Med Chem Res
DOI 10.1007/s00044-013-0870-3

MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Synthesis and antiseizure evaluation of isoindoline-1,3-dione derivatives in mice

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Received: 18 April 2013 / Accepted: 23 October 2013

Abstract Epilepsy is the most common serious chronic noninfective neurological condition in the world. Despite the presence of various antiepileptic drugs in the market for epileptic patients, the necessity for development and discovery of novel antiepileptic drugs is felt. In fact, only 60–70 % of patients respond to the current drugs, and a high incidence of adverse effects is also observed. In the present study, a new series of phthalimide derivatives (compounds **3a**–**3m**) were synthesized through the reaction of phthalic anhydride and various derivatives of aniline in toluene solvent (Reflux, 24 h). Antiepileptic activity of synthesized compounds (**3a**–**3m**) was investigated using two experimental models namely, maximal electroshock (MES) and pentylenetetrazole (PTZ), and the obtained results were compared with diazepam as reference drug. Neurotoxicity of compounds was also evaluated using rotarod model. Compound **3m** with *para* methoxy substituent exhibited the anticonvulsant activity at 15.1 ± 1.53 (12.23–17.96) mg/kg dose in MES model compared to other derivatives. Unfortunately, none of the tested compounds rendered acceptable protection in subcutaneous PTZ model.

Keywords Synthesis · Phthalimide · Antiseizure · Mice

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Introduction

Epilepsy is a condition that is characterized by multiple or recurrent seizures due to sudden neuronal firing in the brain (Prakash *et al*., 2010). A single seizure does not categorize as epilepsy. Epilepsy is the most common serious chronic noninfective neurological condition in the world. In fact, the disease is not a psychological disorder and represents with unconsciousness and muscle spasms in patient. Totally, there are two types of epilepsy according to the involvement of the brain parts. Epilepsy that contains the some parts of the brain is called partial seizure. Whereas, the epilepsy that presents in all parts of the brain is classified as generalized seizure. This disorder affects at least 50 million people worldwide and about 4 % of persons experience one epileptic attack over their lifetime. The exact etiology of epilepsy is not clear. But, some conditions like developmental problems before birth, trauma at birth, head injury, tumor, structural problems, vascular problems, metabolic abnormalities, infections, and idiopathic causes may have a pivotal role in the origin of epilepsy (Noronha *et al*., 2007; Kumar *et al*., 2010; Rajak *et al*., 2010; Kurian *et al*., 2006; Jain *et al*., 2011). Nowadays, the role of various neurological systems such as GABAergic, glutamatergic (NMDA and AMPA receptors), and also ion channels (Calcium, sodium, potassium, and chloride channels) in the pathophysiology of the epilepsy has been disclosed (Meldrum and Rogawski, 2007).

Published online: 05 November 2013

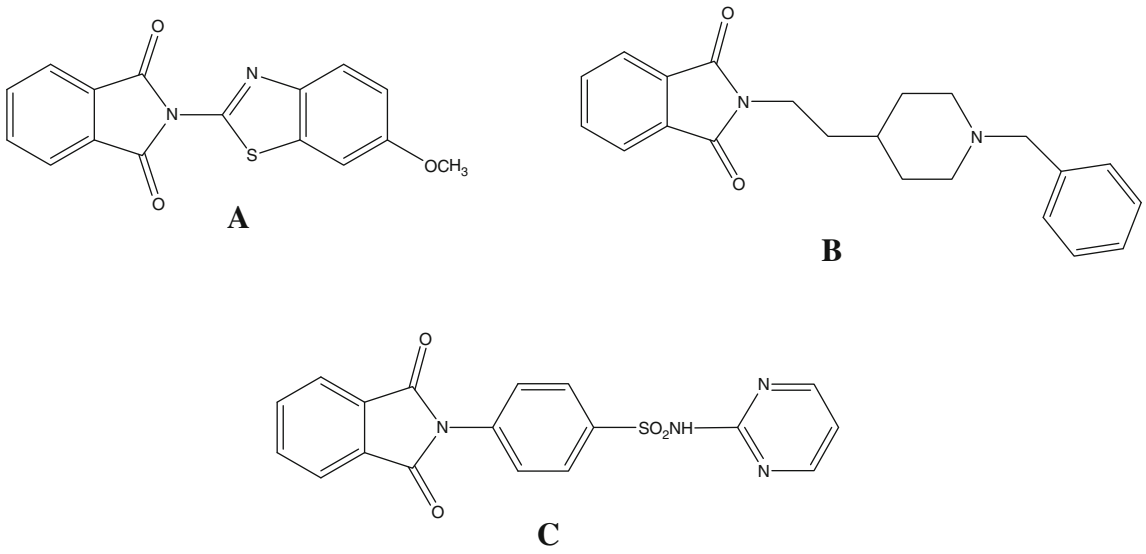
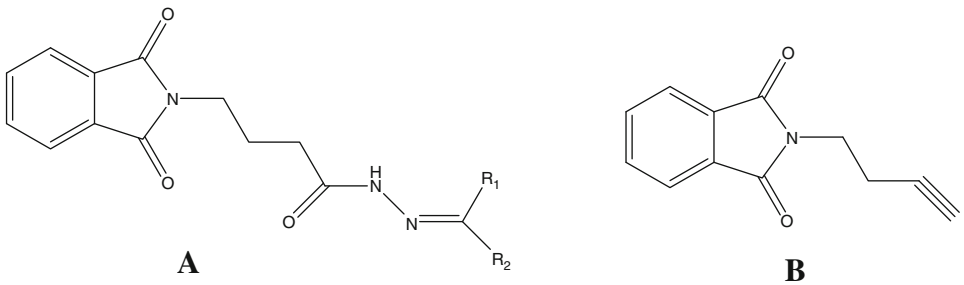


Fig. 1 Structures of some phthalimide-containing compounds. **a** As apoptosis inducer and anticancer agent, **b** acetylcholinesterase inhibitor, **c** antitubercular agent

Fig. 2 Some representatives of anticonvulsant agents with phthalimide structure



Only 60–70 % of patients with epilepsy exert an adequate and acceptable response to currently in use antiepileptic drugs (Prakesh *et al*., 2010). In the other words, there is to some extent resistance to the present drugs in epileptic patients. On the other hands, current antiepileptic drugs cause significant adverse reactions such as CNS effects (drowsiness, ataxia), ophthalmologic side effects (diplopia, nysthagmus), gastrointestinal disturbances (nausea, vomiting), hepatotoxicity, and hamatological (agranulocytosis, megaloblastic anemia) and also the probability of the occurrence of drug interactions is one of the other problems of the current drug therapy in epilepsy (Kwan and Brodie, 2000, 2004; Meldrum and Rogawski, 2007; Leppik, 1994; Perucca, 1996; Alsoud *et al*., 2003).

The recent literatures have been reported that phthalimide (or isoindoline 1,3-dione) derivatives have several biological and pharmacological activities like anticancer, anti-Alzheimer, antiangiogenic, antitubercular, etc. (Fig. 1) (Kok *et al*., 2008; Yang *et al*., 2010; Machado *et al*., 2005; Santos *et al*., 2009; Lee *et al*., 2006; Lima *et al*., 2002; Najda-Bernatowicz *et al*., 2009). Antiepileptic effect is another beneficial effect of the phthalimide derivatives (Fig. 2) (Ragavendran *et al*., 2007; Malgorzata and Katarzyna, 2009; Iniaghe and Usifoh, 2010). Hence, in the present research, we encouraged on the preparation of new phthalimide derivatives with potential antiepileptic activity, and then assessment of antiepileptic activity was carried out using seizure-induced models in mice by pentylenetetrazole (PTZ) and maximal electroshock seizure (MES).

Experimental

Chemistry

All of the chemical substances consist of starter materials, reagents, and solvents were prepared from commercial vendors like Merck and Sigma-Aldrich companies. The purity of the prepared compounds was proved by thin layer chromatography (TLC) using various solvents of different polarities. Merck silica gel 60 F_{254} sheets were applied for analytical TLC. ^{1}H-NMR spectra were recorded using Bruker 400 MHz (for hydrogen, ^{1}H) and 250 MHz (for

carbon, ^{13}C) spectrometer, and chemical shifts are expressed as δ (ppm) with tetramethylsilane (TMS) as internal standard. The IR spectra were obtained on a Shimadzu 470 spectrophotometer using potassium bromide (KBr) disks. Melting points were determined using electrothermal 9001 melting point analyzer apparatus on capillary open tubes and are uncorrected. The mass spectra were run on a Finigan TSQ-70 spectrometer (Finigan, USA) at 70 eV. Mice were purchased from Pasteur Institute of Iran. Elemental analyses were carried out on a CHNO rapid elemental analyzer (GmbH-Germany) for C, H, N, and O, and the results are within ±0.4 % of the theoretical values.

General procedure for synthesis of compounds **3a–3l**

In a flat bottom flask, equimolar quantities of phthalic anhydride and appropriate aniline derivative were mixed in toluene solvent (Scheme 1). The reaction mixture were refluxed in toluene for 24 h. Thin layer chromatography (TLC) was carried out for confirming the reaction end. After completion, the reaction mixture was cooled, and the toluene solvent was removed under reduced pressure using rotary evaporator apparatus. Ethyl acetate/water (100/100 mL) was added to the residue, and the aqueous layer was removed. The organic phase was washed two times by 100 mL diluted sulfuric acid (2 %), sodium bicarbonate (5 %), and saturated sodium chloride (brine). Organic layer was separated and dried over anhydrous sodium sulfate. Then, sodium sulfate was omitted by filtration, and ethyl acetate was evaporated by rotary evaporation (Ragavendran *et al*., 2007).

*2-Phenylisoindoline-1,3-dione (**3a**)*

m.p: 200 °C, Yield: 44 %, MW: 223 g/mol, white powder, IR (KBr, cm^{-1}) $\bar{\upsilon}$: 3452 (N–H, stretch), 3051 (C–H, stretch, aromatic), 1701 (C=O, stretch), 1593 (C=C, stretch, aromatic), 1496 (C=C, stretch, aromatic). ^{1}HNMR ($CDCl_3$, 400 MHz) δ: 7.48 (m, 4H, Phenyl), 7.83 (dd, 2H, $J = 8$ Hz, $J = 4$ Hz, $H_{5,6}$-phthalimide), 7.99 (dd, 2H, $J = 8$ Hz, $J = 4$ Hz, $H_{4,7}$-phthalimide). ^{13}CNMR (DMSO-d_6, 62.5 MHz) δ: 123.7 ($C_{4,7}$), 126.6 ($C_{2,6}$), 128.1 (C_4), 129.1 ($C_{3,5}$), 131.6 ($C_{3a,7a}$-), 131.7 (C_1), 134.4 ($C_{5,6}$), 167.2 (C=O). MS (*m*/*z*, %): 223 (M^+, 100), 179 (80), 152 (5), 104 (15), 76 (45), 64 (5), 51 (5). Elemental anal. for $C_{14}H_9NO_2$, Calculated: C, 75.33 H, 4.06; N, 6.27; O, 14.33. Found: C, 75.12; H, 4.26; N, 6.07; O, 13.97.

*2-(2-Chlorophenyl)isoindoline-1,3-dione (**3b**)*

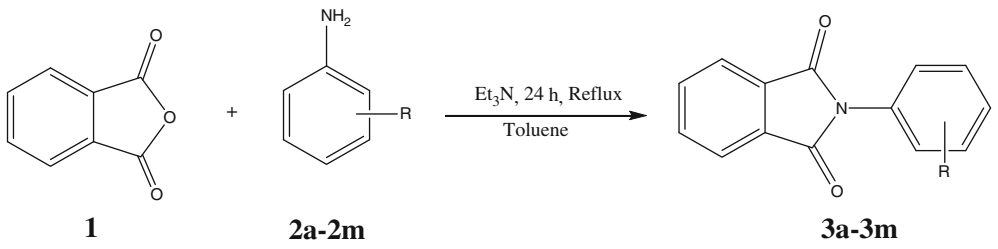
m.p: 197 °C, Yield: 72 %, MW: 257 g/mol, white powder, IR (KBr, cm^{-1}) $\bar{\upsilon}$: 3062 (C–H, aromatic), 1712 (C=O, stretch). ^{1}HNMR: 7.18–7.41 (m, 4H, 2-chlorophenyl), 7.85 (dd, 2H, $J = 8$ Hz, $J = 4$ Hz, $H_{5,6}$-phthalimide), 7.99 (dd, 2H, $J = 8$ Hz, $J = 4$ Hz, $H_{4,7}$-phthalimide). ^{13}CNMR (DMSO-d_6, 62.5 MHz) δ: 123.6 ($C_{4,7}$), 127.1 (C_3), 128.5 (C_6), 129.2 (C_6), 131.2 (C_4), 131.5 ($C_{3a,7a}$), 133.1 (C_4), 135.8 ($C_{5,6}$), 138.7 (C_1), 166.8 (C = O). MS (*m*/*z*, %): 259 (M^++2, 30), 257 (M^+, 100), 215 (20), 213 (55), 178 (30), 104 (20), 76 (48), 63 (10). Elemental anal. for $C_{14}H_8ClNO_2$, Calculated: C, 65.26; H, 3.13; N, 5.44; O, 12.42. Found: C, 65.22; H, 3.03; N, 5.49; O, 12.32.

*2-(3-Chlorophenyl)isoindoline-1,3-dione (**3c**)*

m.p: 162 °C, Yield: 48 %, MW: 257 g/mol, white powder, IR (KBr, cm^{-1}) $\bar{\upsilon}$: 3074 (CH, stretch, aromatic), 1720 (C=O, stretch), 1589 (C=C, stretch, aromatic), 1481 (C=C, stretch, aromatic). ^{1}HNMR ($CDCl_3$, 400 MHz) δ: 7.4–7.48 (m, 3H, 3-chlorophenyl), 7.53 (s, H_2-3-chlorophenyl), 7.85 (dd, 2H, $J = 8$ Hz, $J = 4$ Hz, $H_{5,6}$-phthalimide), 7.99 (dd, 2H, $J = 8$ Hz, $J = 4$ Hz, $H_{4,7}$-phthalimide). ^{13}CNMR (DMSO-d_6, 62.5 MHz) δ: 118.6 (C_2), 120.8 (C_6), 124.8 ($C_{4,7}$), 126.6 (C_4), 129.9 (C_5), 131.9 ($C_{3a,7a}$), 132.5 (C_1), 134.3 (C_3), 135.9 ($C_{5,6}$), 166.6 (C=O). Elemental anal. for $C_{14}H_8ClNO_2$, Calculated: C, 65.26; H, 3.13; N, 5.44; O, 12.42. Found: C, 65.28; H, 3.19; N, 5.34; O, 12.49.

*2-(4-Chlorophenyl)isoindoline-1,3-dione (**3d**)*

m.p: 190 °C, Yield: 80 %, MW: 257 g/mol, white powder, IR (KBr, cm^{-1}) $\bar{\upsilon}$: 3062 (C–H, stretch, aromatic), 1712 (C=O, stretch). ^{1}HNMR ($CDCl_3$, 400 MHz) δ: 7.45 (d, 2H, $J = 8$ Hz, $H_{2,6}$-4-chlorophenyl), 7.51 (d, 2H, $J = 8$ Hz, $H_{3,5}$-4-chlorophenyl), 7.83 (dd, 2H, $J = 8$ Hz, $J = 4$ Hz,



Scheme 1 Synthetic pathway of compounds **3a–3m**

$H_{5,6}$-phthalimide), 7.99 (dd, 2H, $J = 8$ Hz, $J = 4$ Hz, $H_{4,7}$-phthalimide). ^{13}CNMR (DMSO-d_6, 62.5 MHz) δ: 123.8 ($C_{2,6}$), 127.7 ($C_{4,7}$), 129.3 ($C_{3,5}$), 130.2 ($C_{3a,7a}$), 131.6 (C_1), 133.8 (C_4), 134.6 ($C_{5,6}$), 167.0 (C=O). Elemental anal. for $C_{14}H_8ClNO_2$, Calculated: C, 65.26; H, 3.13; N, 5.44; O, 12.42. Found: C, 65.32; H, 3.09; N, 5.49; O, 12.47.

*2-(2-Fluorophenyl)isoindoline-1,3-dione (**3e**)*

m.p: 181 °C, Yield: 59 %, MW: 291 g/mol, white powder, IR (KBr, cm^{-1}) $\overline{v}$: 3074 (C–H, aromatic), 1720 (C=O, stretch), 1593 (C=C, stretch, aromatic), 1462 (C=C, stretch, aromatic). ^{1}HNMR ($CDCl_3$, 400 MHz) δ: 7.79 (m, 2H, 2-fluorophenyl), 7.84 (m, 2H, 2-fluorophenyl), 7.87 (dd, 2H, $J = 8$ Hz, $J = 4$ Hz, $H_{5,6}$-phthalimide), 8.01 (dd, 2H, $J = 8$ Hz, $J = 4$ Hz, $H_{4,7}$-phthalimide). ^{13}CNMR (DMSO-d_6, 62.5 MHz) δ: 115.7 (d, C_3), 121.0 (d, C_1), 124.1 (C_5), 124.9 ($C_{4,7}$), 129.1 (d, C_4), 129.6 (d, C_6), 133.7 ($C_{3a,7a}$), 136.1 ($C_{5,6}$), 158.3 (d, C_2), 166.7 (C=O). Elemental anal. for $C_{14}H_8FNO_2$, Calculated: C, 69.71; H, 3.34; N, 5.81; O, 13.27. Found: C, 69.75; H, 3.31; N, 5.89; O, 13.25.

*2-(3-Fluorophenyl)isoindoline-1,3-dione (**3f**)*

m.p: 199 °C, Yield: 49 %, MW: 291 g/mol, white powder, IR (KBr, cm^{-1}) $\overline{v}$: 3074 (C–H, stretch, aromatic), 3032 (C–H, stretch, aromatic), 1712 (C=O, stretch), 1597 (C=C, stretch, aromatic), 1492 (C=C, stretch, aromatic). ^{1}HNMR ($CDCl_3$, 400 MHz) δ: 7.15 (t, 1H, $J = 8$ Hz, H_5-3-fluorophenyl), 7.27 (t, 1H, H_6-3-fluorophenyl), 7.32 (d, 1H, $J = 8$ Hz, H_2-3-fluorophenyl), 7.51 (q, 1H, $J = 8$ Hz, H_4-3-fluorophenyl), 7.85 (dd, 2H, $J = 8$ Hz, $J = 4$ Hz, $H_{5,6}$-phthalimide), 8.00 (dd, 2H, $J = 8$ Hz, $J = 4$ Hz, $H_{4,7}$-phthalimide). ^{13}CNMR (DMSO-d_6, 62.5 MHz) δ: 103.7 (d, C_2), 110.5 (C_6), 113.2 (C_4), 123.6 ($C_{4,7}$), 128.2 (d, C_5), 132.3 ($C_{3a,7a}$), 135.1 ($C_{5,6}$), 143.5 (d, C_1), 162.5 (C_3), 165.8 (C=O). MS (*m/z*, %): 241 (M^+, 100), 197 (75), 170 (20), 149 (12), 123 (15), 104 (15), 76 (30). Elemental anal. for $C_{14}H_8FNO_2$, Calculated: C, 69.71; H, 3.34; N, 5.81; O, 13.27. Found: C, 69.61; H, 3.29; N, 5.86; O, 13.21.

*2-(4-Fluorophenyl)isoindoline-1,3-dione (**3g**)*

m.p: 161 °C, Yield: 70 %, MW: 291 g/mol, white powder, IR (KBr, cm^{-1}) $\overline{v}$: 3066 (C–H, stretch, aromatic), 1716 (C=O, stretch), 1604 (C=C, stretch, aromatic), 1465 (C=C, stretch, aromatic). ^{1}HNMR ($CDCl_3$, 400 MHz) δ: 7.23 (t, 2H, $J = 8$ Hz, $H_{2,6}$-4-fluorophenyl), 7. 46 (q, 2H, $J = 4$ Hz, $H_{3,5}$-4-fluorophenyl), 7.83 (dd, 2H, $J = 8$ Hz, $J = 4$ Hz, $H_{5,6}$-phthalimide), 7.99 (dd, 2H, $J = 8$ Hz, $J = 4$ Hz, $H_{4,7}$-phthalimide). ^{13}CNMR (DMSO-d_6, 62.5 MHz) δ: 116.2 (d, $C_{3,5}$), 123.8 ($C_{4,7}$), 127.6 (C_1), 128.4 (d, $C_{2,6}$), 131.7 ($C_{3a,7a}$), 134.5 ($C_{5,6}$), 162.0 (d, C_4), 167.2 (C=O). Elemental anal. for $C_{14}H_8FNO_2$, Calculated: C, 69.71; H, 3.34; N, 5.81; O, 13.27. Found: C, 69.73; H, 3.38; N, 5.84; O, 13.35.

*2-(2-Nitrophenyl)isoindoline-1,3-dione (**3h**)*

m.p: 139 °C, Yield: 74 %, MW: 268 g/mol, orange powder, IR (KBr, cm^{-1}) $\overline{v}$: 2924 (C–H, stretch, asymmetric, aliphatic), 1720 (C=O, stretch), 1519 (NO_2, stretch, asymmetric), 1346 (NO_2, stretch, asymmetric). ^{1}HNMR ($CDCl_3$, 400 MHz) δ: 7.57 (d, 1H, $J = 8$ Hz, H_6-2-nitrophenyl), 7.67 (t, 1H, $J = 8$ Hz, H_4-2-nitrophenyl), 7.85 (t, 1H, $J = 8$ Hz, H_5-2-nitrophenyl), 7.86 (dd, 2H, $J = 8$ Hz, $J = 4$ Hz, $H_{5,6}$-phthalimide), 8.02 (dd, 2H, $J = 8$ Hz, $J = 4$ Hz, $H_{4,7}$-phthalimide), 8.23 (d, 1H, $J = 8$ Hz, H_3-2-nitrophenyl). ^{13}CNMR (DMSO-d_6, 62.5 MHz) δ: 123.6 ($C_{4,7}$), 125.5 (C_6), 126.2 (C_3), 128.7 (C_4), 131.3 (C_1), 133.6 (C_5), 134.1 ($C_{3a,7a}$), 135.9 ($C_{5,6}$), 148.3 (C_2), 164.1 (C=O). MS (*m/z*, %): 268 (M^+, 5), 222 (100), 194 (5), 166 (15), 140 (12), 104 (12), 76 (22). Elemental anal. for $C_{14}H_8N_2O_4$, Calculated: C, 62.69; H, 3.01; N, 10.44; O, 23.86. Found: C, 62.72; H, 3.11; N, 10.48; O, 23.81.

*2-(3-Nitrophenyl)isoindoline-1,3-dione (**3i**)*

m.p: 239 °C, Yield: 36 %, MW: 268 g/mol, yellow powder, IR (KBr, cm^{-1}) $\overline{v}$: 3097 (C–H, stretch, aromatic), 1724 (C=O, stretch), 1535 (NO_2, stretch, asymmetric), 1350 (NO_2, stretch, symmetric). ^{1}HNMR ($CDCl_3$, 400 MHz) δ: 7.73 (t, 1H, $J = 8$ Hz, H_5-3-nitrophenyl), 7.88 (dd, 2H, $J = 8$ Hz, $J = 4$ Hz, $H_{5,6}$-phthalimide), 7.91 (d, 1H, $J = 8$ Hz, H_6-3-nitrophenyl), 8.04 (dd, $J = 8$ Hz, $J = 4$ Hz, $H_{5,6}$-phthalimide), 8.30 (d, 1H, $J = 8$ Hz, H_4-3-nitrophenyl), 8.47 (s, 1H, H_2-3-nitrophenyl). ^{13}CNMR (DMSO-d_6, 62.5 MHz) δ: 113.6 (C_2), 117.9 (C_4), 122.7 ($C_{4,7}$), 127.9 (C_6), 129.7 (C_5), 131.6 ($C_{3a,7a}$), 135.0 ($C_{5,6}$), 138.2 (C_1), 148.03 (C_3), 167 (C=O). Elemental anal. for $C_{14}H_8N_2O_4$, Calculated: C, 62.69; H, 3.01; N, 10.44; O, 23.86. Found: C, 62.74; H, 3.09; N, 10.39; O, 23.82.

*2-(4-Nitrophenyl)isoindoline-1,3-dione (**3j**)*

m.p: 265 °C, Yield: 21 %, MW: 268 g/mol, yellow powder, IR (KBr, cm^{-1}) $\overline{v}$: 3120 (C–H, stretch, aromatic), 1708 (C=O, stretch), 1597 (C=C, Stretch, aromatic), 1525 (NO_2, stretch, asymmetric), 1346 (NO_2, stretch, symmetric). ^{1}HNMR ($CDCl_3$, 400 MHz) δ: 7.62 (d, 1H, $J = 8$ Hz, $H_{2,6}$-4-nitrophenyl), 7.78 (dd, 2H, $J = 8$ Hz, $J = 4$ Hz, $H_{5,6}$-phthalimide), 7.91 (dd, 2H, $J = 8$ Hz, $J = 4$ Hz, $H_{4,7}$-phthalimide), 8.21 (d, 1H, $J = 8$ Hz, $H_{3,5}$-4-nitrophenyl). ^{13}CNMR (DMSO-d_6, 62.5 MHz) δ: 117.9 ($C_{2,6}$), 124.1 ($C_{4,7}$), 126.5 ($C_{3,5}$), 132.2 ($C_{3a,7a}$), 135.4 ($C_{5,6}$), 138.7 (C_1), 145.7 (C_4-4-nitrophenyl), 166.7 (C=O). Elemental anal. for

$C_{14}H_8N_2O_4$, Calculated: C, 62.69; H, 3.01; N, 10.44; O, 23.86. Found: C, 62.79; H, 3.11; N, 10.41; O, 23.92.

*2-(2-Methoxyphenyl)isoindoline-1,3-dione (**3k**)*

m.p: 158 °C, Yield: 62 %, MW: 253 g/mol, creamy powder, IR (KBr, cm^{-1}) $\bar{\upsilon}$: 3066 (C–H, stretch, aromatic), 2927 (C–H, stretch, asymmetric, aliphatic), 2843 (C–H, stretch, symmetric, aliphatic), 1712 (C=O, stretch), 1597 (C=C, stretch, aromatic), 1465 (C=C, stretch, aromatic). ^{1}HNMR ($CDCl_3$, 400 MHz) δ: 7.1 (m, 2H, H_3, H_5-2-methoxyphenyl), 7.3 (d, 1H, H_6-2-methoxyphenyl), 7.47 (t, 1H, $J = 8$ Hz, H_5-2-methoxyphenyl), 7.81 (dd, 2H, $J = 8$ Hz, $J = 4$ Hz, $H_{5,6}$-phthalimide), 7.98 (dd, 2H, $J = 8$ Hz, $J = 4$ Hz, $H_{4,7}$-phthalimide). ^{13}CNMR (DMSO-d_6, 62.5 MHz) δ: 55.8 (-O$\underline{C}H_3$), 112.1 (C_3), 120.2 (C_5), 120.8 (C_6), 123.6 ($C_{4,7}$), 129.9 (C_4), 130.6 (C_1), 132.2 ($C_{3a,7a}$), 134.1 ($C_{5,6}$), 155.4 (C_2), 167.3 (C=O). Elemental anal. for $C_{15}H_{11}NO_3$, Calculated: C, 71.14; H, 4.38; N, 5.53; O, 18.95. Found: C, 71.19; H, 4.42; N, 5.51; O, 18.85.

*2-(3-Methoxyphenyl)isoindoline-1,3-dione (**3l**)*

m.p: 117 °C, Yield: 84 %, MW: 253 g/mol, creamy powder, IR (KBr, cm^{-1}) $\bar{\upsilon}$: 3016 (C–H, stretch, aromatic), 2924 (C–H, stretch, asymmetric, aliphatic), 2846 (C–H, stretch, symmetric, aliphatic), 1724 (C=O, stretch), 1612 (C=C, stretch, aromatic), 1462 (C=C, stretch, aromatic). ^{1}HNMR ($CDCl_3$, 400 MHz) δ: 6.98 (d, 1H, $J = 8$ Hz, H_4-3-methoxyphenyl), 7.01 (s, 1H, H_2-3-methoxyphenyl), 7.07 (d, 1H, $J = 8$ Hz, H_6-3-methoxyphenyl), 7.44 (t, 1H, $J = 8$ Hz, H_5-3-methoxyphenyl), 7.82 (dd, 2H, $J = 8$ Hz, $J = 4$ Hz, $H_{5,6}$-phthalimide), 7.99 (dd, 2H, $J = 8$ Hz, $J = 4$ Hz, $H_{4,7}$-phthalimide). ^{13}CNMR (DMSO-d_6, 62.5 MHz) δ: 55.4 (-O$\underline{C}H_3$), 105.7 (C_2), 109.4 (C_6), 113.7 (C_4), 124.5 ($C_{5,7}$), 129.8 (C_5), 131.9 ($C_{3a,7a}$), 159.6 (C_1), 166.4 (C=O). MS (*m/z*, %): 253 (M^+, 100), 224 (10), 209 (12), 179 (10), 166 (5), 123 (12), 104 (22), 76 (30). Elemental anal. for $C_{15}H_{11}NO_3$, Calculated: C, 71.14; H, 4.38; N, 5.53; O, 18.95. Found: C, 71.09; H, 4.29; N, 5.59; O, 19.07.

*2-(4-Methoxyphenyl)isoindoline-1,3-dione (**3m**)*

m.p: 150 °C, Yield: 95 %, MW: 253 g/mol, green powder, IR (KBr, cm^{-1}) $\bar{\upsilon}$: 3066 (C–H, stretch, aromatic), 1708 (C=O, stretch), 1612 (C=C, stretch, aromatic), 1462 (C=C, stretch, aromatic). ^{1}HNMR ($CDCl_3$, 400 MHz) δ: 7.05 (d, 1H, $J = 8$ Hz, $H_{3,5}$-4-methoxyphenyl), 7.36 (d, 1H, $J = 8$ Hz, $H_{2,6}$-4-methoxyphenyl), 7.81 (dd, 2H, $J = 8$ Hz, $J = 4$ Hz, $H_{5,6}$-phthalimide), 7. 97 (dd, 2H, $J = 8$ Hz, $J = 4$ Hz, $H_{4,7}$-phthalimide). ^{13}CNMR (DMSO-d_6, 62.5 MHz) δ: 55.9 (-O$\underline{C}H_3$), 114.7 ($C_{3,5}$), 116.8 ($C_{2,6}$), 124.6 ($C_{4,7}$), 125.5 (C_1), 130.2 ($C_{3a,7a}$), 136.4 ($C_{5,6}$), 157.6 (C_4), 165.9 (C=O). Elemental anal. for $C_{15}H_{11}NO_3$, Calculated: C, 71.14; H, 4.38; N, 5.53; O, 18.95. Found: C, 71.10; H, 4.36; N, 5.57; O, 18.89.

Anticonvulsant activity evaluation

The anticonvulsant activity was assessed using male albino mice weighing 25–30 g. Test compounds were evaluated by two anticonvulsant models, namely, MES as well as subcutaneous PTZ. All synthesized compounds (**3a**–**3m**) were dissolved in dimethylsulfoxide (DMSO 5 %) and injected intraperitoneally (i.p.) to the mice. Diazepam was utilized as reference drug for comparison of the obtained results (Jain *et al*., 2011; Tabatabai *et al*., 2013; Jiang *et al*., 2012).

Maximal electroshock seizure (MES)

Test compounds were administered intraperitoneally (i.p.) at dose level of 30, 100, and 300 mg/kg. Anticonvulsant activity was investigated at 0.5 h interval of administration. Maximal electroshock seizure was induced in mice via ear clip electrode. The seizure was elicited with 60 Hz and 50 mA for 0.2 s. Abolition of hind limb tonic extensor component of the seizure in half or more of the animals is considered as protection.

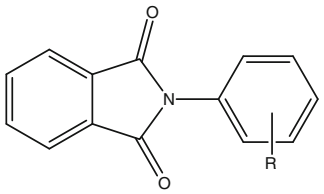
Pentylenetetrazole (PTZ)

Test compounds were administered intraperitoneally (i.p.) with 30, 100, and 300 mg/kg doses. A subcutaneous injection of PTZ (85 mg/kg) was administered as convulsive dose (CD_{97}) in mice at 0.5-h interval after injection of intended compounds. The animals were followed over 30 min. The absence of clonic spasm in half or more of the animals in the observed time period indicated a compound's ability to abolish the effect of PTZ on seizure threshold.

Neurotoxicity evaluation

Minimal motor impairment of tested compounds was also investigated by rotarod model. The mice were trained to stay on a rotarod of diameter 3.2 cm that rotates at 6 rpm. Neurotoxicity was determined by the inability of the animal to maintain equilibration on the rod for at least 1 min in each of the three trials. The dose at which 50 % of the animals enabled to balance themselves and fell off the rotating rod was recorded. Formerly, mice have received 30, 100, and 300 mg/kg doses of test intended compounds intraperitoneally (i.p.). Thirty minutes after i.p. injection, the mice were placed on the rotarod. Failure of the mice to keep itself on the rotating rod after 1 min in each of the three trials is the indication of the neurotoxicity.

Table 1 Obtained results of MES test



Compounds	R	% Seizure			ED_{50} (mg/kg)	TD_{50} (mg/kg)	PI
		30 mg/kg	100 mg/kg	300 mg/kg			
3a	H	100	25	0	66.16 ± 8.31 (45.51–86.81)[a]	>300	>4.53
3b	2-Cl	100	75	25	167.66 ± 2.51 (161–173.92)	NO	–
3c	3-Cl	100	100	25	190 ± 5 (117.58–202.42)	300	1.57
3d	4-Cl	75	25	25	55 ± 9.46 (30.157–79.84)	>300	>5.54
3e	2-F	100	75	0	>300	233.33 ± 12.58 (202.07–264.59)	<0.77
3f	3-F	100	0	25	39.66 ± 4.05 (28.46–50.86)	31.66 ± 4.7 (19.92–43.4)	0.79
3g	4-F	100	100	75	>300	181.66 ± 11.015 (154.3–209.03)	<0.60
3h	2-NO_2	75	25	0	53.5 ± 4.09 (43.33–63.66)	>300	>5.6
3i	3-NO_2	100	75	25	174 ± 9.29 (151.25–197.42)	74.21 ± 4.9 (64.29–84.37)	0.42
3j	4-NO_2	100	25	25	62.31 ± 2.05 (38.12–75.31)	175.66 ± 5.5 (161.98–189.35)	2.81
3k	2-OCH_3	100	100	25	194.6 ± 10.5 (168.5–220.7)	222.1 ± 7.93 (201.28–240.72)	1.14
3l	3-OCH_3	100	100	75	>300	NO	–
3m	4-OCH_3	25	25	0	15.1 ± 1.53 (12.23–17.96)	89.33 ± 5.1 (76.58–102.08)	5.91
Diazepam					1.8 (1.1–2.6)		

[a] $n = 8$, 95 %, Confidence limits in parentheses

Results and discussion

A new series of phthalimide(isoindoline 1,3-dione)-based compounds were synthesized, and their antiseizure activity was investigated using two experimental procedures namely, MES, and subcutaneous PTZ in mice. In the present synthesized derivatives **3a**–**3m**, various electron withdrawing as well as electron-donating substituents were applied to study the role of electronic effects of the moiety on the phenyl ring. In addition, the probable neurotoxicity of synthesized derivatives was explored by ratarod model.

Maximal electroshock seizure (MES)

All synthesized derivatives **3a**–**3m** were tested by MES model, and obtained results were compared with diazepam as reference drug. According to the Table 1, an intrapritoneally injection of compounds **3a**–**3m** was administered with 30, 100, and 300 mg/kg doses to the mice, and the percent of mice that presented seizure was recorded and effective dose to protect the 50 % of the mice from the induced seizure was calculated. Among the tested compounds, compounds **3e**, **3g**, and **3l** did not show an acceptable anticonvulsant activity. In fact, up to injected dose 300 mg/kg did not observed any protection against MES-induced seizure. Comparison of ED_{50} of chlorinated derivatives shows that *para* positioning of this moiety (compound **3d**) is favorable for anticonvulsant effect, while compared to other positions of the phenyl ring (compounds **3c** and **3d**). Decrease in polarity of compound **3d** in comparison with compounds **3b** and **3c** may be the reason for this event. Because the polarity is a limiting factor for passing the blood brain barrier (BBB) and therefore, decrease the penetration of the intended compound. Replacement of the chlorine atom with fluorine abolished the anticonvulsant potency when fluorine atom positioned at *ortho* and *para*. Position 3 (*meta*) of the phenyl ring was so beneficial for enhancing the antiseizure potency with fluorine moiety. Comparison of compounds **3c** (3-Cl) and **3f** (3-F) revealed that electronic effect induced by fluorine is likely so important that lipophilicity induced via chlorine moiety. Although chlorine atom generates electron withdrawing effect, fluorine can play this role in a better manner. Substitution of the nitro moiety at different positions of the phenyl ring demonstrated that the *ortho* and *para* positions are the best positions for this moiety to obtain an acceptable antiseizure potency. It is obvious that electronic effect of the nitro substituent could better induce

Table 2 Obtained results of tested compounds in PTZ model

Compounds	R	Duration of seizure time			Onset of seizure time			% Death			% Seizure
		30 mg/kg	100 mg/kg	300 mg/kg	30 mg/kg	100 mg/kg	300 mg/kg	30 mg/kg	100 mg/kg	300 mg/kg	100 mg/kg
3a	H	3.75 ± .577	11 ± 3.55	15.75 ± 4.9	3.25 ± 0.95	4.5 ± 1.29	6 ± 0.81	100	100	25	100
3b	2-Cl	8.25 ± 3.5	14.25 ± 2.21	24.5 ± 1.29	1.5 ± 0.57	6.5 ± 1.29	9.5 ± 6.55	100	75	100	100
3c	3-Cl	11.25 ± 4.11	7 ± 3.46	9 ± 7.43	3.5 ± 3.10	3.75 ± 2.08	4.75 ± 1.70	100	75	25	100
3d	4-Cl	10.5 ± 5.19	12.75 ± 4.5	19.25 ± 5.2	3 ± 0.81	3.25 ± 0.95	8.25 ± 2.5	100	75	75	100
3e	2-F	5.5 ± 4.04	9 ± 2	12 ± 4.08	2 ± 0.81	5 ± 1.82	4.75 ± 0.95	100	100	75	100
3f	3-F	10.5 ± 5.91	11 ± 4.69	14 ± 7.61	5 ± 0.81	4 ± 0.81	4.75 ± 2.21	100	100	75	100
3g	4-F	13.25 ± 1.3	23.75 ± 2.6	23.5 ± 7.04	3 ± 0.81	8.5 ± 1.29	15.25 ± 4.64	25	25	0	100
3h	2-NO_2	7 ± 2.82	22.5 ± 2.08	18 ± 8.79	2 ± 0.81	7.5 ± 1.29	8.75 ± 2.62	100	0	75	100
3i	3-NO_2	6 ± 1.82	16.5 ± 4.24	18.25 ± 2.7	3.5 ± 1.29	4.5 ± 1.29	10.5 ± 5.8	100	100	75	100
3j	4-NO_2	5.75 ± 4.27	11.75 ± 3.5	15.5 ± 7.32	3.5 ± 1.29	4 ± 1.41	6.25 ± 1.5	75	75	75	100
3k	2-OCH_3	9.75 ± 6.7	9.75 ± 4.85	13.5 ± 5.74	2.75 ± 1.25	5.75 ± 2.21	7.25 ± 2.21	100	100	75	100
3l	3-OCH_3	7.25 ± 4.71	18.75 ± 2.21	22.25 ± 5.2	3 ± 0.81	7.25 ± 1.89	10.25 ± 1.25	100	75	25	100
3m	4-OCH_3	11.75 ± 9.6	22.25 ± 9.8	23.75 ± 4.3	3 ± 0.81	5.25 ± 0.95	13.5 ± 2.64	100	100	0	100
Negative control		3.75 ± .95			2.5 ± 1.29			100 %			100
Diazepam		NC			NC			0 %			0

NC no convulsion

at *ortho* and *para* positions than *meta* position. Introduction of the methoxy group as electron-donating moiety on the phenyl ring was also investigated. Methoxy moiety caused a dramatic increase in activity, while substituted at *para* position. Totally, although electron withdrawing effects such as seen about chlorine, fluorine, and nitro substituents enhanced the anticonvulsant effect, electron-donating effect of methoxy group caused a more enhancement in potency.

Pentylenetetrazole (PTZ)

According to Table 2, all compounds **3a–3m** were tested using PTZ model in mice. Tested compounds injected to mice at 30, 100, and 300 mg/kg doses intraperitoneally, and then subcutaneous PTZ was injected at 85 mg/kg. Onset of seizure time as well as duration of seizure time was recorded. Diazepam was utilized as a reference anticonvulsant drug. Unfortunately, none of the tested compounds demonstrated an acceptable protection against PTZ-induced convulsion.

Neurotoxicity evaluation

All derivatives **3a–3m** were tested for neurotoxicity using rotarod model, and the protective index (PI) was also calculated. In fact, the PI is the ratio of TD_{50}/ED_{50}. According to Table 1, compound **3m** exerted the highest protective index (PI = 5.91) compared to other derivatives. Interestingly, compound **3m** that showed the lowest ED_{50} in MES, also exhibited the highest TD_{50}.

Acknowledgments Authors appreciate from the research council of Kermanshah University of Medical Sciences for financial supports. This work was performed in partial fulfillment of the requirement for PharmD of Ms Tahereh Karimi.

Conflict of interest The authors have declared no conflict of interests.

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