d, $J = 6.6$ Hz, $2 \times CH_3$), 3.13-3.17 (1H, m, CH), 4.08 (2H, d, $J = 5.5$ Hz, CH_2), 7.14–7.17 (2H, m, H-4 and H-5), 7.28–7.30 (2H, m, H-3 and H-6), 7.45–7.49 (2H, m, H-3′ and H-5′), 7.51–7.55 (1H, m, H-4′), 7.89 (2H, dd, $J = 1.6$; 7.0 Hz, H-2′ and H-6′), 8.82 (1H, *t*, $J = 5.6$ Hz, N<u>H</u>–CH_2), 9.35 (1H, bs, N<u>H</u>–phenyl); ^{13}C NMR (DMSO-d_6): δ 23.9, 27.7, 43.8, 126.2, 126.4, 126.8, 127.1, 128.0, 129.0, 132.1, 134.7, 135.3, 143.6, 167.4, 169.1 ppm; MS (API-ES) *m/z* (%):295 ($M-H^-$, 100); Anal. calcd. for $C_{18}H_{20}N_2O_2$: C, 72.95; H, 6.80; N, 9.45. Found: C, 73.16; H, 6.77; N, 9.50.

*N-(2-(2,6-dimethylphenylamino)-2-oxoethyl)benzamide (**7**)*

Yield 80 %; mp 224 °C; IR υ_{maks} (KBr): 3417, 3249, 1668, 1627, 1569, 1536 cm^{-1}; ^1H NMR (DMSO-d_6): δ 2.13 (6H, s, $2 \times CH_3$), 4.05 (2H, d, $J = 5.9$ Hz, CH_2), 7.03–7.04 (3H, m, H-3, H-4 and H-5), 7.45 (2H, d, $J = 7.0$ Hz, H-3′ and H-5′), 7.48–7.55 (1H, m, H-4′), 7.89 (2H, dd, $J = 1.6$;

7.0 Hz, H-2′ and H-6′), 8.83 (1H, *t*, $J = 5.9$ Hz, $CH_2N\underline{H}$), 9.27 (1H, bs, N<u>H</u>–phenyl); ^{13}C NMR (DMSO-d_6): δ 18.7, 43.5, 127.1, 128.1, 128.3, 128.9, 132.0, 134.8, 135.7, 136.1, 167.4, 168.4 ppm; MS (API-ES) *m/z* (%): 283 ($M+H^+$, 100); Anal. calcd. for $C_{17}H_{18}N_2O_2$. $1H_2O$: C, 68.00; H, 6.67; N, 9.33. Found: C, 67.91; H, 6.99; N, 9.34.

*N-(2-((4-methoxyphenyl)amino)-2-oxoethyl)benzamide (**8**)*

Yield 30 %; mp 227 °C (lit.[26] 217–218 °C); IR υ_{maks} (KBr): 3269, 3057, 1675, 1639, 1538, 1515 cm^{-1}; ^1H NMR (DMSO-d_6): δ 3.72 (3H, s, OCH_3), 4.03 (2H, d, $J = 5.9$ Hz, CH_2), 6.88 (2H, d, $J = 9.0$ Hz, H-3 and H-5), 7.47–7.57 (5H, m, H-2′–H-6′), 7.89 (2H, d, $J = 8.6$ Hz, H-2 and H-6), 8.80 (1H, *t*, $J = 5.9$ Hz, $CH_2N\underline{H}$), 9.89 (1H, bs, N<u>H</u>–phenyl); ^{13}C NMR (DMSO-d_6): δ 43.9, 55.8, 114.6, 121.4, 128.0, 129.0, 132.0, 132.8, 134.7, 155.9, 167.3, 168.0 ppm; MS (API-ES) *m/z* (%): 285 ($M+H^+$, 100); Anal. calcd. for $C_{16}H_{16}N_2O_3$. $0.4H_2O$: C, 65.86; H, 5.76; N, 9.60. Found: C, 66.00; H, 5.83; N, 9.53.

*2-Chloro-N-(2-oxo-2-(phenylamino)ethyl)benzamide (**9**)*

Yield 40 %; mp 182 °C; IR υ_{maks} (KBr): 3255, 3062, 1691, 1645, 1551, 1498 cm^{-1}; ^1H NMR (DMSO-d_6): δ 4.03 (2H, d, $J = 5.9$ Hz, CH_2), 7.04 (1H, *tt*, $J = 1.2$; 7.4 Hz, H-4), 7.30–7.34 (2H, m, H-3 and H-5), 7.40–7.54 (4H, m, H-2, H-6, H-3′ and H-5′), 7.59–7.61 (2H, m, H-4′ and H-6′), 8.70 (1H, *t*, $J = 5.9$ Hz, $CH_2N\underline{H}$), 10.01 (1H, bs, N<u>H</u>–phenyl); ^{13}C NMR (DMSO-d_6): δ 43.7, 119.8, 124.0, 127.7, 129.5, 130.0, 130.4, 130.7, 131.7, 137.0, 140.0, 167.4, 168.0 ppm; MS (API-ES) *m/z* (%): 289 ($M+H^+$, 49), 291(M+H+2, 18); Anal. calcd. for $C_{15}H_{13}ClN_2O_2$: C, 62.40; H, 4.54; N, 9.70. Found: C, 62.27; H, 4.22; N, 9.84.

*4-Methyl-N-(2-oxo-2-(phenylamino)ethyl)benzamide (**10**)*

Yield 33 %; mp 222 °C; IR υ_{maks} (KBr): 3269, 1680, 1641, 1599, 1543 cm^{-1}; ^1H NMR (DMSO-d_6): 2.35 (3H, s, CH_3), 4.03 (2H, d, $J = 5.9$ Hz, CH_2), 7.02 (1H, *t*, $J = 7.4$ Hz, H-4), 7.26–7.30 (4H, m, H-2, H-3, H-5 and H-6), 7.58 (2H, d, $J = 7.8$ Hz, H-3′ and H-5′), 7.79 (2H, d, $J = 7.8$ Hz, H-2′ and H-6′), 8.72 (1H, *t*, $J = 5.9$ Hz, $CH_2N\underline{H}$), 10.02 (1H, bs, N<u>H</u>–phenyl); ^{13}C NMR (DMSO-d_6): δ 21.6, 43.9, 119.8, 123.9, 128.0, 129.4, 129.5, 131.9, 139.6, 142.0, 167.2, 168.6 ppm; MS (API-ES) *m/z* (%):269 ($M+H^+$, 29); Anal. calcd. for $C_{16}H_{16}N_2O_2$: C, 71.62; H, 6.01; N, 10.44. Found: C, 71.25; H, 5.83; N, 10.33.

*4-Nitro-N-(2-oxo-2-(phenylamino)ethyl)benzamide (**11**)*

Yield 59 %; mp 219 °C (lit.[24] 213.5–215.5 °C); IR υ_{maks} (KBr): 3342, 3263, 1684, 1645, 1599, 1547 cm^{-1}; ^1H NMR (DMSO-d_6): δ 4.09 (2H, d, $J = 5.5$ Hz, CH_2), 7.03 (1H, *t*, $J = 7.4$ Hz, H-4), 7.29 (2H, *t*, $J = 7.8$ Hz, H-3 and H-5), 7.58 (2H, d, $J = 8.8$ Hz, H-2 and H-6), 8.12 (2H, d, $J = 8.9$ Hz, H-3′and H-5′), 8.33 (2H, d, $J = 8.9$ Hz, H-2′ and H-6′), 9.18 (1H, *t*, $J = 5.9$ Hz, $CH_2N\underline{H}$), 10.07 (1H, bs, N<u>H</u>–phenyl); ^{13}C NMR (DMSO-d_6): δ 44.1, 119.9, 124.0, 124.3, 129.4, 129.5, 139.6, 140.3, 149.8, 165.8, 168.1 ppm; MS (API-ES) *m/z* (%): 300 ($M+H^+$, 100); Anal. calcd. for $C_{15}H_{13}N_3O_4$: C, 60.20; H, 4.38; N, 14.04. Found: C, 60.00; H, 4.05; N, 13.95.

Anticonvulsant activity screening

The compounds were tested for their anticonvulsant activity against maximal electroshock (MES) and subcutaneous Metrazole (scMet)-induced seizure threshold tests. The acute neurologic 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 Neurologic Disorders, and Stroke (NINDS) (Krall *et al.*, 1978). Stimulator (Grass S88, Astro-Med. Inc. Grass Instrument Division, W. Warwick, RI, USA), constant current unit (Grass CCU1A, Grass Medical Instrument, Quincy, MA, USA), and corneal electrodes were used for the evaluation of anticonvulsant activity against MES test. All the synthesized compounds were suspended in 30 % aqueous of PEG 400 and administered to the mice intraperitoneally in a volume of 0.01 mL g^{-1} at body weight. Twelve 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 animals 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).

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 elicit 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.

scMet-induced seizure test

85 mg kg^{-1} 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 threshold 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. Neurologic toxicity was defined as the failure of the animal to remain on the rod for 1 min.

Results and discussion

Chemistry

In this study, eleven *N*-benzoylglycinanilide derivatives have been synthesized to evaluate anticonvulsant activity profile. The target compounds were prepared by a two-step synthesis. In the first step, anilides were prepared by reacting *N*-Boc-glycine with substituted anilines or aniline and then, Boc group was removed from molecules (Ho *et al.*, 1998). In the second step, those intermediates were reacted with appropriate benzoyl chlorides to furnish the target compounds (Pabuccuoglu and Hesse, 1997). The synthetic pathways are given in Scheme 1. The structures of the synthesized compounds were confirmed by spectral (IR, ^{1}H NMR, ^{13}C NMR, and API-ES Mass) and elemental analyses.

According to the literature survey, compounds **1**, **3**, **4**, **8**, and **10** are reported derivatives (Fox and Wax, 1950; Albertson, 1951; Abernathy *et al.*, 1958; Bird and Twibell, 1971) and **2**, **5**–**7**, and **9** are listed substances in the literature with the CAS registry numbers 941476-87-5, 1017100-89-8, 1089403-42-8, 747411-49-0, and 903348-90-3, 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.

In the IR spectra, the presence of C=O stretching bands were the confirmative signals for the constructed functional groups in the title compounds (Hesse *et al.*, 1997; Nakanishi and Solomon, 1977). For instance, amide C=O stretching bands of anilide and benzamide groups were observed between 1666–1691 and 1627–1648 cm^{-1}, respectively. N–H stretching bands of the secondary amide structure of the title compounds were observed between 3050 and 3417 cm^{-1}.

^{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 phenyl protons in each compound differed depending on the nature of the substituents.

^{13}C NMR spectra, the resonance signals did not account for the total number of carbon atoms in individual compounds since as expected certain signals represent more than one carbon atom.

The structure of the title compounds was further verified by API-ES Mass spectra. The spectra of the compounds were obtained by positive or negative ionization and the *m*/*z* values of molecular ion peaks were in complete agreement with the calculated molecular weight.

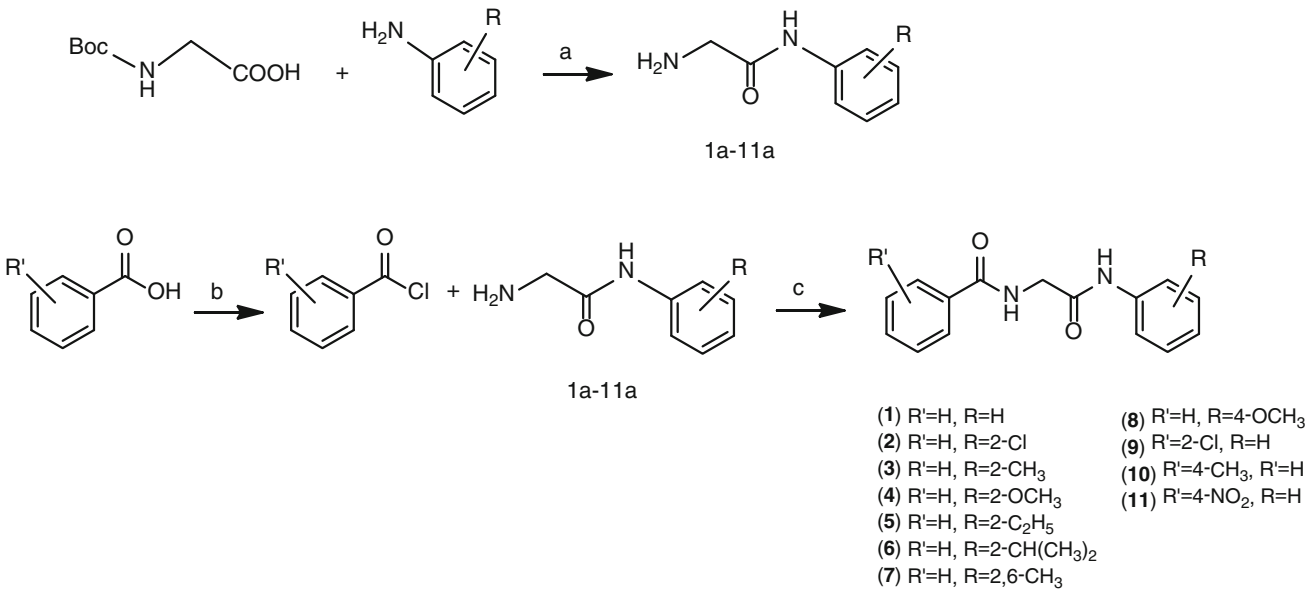
Anticonvulsant activity screening

Preliminary anticonvulsant evaluation (Phase 1) of the synthesized compounds was performed by following the standard procedure provided by the antiepileptic drug development program. The initial evaluation includes qualitative assays by MES and scMet seizure tests, the most widely employed two seizure models for early identification of candidate anticonvulsants. The MES test generally accepted as a model which employs an electrical stimulus to induce generalized tonic–clonic seizures and identifies compounds preventing the spread of seizures. The scMet test, producing myoclonic seizures induced chemically, is capable recognizing the agents that act by raising the seizure threshold. The rotarod test was used to determine the possible neurotoxic effects of the compounds under anticonvulsant screening.

Anticonvulsant activity profile of synthesized compounds was established after intraperitoneally (i.p) injections into mice and evaluated in the MES, scMet at doses of 30, 100, and 300 mg kg^{-1} at two different time intervals (0.5 and 4 h). Neurotoxicity of the compounds was determined at same doses by rotarod test. The results are shown in Table 1.

The initial anticonvulsant screening results indicated that all the compounds, except compounds **2**, **4**, and **5**, were effective in MES or scMet tests. None of the compounds showed toxicity in the rotarod neurotoxicity screening at doses studied.

Compounds **1**, **3**, **6**–**8,** and **9** exhibited activity in the MES test. Among these compounds, **1**, **3**, and **6** showed activity at doses of 300 mg kg^{-1} at 0.5 and they lost their



Scheme 1 Syntheses of the compounds **1–11**. *a* NMM, IBCF, rt, 4 h; TFA, rt, 30 min; 10 % NaOH, CH_2Cl_2; *b* $SOCl_2$, reflux, 1 h; *c* CH_2Cl_2, 0–4 °C, 30 min; rt 2 h

Table 1 Phase 1 anticonvulsant and neurotoxicity screening data of title compounds

Compound	MES[a]						scMet[b]						Toxicity[c]					
	0.5 h (mg kg^{-1})			4 h (mg kg^{-1})			0.5 h (mg kg^{-1})			4 h (mg kg^{-1})			0.5 h (mg kg^{-1})			4 h (mg kg^{-1})		
	30	100	300	30	100	300	30	100	300	30	100	300	30	100	300	30	100	300
1	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
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	**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
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	0/1	0/1	0/1	0/1	0/1	0/4	0/4	0/4	0/2	0/2	0/2
6	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
7	0/1	0/1	0/1	0/1	**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
8	0/1	**1/1**	**1/1**	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
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	**1/1**	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	**1/1**	0/4	0/4	0/4	0/2	0/2	0/2

0/1 No activity at dose level, *1/1* noticeable activity at dose level [bold], *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

[a] Maximal electroshock test

[b] Subcutaneous metrazole test

[c] Rotarod test

potencies at 4 h indicating a rapid onset of action or fast inactivation. Compound **9** displayed protection at 300 mg kg^{-1} dose level at 4 h period. Compound **7** showed activity at 100 and 300 mg kg^{-1} doses at 4 h. The most active compound in the series was compound **8** which demonstrated activity at 100 and 300 mg kg^{-1} doses at 0.5 h and 300 mg kg^{-1} at 4 h. These results suggested that compound **8** has quick onset and long duration of action.

According to the results, only two compounds (**10** and **11**) in the series were found to be active in the scMet test. Thus, compound **10** was active at the dose of 300 mg kg^{-1} only at 0.5 h. Compound **11** was active at same dose level both at 0.5 and 4 h.

Under the set of compounds studied, it can be concluded that, in *N*-benzoylglycinanilide derivatives, substitution of *N*-phenyl ring enhances anti-MES activity, whereas

substitution of phenyl ring in benzoyl residue strengthens anti-scMet activity, depending on the substitution pattern and the nature.

Monosubstitution at the 2 position of *N*-phenyl ring does not yield superior compounds in comparison to nonsubstituted one. However, disubstitution at 2,6-positions enhances and retards the activity. Surprisingly, methoxy substitution in para position of *N*-phenyl ring creates the most active compound in the series at 0.5 h in MES test.

Substitution of phenyl ring in benzoyl residue in *N*-benzoylglycinanilide derivatives seems to determine the activity profile. For example, 2-substituted derivative (compound **9**) was active in MES test, whereas 4-substituted derivatives (compounds **10** and **11**) were active only in scMet test.

Conclusion

In this study, a series of *N*-benzoylglycinanilide derivatives was designed, synthesized, and their anticonvulsant activities were evaluated. The preliminary anticonvulsant screening results have demonstrated that *N*-benzoylglycinanilide template has certain anticonvulsant activity and can serve as a starting structure for further studies to design new effective anticonvulsant molecules.

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Conflict of interest The authors report no conflicts of interest.

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