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Original Article

Synthesis and evaluation of anticonvulsant activity of (*Z*)-4-(2-oxoindolin-3-ylideneamino)-*N*-phenylbenzamide derivatives in mice

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Abstract

Due to resistance of some epileptic patients to the current medications and the general incidence of severe side effects of these drugs, development and discovery of novel antiepileptic drugs is crucial. Isatin-based derivatives are promising compounds as antiepileptic agents. In this study a new series of isatin-containing derivatives were synthesized via the imine formation between isatin and *p*-aminobenzoic acid. Subsequently, the obtained acidic compound was utilized to prepare the final amidic derivatives (**4a-4l**) through the reaction with various aniline derivatives. Then, their anti-seizure activity was investigated using maximal electroshock seizure (MES) as well as pentylenetetrazole (PTZ) models in mice. Neurotoxicity of target compounds was also determined by rotarod test. Tested isatin-based derivatives exhibited a favorable protection in both MES and PTZ procedures with high safety levels in neurotoxicity test. The introduced derivatives have demonstrated remarkable activity in mice and could be suggested as potential anticonvulsant lead compounds. All methoxylated derivatives (**4j**, **4k**, **4l**) showed a significant anti-seizure activity in MES model. Compounds **4j** (2-OCH_3) and **4l** (4-OCH_3) also demonstrated a potent anti-seizure activity against PTZ. Compound **4k** (*m*-OCH_3) did not induce protection towards PTZ-induced convulsion.

Keywords: Synthesis; Isatin; Anticonvulsant; PTZ; MES.

INTRODUCTION

Epilepsy is a common central nervous system (CNS) disorder that is characterized by presentation of recurrent spontaneous convulsions as well as by emergence of some sensory, motor or autonomic symptoms. According to the epidemiological surveys, approximately 1% of the world's population suffers from epilepsy (1). The disease has a very complex nature, and diverse pathological factors can cause the disease. Epilepsy is a condition that can occur in both sexes and at all age groups (2). Many of the currently used antiepileptic drugs have severe adverse effects that limit their use in short or long term treatment. These include CNS (ataxia, drowsiness), gastrointestinal (gingival hyperplasia, nausea, vomiting, hepatic dysfunction), hematological (megaloblastic anaemia), bone related (osteomalacia), and dermatological (rash, Stevens-Johnson syndrome) disorders. Furthermore, about 30% of epileptic patients do not show adequate response to the current drugs (3-5). Many antiepileptic agents exert their anticonvulsant effects via a variety of mechanisms. Inhibition of ion channels such as sodium channels (phenytoin, carbamazepine), calcium channels (ethosuximide), and potassium channels (retigabine) as well as $GABA_A$ receptors (benzodiazepines, barbiturates) are common mechanisms of conventional antiepileptic drugs.

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On the other hand, effect on glutamatergic receptors (lamotrigine, topiramate, felbamate) is performed by means of newer drugs.
icacy of these medications is in question for controlling and treatment of some types of epilepsy. Hence, in antiepileptic drug development research there is a deep need and demand for the development of more efficacious agents with fewer side effects (6-9).

Isatin, an oxidation product of indigo, is a bright orange-colored compound with a broad range of biological activities. Presence of significant amounts of isatin in such tissues as brain, proposes that it probably has a role in CNS functions. Isatin is demonstrated to have anxiogenic as well as sedative effects. It also increases the levels of monoamines in CNS (10). Inhibition of monoamine oxidase (MAO) enzymes especially MAO_B is shown in some studies. Moreover, isatin is also formed in the tryptophan metabolic pathway (11,12). The chemical structure of isatin attracted the attention of many medicinal chemists for derivatization. A large number of pharmacological effects such as anticancer, antidepressant, anticonvulsant, anti-acetylcholinesterase, antibacterial, antifungal, anti-HIV, antitubercular, and anti-inflammatory properties have been observed from the compounds with isatin substructure (12-14). Anticonvulsant activity is one of the main promising biological activities that are shown to possess some isatin-based compounds (Fig. 1) (13-19). In continuation of our previous studies on isatin-based anticonvulsant derivatvies (19), we designed a new series of anticonvulsant agents based on isatin as a substructure. As illustrated in Fig. 2, similar pharmacophoric sections could be defined for isatin derivatives which are already presented in phenytoin and carbamazepine as standard anticonvulsant agents.

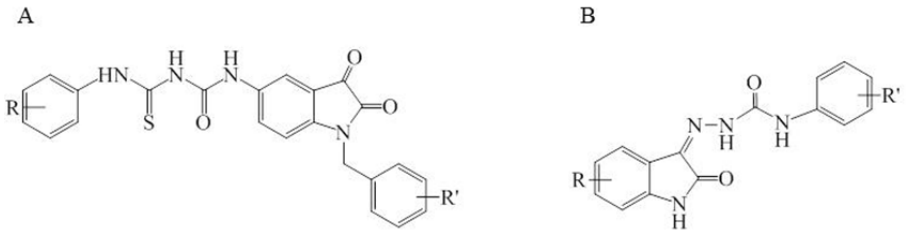


Fig 1. Structures of some isatin-based compounds as anticonvulsant agents.

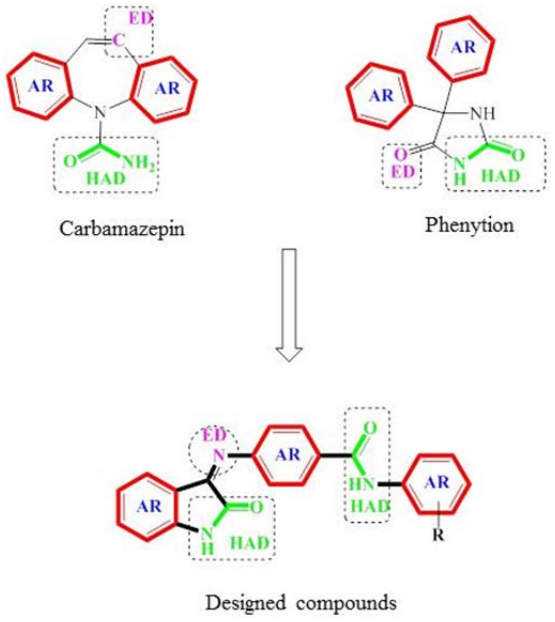


Fig. 2. Pharmacophoric necessities of anticonvulsant drugs. Hydrogen bond acceptor/donor represented in green (HAD), electron donor represented in violet (ED) and aromatic ring represented in red (AR represented) for hydrophobic interaction. Target compounds were designed according to this theory.

The insufficient data and knowledge about the physiopathology and cellular mechanisms of epilepsy in human and the multiple pharmacological mechanisms for antiepileptic drugs makes it difficult to design and develop novel drugs (3). Pharmacophoric studies of antiepileptic drugs have revealed that three main parts are present in chemical scaffolds of efficacious drugs for presentation of antiepileptic activity. The main requisite parts are: hydrogen bond donor/acceptor moiety, electron donor group , and hydrophobic region that is produced by aromatic rings such as phenyl residue (Fig. 2) (1,20). Based on this information, we designed a new series of isatin-based antiepileptic agents and subsequently synthesized the designed compounds and investigated their activity using pentylenetetrazole (PTZ) and maximal electroshock seizure (MES) protocols in mice.

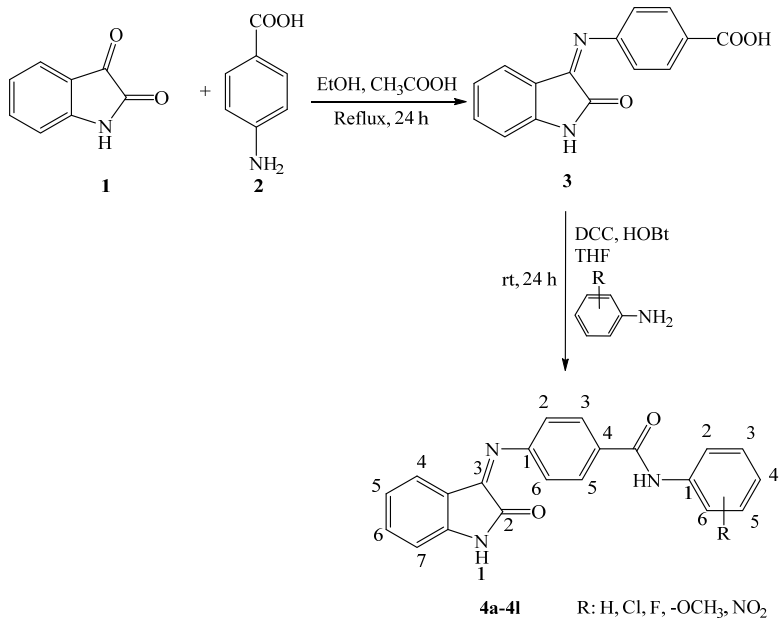
MATERIALS AND METHODS

Chemistry

All solvents as well as starting materials and corresponding reagents were purchased either from Merck or Sigma-Aldrich companies or from their branches. Aluminum based TLC sheets were applied for thin layer chromatography (TLC). Silica gel (70-230 mesh) was utilized for purification of the intermediate and final compounds. Nuclear magnetic resonance (NMR) Bruker 250 MHz apparatus was used for hydrogen-1 NMR (^{1}HNMR) spectra acquisition. All prepared compounds were dissolved in deutrated dimethylsulfoxide (DMSO-d_6) and tetramethylsilane was used as internal standard. Chemical shifts for each proton were presented as δ (ppm) related to tetramethylsilane. Potassium bromide (KBr) disk was prepared for infrared (IR) spectra in Shimadzu 470 spectrophotometer. Mass spectroscopy was performed using a Finigan TSQ-70 spectrometer (Finigan, USA) at 70 eV and mass of each fragment were provided with its frequency percentage. Melting points for final compounds was also obtained using melting point analyzer (electrothermal 9001A) with drugs nested in open capillary tubes.

Synthesis of (Z)-4-((2-oxoindolin-3-ylidene) amino)benzoic acid (3)

In a flat-bottom flask, 3 g (0.020 mmol) of isatin (MW: 147 g/mol) was mixed with 2.80 g (0.020 mmol) *p*-aminobenzoic acid (PABA, MW: 137 g/mol) and the resultant mixture was dissolved in absolute ethanol (40 mL). Catalytic amount (1 mL) of glacial acetic acid was added to the reaction medium and refluxing condition was applied overnight (Scheme 1).



Scheme 1. Synthetic pathway for preparation of compounds **4a-4l**.

TLC was carried out for reaction monitoring and also for determination of the reaction end. Then, ethanol was removed under reduced pressure and water/ethylacetate (50/50) was added to the residue. Organic layer was separated and washed twice by diluted sulfuric acid and consequently two times by brine. Ethylacetate was evaporated using rotary evaporator apparatus and the obtained precipitate was washed by *n*-hexane and diethyl ether (Et_2O). Column chromatography (ethylacetate/petroleum ether 90:10) was applied for final purification (17).

***General procedure for synthesis of (Z)-4-((2-oxoindolin-3-ylidene)amino)-N-phenyl benzamide derivatives* (4a-4l)**

In a flat bottom flask, 0.200 g (0.75 mmol) of compound **3**, were treated with 0.155 g (0.75 mmol) dicyclohexyl carbodiimide (DCC) and 0.102 g (0.75 mmol) hydroxybenzotriazole in 20 mL tetrahydrofuran (THF). Equimolar quantity of appropriate aniline derivative was also added to the reaction medium. The reaction container was put in an ice bath and stirred for 1 h. Then, stirring was continued for 24 h at room temperature.

TLC was used to determine the end of the reaction. Reaction mixture was filtered and solvent was removed using rotary evaporator. Ethylacetate/water (50/50 mL) was added to the obtained residue and aqueous layer was discarded. Organic layer was washed tree times with diluted sulfuric acid 1%, sodium bicarbonate 2% and finally two times by brine. Organic phase was dried over anhydrous sodium sulfate and then filtered. Ethylacetate was evaporated and obtained powder was washed with *n*-hexane and (Et_2O). The obtained powder was purified by column chromatography (ethylacetate/petroleum ether 90:10) (21-26).

Anticonvulsant and neurotoxicity identification tests

The new mentioned compounds were undertaken for the anticonvulsant screening procedure by the anticonvulsant drug development program protocol (27). Adult male NMRI mice weighing between 20-25 g (purchased from Iran Pasteur Institute and nurtured in a temperature-controlled room under 12-h light/dark cycle) were used as experimental animals. Animals were treated in accordance with the ethical principles approved in ethical committee of Kermanshah University of Medical Sciences (ID No. 92281). The screening was performed using PTZ (Sigma Aldrich, Germany), and MES models of seizures. Drugs were dissolved in 0.5% methylcellulose and administered intraperitoneally (i.p.) in a volume of 0.01 mL/g body weight. In the preliminary screening procedure (phase I) drugs were given at doses of 30, 100, or 300 mg/kg at 0.5 h and 4 h time intervals. Neurotoxicity screening was also performed using the rotarod test. PTZ was dissolved in 0.9% saline and used at the dose of 85 mg/kg (subcutaneous). In the MES test, 60-Hz current (50 mA) was delivered for 0.2 second through corneal electrodes in each mice (the MES device was made by Borj Sanat corporation, Tehran, Iran). Phase I tests were performed according to the standard procedure provided by the antiepileptic drug development program (27).

In the MES test after the shock was delivered animals were observed to see if they show a tonic extension in their hind limbs. A tonic extension is defined as the full extension (180°) of the hind limbs with the plane of the body. Absence of this component means that the test compound was able to prevent the spreading of seizure discharge in the brain.

In the PTZ test animals were observed for 30 minutes after the PTZ injection and absence of a clonic spasm persisting for at least 5 seconds meant that the test substance has the ability to raise the seizure threshold.

In the rotorod test the speed of the rod was set at 6 rpm. If the animal was unable to maintain its balance in a 1 min challenge test the substance was considered neurotoxic.

Identification of median effective dose and median neurotoxic dose

Median effective dose (ED_{50}) was determined for some of the most active agents in phase I tests. For ED_{50} determination (phase II), groups of eight mice were injected with doses of each compound around the best

dose determined in the screening tests. The statistical method of Litchfield and Wilcoxon was used for determination of ED_{50} (28). Median neurotoxic dose (TD_{50}) was determined using the results of the rotorod test and the same statistical method. Briefly the best determined dose was applied to a group of animals (n = 8) and the number of responding cases were recorded. Then another dose, one half or double the initial dose, was administered to another group of animals. This procedure was repeated until we had at least two doses with 0% animal response and two doses with 100% animal response. As mentioned above the results were analyzed through the statistical log-probit method of Litchfield and Wilcoxon.

RESULTS

Chemistry

According to the Table 1, all target compounds were prepared with an average yield. An imine derivative of isatin (compound **3,** orange powder) was synthesized via the reaction of isatin with *p*-aminobenzoic acid in the presence of catalytic amounts of glacial acetic acid. Final compounds were obtained through the reaction of compound **3** with various derivatives of aniline bearing electron withdrawing as well as electron donating substituents. The corresponding amidation reaction was carried out using dicyclohexyl carbodiimide and hydroxybenzotriazole. Purification was done using column chromatography. Melting points of all synthesized derivatives were measured by melting point analyzer and a range of 166.8-189.6 °C was observed for these series except for compounds **4c** and **4e-4g** that were decomposed over 200 °C. Spectroscopic methods such as ^{1}HNMR, IR, and MS were applied for characterization of synthesized compounds. All compounds were dissolved in DMSO-d_6 for NMR spectra acquisition. Wave numbers for typical functional groups were reported in IR spectra interpretation. Stretching vibrations of carbonyl groups of isatin residue and also amide bond in final derivatives were the main peaks in IR spectra. The main fragments in MS spectra were provided with related abundance. Molecular ion (M^+) peak in some cases were weak and did not observe clearly.

(Z)-4-((2-oxoindolin-3-ylidene)amino)benzoic acid (3)

^{1}HNMR (250 MHz, DMSO-d_6) δ (ppm): 6.67 (d, 2H, *J* = 7.5 Hz, $H_{3,5}$-phenyl), 6.93 (m, 1H, H_5-isatin), 7.07 (m, 1H, H_6-isatin), 7.53 (m, 1H, H_7-isatin), 7.76 (d, 2H, *J* = 7.5 Hz, $H_{2,6}$-phenyl), 8.11 (d, 1H, *J* = 7.5 Hz, H_4-isatin), 10.90 (brs, NH-isatin), 11.10 (brs, -COOH). IR (KBr, cm^{-1}) ῡ: 3275 (stretch, NH), 1743 (stretch, C=O, acid), 1689 (stretch, C=O, isatin). MS (m/z, %): 266 (75), 238 (100), 221 (15), 211 (12), 148 (15), 137 (15), 121 (25), 90 (15), 76 (20).

Table 1. Properties of compounds **4a-4l**.

Compound	R	Molecular formula	Molecular weight (g/mol)	Melting point (°C)	Yield (%)
4a	H	$C_{21}H_{14}N_3O_2$	341	188.5	80
4b	2-Cl	$C_{21}H_{14}ClN_3O_2$	376	187.3	32
4c	3-Cl	$C_{21}H_{14}ClN_3O_2$	376	> 200 (decomposed)	39
4d	4-Cl	$C_{21}H_{14}ClN_3O_2$	376	189.6	51
4e	2-F	$C_{21}H_{14}FN_3O_2$	360	> 200 (decomposed)	37
4f	3-F	$C_{21}H_{14}FN_3O_2$	360	> 200 (decomposed)	43
4g	4-F	$C_{21}H_{14}FN_3O_2$	360	> 200 (decomposed)	61
4h	3-NO_2	$C_{21}H_{14}N_4O_4$	387	189.6	39
4i	4-NO_2	$C_{21}H_{14}N_4O_4$	387	191.3	59
4j	2-OCH_3	$C_{22}H_{17}N_3O_3$	372	186.4	88
4k	3-OCH_3	$C_{22}H_{17}N_3O_3$	372	186.4	39
4l	4-OCH_3	$C_{22}H_{17}N_3O_3$	372	166.8	27

*(Z)-4-((2-oxoindolin-3-ylidene)amino)-N-phenylbenzamide (**4a**)*

^{1}HNMR (250 MHz, DMSO-d_6) δ (ppm): 6.80 (m, 5H, aromatic), 6.92 (d, 2H, *J* = 7.5 Hz, $H_{3,5}$-phenyl), 7.07 (t, 1H, H_5-isatin), 7.33 (t, 1H, H_6-isatin), 7.49 (d, 1H, *J* = 7.5 Hz, H_7-isatin), 7.69 (d, 2H, *J* = 7.5 Hz, $H_{2,6}$-phenyl), 7.78 (d, 1H, *J* = 7.5 Hz, H_4-isatin), 9.44 (brs, NH-isatin), 10.97 (brs, NH-amide). IR (KBr, cm^{-1}) ῡ: 3251 (stretch, NH), 1747 (C=O, isatin), 1647 (C=O, amide).

*(Z)-N-(2-chlorophenyl)-4-((2-oxoindolin-3-ylidene)amino)benzamide (**4b**)*

^{1}HNMR (250 MHz, DMSO-d_6) δ (ppm): 6.86 (d, 2H, *J* = 8 Hz, $H_{3,5}$-phenyl), 6.86-7.08 (m, 4H, 2-chlorophenyl), 7.38 (d, 1H, *J* = 7.5 Hz, H_4-isatin), 7.48 (t, 1H, *J* = 7.5 Hz, H_5-isatin), 7.69 (d, 1H, *J* = 7.5 Hz, H_8-isatin), 7.95 (d, 1H, *J* = 7.5 Hz, H_7-isatin), 7.97 (d, 2H, *J* = 8 Hz, $H_{2,6}$-phenyl), 9.44 (brs, NH-isatin), 10.97 (brs, NH-amide). IR (KBr, cm^{-1}) ῡ: 3325 (stretch, NH), 1735 (C=O, isatin), 1624 (C=O, amide).

*(Z)-N-(3-chlorophenyl)-4-((2-oxoindolin-3-ylidene)amino)benzamide (**4c**)*

^{1}HNMR (250 MHz, DMSO-d_6) δ (ppm): 6.61 (s, 1H, H_2-3-chlorophenyl), 6.70 (d, 2H, *J* = 7.5 Hz, $H_{3,5}$-phenyl), 6.82 (d, 1H, H_6-2-chlorophenyl),7.23 (m, 2H, 2-chlorophenyl), 7.38 (t, 1H, *J* = 7.5 Hz, H_5-isatin), 7.51 (t, 1H, *J* = 7.5 Hz, H_6-isatin), 7.68 (d, 1H, *J* = 7.5 Hz, H_7-isatin), 7.71 (d, 2H, *J* = 7.5 Hz, $H_{2,6}$-phenyl), 7.95 (d, 1H, *J* = 7.5 Hz, H_4-isatin), 10.23 (brs, NH-isatin), 10.51 (brs, NH-amide). IR (KBr, cm^{-1}) ῡ: 3325 (stretch, NH), 1735 (C=O, isatin), 1624 (C=O, amide).

*(Z)-N-(4-chlorophenyl)-4-((2-oxoindolin-3-ylidene)amino)benzamide (**4d**)*

^{1}HNMR (250 MHz, DMSO-d_6) δ (ppm): 6.73 (d, 2H, *J* = 7.5 Hz, $H_{3,5}$-phenyl), 7.04-7.18 (m, 2H, aromatic), 7.29-7.49 (m, 4H, aromatic), 7.59 (d, 2H, *J* = 7.5 Hz, $H_{2,6}$-phenyl), 7.80-7.96 (m, 2H, aromatic), 10.45 (brs, NH-isatin), 11.02 (brs, NH-amide). IR (KBr, cm^{-1}) ῡ: 3325 (stretch, NH), 1735 (C=O, isatin), 1624 (C=O, amide). MS (*m/z*, %): 357 (M^+, 2), 246 (45), 218 (20), 146 (20), 136 (40), 127 (100), 120 (90), 92 (75), 65 (70).

*(Z)-N-(2-fluorophenyl)-4-((2-oxoindolin-3-ylidene)amino)benzamide (**4e**)*

^{1}HNMR (250 MHz, DMSO-d_6) δ (ppm): 6.75 (d, 2H, *J* = 7.5 Hz, $H_{3,5}$-phenyl), 6.90 (t, 1H, H_6-4-fluorophenyl), 7.05 (t, 1H, *J* = 7.5 Hz, H_5-isatin), 7.41 (t, 1H, *J* = 7.5 Hz, H_6-isatin), 7.46-7.66 (m, 3H, 4-fluorophenyl), 7.56 (d, 2H, *J* = 7.5 Hz, $H_{2,6}$-phenyl), 7.88-8.07 (m, 4H, aromatic), 10.10 (brs, NH-isatin), 10.95 (brs, NH-amide). IR (KBr, cm^{-1}) ῡ: 3325 (stretch, NH), 1732 (C=O, isatin), 1624 (C=O, amide).

*(Z)-N-(3-fluorophenyl)-4-((2-oxoindolin-3-ylidene)amino)benzamide (**4f**)*

^{1}HNMR (250 MHz, DMSO-d_6) δ (ppm): 6.80 (d, 2H, $H_{3,5}$-phenyl), 6.95 (m, 1H, aromatic), 7.10 (t, 1H, H_4-3-fluorophenyl), 7.26 (t, 1H, H_6-isatin), 7.27 (t, 1H, 3-fluorophenyl), 7.50 (dd, 1H, 3-fluorophenyl), 7.63 (d, 2H, *J* = 7.5 Hz, $H_{2,6}$-phenyl), 7.73-7.95 (m, 2H, aromatic), 9.85 (brs, NH-isatin), 10.95 (brs, NH). IR (KBr, cm^{-1}) ῡ: 3325 (stretch, NH), 1732 (C=O, isatin), 1651 (C=O, amide).

*(Z)-N-(4-Fluorophenyl)-4-((2-oxoindolin-3-ylidene)amino)benzamide (**4g**)*

^{1}HNMR (250 MHz, DMSO-d_6) δ (ppm): 6.92 (d, 2H, *J* = 7.5 Hz, $H_{2,6}$-phenyl), 7.06 (t, 2H, *J* = 7.5 Hz, $H_{2,6}$-4-fluorophenyl), 7.39 (t, 1H, H_6-isatin), 7.47-7.58 (m, 4H, aromatic), 7.65 (d, 1H, *J* = 7.5 Hz, H_7-isatin), 7.96 (d, 1H, *J* = 7.5 Hz, H_4-isatin), 10.11 (brs, NH-isatin), 10.16 (brs, NH-isatin), 10.97 (brs, NH-amide). IR (KBr, cm^{-1}) ῡ: 3325 (stretch, NH), 1732 (C=O, stretch, isatin), 1620 (C=O, stretch, amide). MS (*m/z*, %): 359 (M^+, weak), 224 (20), 147 (40), 119 (100), 110 (45), 91 (50), 76 (30).

*(Z)-N-(3-Nitrophenyl)-4-((2-oxoindolin-3-ylidene)amino)benzamide (**4h**)*

^{1}HNMR (250 MHz, DMSO-d_6) δ (ppm): 6.76 (d, 2H, *J* = 7.5 Hz, $H_{3,5}$-phenyl), 6.92 (d, 1H, *J* = 7.5 Hz, H_7-isatin), 7.05 (t, 1H, *J* = 7.5 Hz, H_5-isatin), 7.25 (t, 1H, *J* = 7.5 Hz,

H_6-isatin), 7.43-7.58 (m, 3H, aromatic), 7.69 (s, 1H, H_2-3-nitrophenyl), 7.96 (d, 2H, *J* = 7.5 Hz, $H_{2,6}$-phenyl), 8.05 (d, 1H, H_4-3-nitrophenyl), 10.12 (brs, NH-isatin), 10.92 (brs, NH-amide). IR (KBr, cm^{-1}) ῡ: 3344 (stretch, NH), 1716 (C=O, stretch, isatin), 1608 (C=O, stretch, amide), 1523 (asymmetric stretch, NO_2), 1338 (symmetric stretch, NO_2).

*(Z)-N-(4-Nitrophenyl)-4-((2-oxoindolin-3-ylidene)amino)benzamide (**4i**)*

^{1}HNMR (250 MHz, DMSO-d_6) δ (ppm): 6.63 (d, 2H, *J* = 7.5 Hz, $H_{3,5}$-phenyl), 6.74 (d, 1H, *J* = 7.5 Hz, H_7-isatin), 6.92 (d, 2H, *J* = 7.5 Hz, $H_{2,6}$-4-nitrophenyl), 7.05 (t, 1H, *J* = 7.5 Hz, H_5-isatin), 7.40 (t, 1H, *J* = 7.5 Hz, H_6-isatin), 7.52 (d, 2H, *J* = 7.5 Hz, $H_{3,5}$-4-nitrophenyl), 7.63 (d, 1H, *J* = 7.5 Hz, H_4-isatin), 7.94 (d, 2H, *J* = 7.5 Hz, $H_{2,6}$-phenyl), 9.91 (brs, NH-isatin), 10.95 (brs, NH-amide). IR (KBr, cm^{-1}) ῡ: 3360 (stretch, NH), 1735 (C=O, stretch, isatin), 1627 (C=O, stretch, amide), 1500 (asymmetric stretch, NO_2), 1303 (symmetric stretch, NO_2). MS (*m/z*, %): 386 (M^+, weak), 267 (25), 239 (30), 224 (45), 145 (30), 138 (100), 120 (75), 120 (70), 110 (60), 92 (45), 76 (20).

*(Z)-N-(2-Methoxyphenyl)-4-((2-oxoindolin-3-ylidene)amino)benzamide (**4j**)*

^{1}HNMR (250 MHz, DMSO-d_6) δ (ppm): 3.81 (s, 3H, -OCH_3), 6.68 (d, 1H, H_3-2-methoxyphenyl), 6.87 (d, 1H, *J* = 7.5 Hz, $H_{2,6}$-phenyl), 7.02 (t, 1H, H_5-2-methoxyphenyl), 7.32 (t, 1H, H_4-2-methoxyphenyl), 7.42 (t, 1H, H_5-isatin), 7.46 (d, 1H, H_6-isatin), 7.54 (t, 1H, H_4-isatin), 7.60 (d, 1H, *J* = 7.5 Hz, $H_{3,5}$-phenyl), 7.88 (d, 1H, *J* = 7.5 Hz, H_7-isatin), 7.99 (d, 1H, *J* = 7.5 Hz, H_6-2-methoxyphenyl), 9.89 (brs,, NH-isatin), 11.04 (brs, NH). IR (KBr, cm^{-1}) ῡ: 3325 (stretch, NH), 1728 (C=O, stretch, isatin), 1624 (C=O, stretch, amide).

*(Z)-N-(3-Methoxyphenyl)-4-((2-oxoindolin-3-ylidene)amino)benzamide (**4k**)*

^{1}HNMR (250 MHz, DMSO-d_6) δ (ppm): 3.69 (s, 3H, -OCH_3), 6.86 (d, 2H, *J* = 7.5 Hz, $H_{3,5}$-phenyl), 7.03 (t, 1H, *J* = 7.5 Hz, H_5-3-methoxyphenyl), 7.36 (t, 1H, *J* = 7.5 Hz, H_5-isatin), 7.46 (d, 2H, *J* = 7.5 Hz, $H_{2,6}$-phenyl), 7.50 (t, 1H, *J* = 7.5 Hz, H_6-isatin), 7.54 (m, 2H, *J* = 7.5 Hz, 3-methoxyphenyl), 7.66 (d, 1H, *J* = 7.5 Hz, H_7-isatin), 7.93 (d, 1H, *J* = 7.5 Hz, H_4-isatin), 10.07 (brs, NH-isatin), 10.99 (brs, NH-amide). IR (KBr, cm^{-1}) ῡ: 3329 (stretch, NH), 1732 (C=O, isatin), 1624 (C=O, amide).

*(Z)-N-(4-Methoxyphenyl)-4-((2-oxoindolin-3-ylidene)amino)benzamide (**4l**)*

^{1}HNMR (250 MHz, DMSO-d_6) δ (ppm): 3.80 (s, 3H, -OCH_3), 6.82 (d, 2H, *J* = 7.5 Hz, $H_{3,5}$-4-methoxyphenyl), 6.86 (d, 2H, $H_{3,5}$-phenyl), 6.90 (d, 2H, *J* = 7.5 Hz, $H_{2,6}$-4-methoxyphenyl), 7.05 (t, 1H, H_5-isatin), 7.53 (t, 3H, H_6-isatin), 7.64 (d, 1H, *J* = 7.5 Hz, H_7-isatin), 7.73 (d, 2H, $H_{2,6}$-phenyl), 7.82 (d, 1H, *J* = 7.5 Hz, H_7-isatin), 9.45 (brs, NH-isatin), 10.95 (brs, NH-amide). IR (KBr, cm^{-1}) ῡ: 3325 (stretch, NH), 1732 (C=O, stretch, isatin), 1624 (C=O, stretch, amide). MS (*m/z*, %): 371 (M^+, weak), 329 (20), 224 (45), 165 (20), 143 (30), 123 (95), 99 (35), 56 (100).

Anticonvulsant assay

As seen in Table 2 the molecules are divided into four classes based on their results in MES test. (1) anti-seizure activity at 100 mg/kg or less, (2) anti-seizure effects at doses higher than 100 mg/kg, (3) compound having no activity at any doses up to 300 mg/kg, and (4) agents inactive at 300 mg/kg and toxic at 30 mg/kg or less (27). Although most agents are classified as group 1 compounds the screening results show that compounds **4a, 4b,** and **4h** have anti-seizure effects at the minimum dose of 30 mg/kg and compounds **4g** and **4l** have anti-seizure activity at the minimum dose of 100 mg/kg. These are actually the most active compounds which were selected for further evaluation in the phase II tests. The results of phase II tests show (Table 3) that all the selected compounds have a better protective index (PI) than diazepam (PI = 0.07). PI = TD50/ED50 was calculated for the MES test. When there was a solubility limitation the results were reported as being greater than the last meaningful PI (Table 3).

Table 2. Anti-seizure activities after intraperitoneal administration of compounds **4a-4l** in mice.

Compound	R	Class	Dose (mg/kg)	Activity MES[a,*] Time (h)		TOX[b,*] Time (h)		Activity PTZ[c,*] Time (h)	
		1, 2, 3, 4		0.5	4	0.5	4	0.5	4
4a	H	1	30	1/1	1/1	0/4	0/2	1/1	1/1
			100	3/3	3/3	0/8	0/4	1/1	1/1
			300	1/1	1/1	0/4	0/2	1/1	1/1
4b	2-Cl	1	30	1/1	1/1	0/4	0/2	1/1	1/1
			100	3/3	3/3	0/8	0/4	1/1	1/1
			300	1/1	1/1	4/4	2/2	1/1	1/1
4c	3-Cl	3	30	0/1	0/1	0/4	0/2	0/1	0/1
			100	0/3	0/3	0/8	0/4	0/1	0/1
			300	1/1	1/1	0/4	0/2	1/1	1/1
4d	4-Cl	1	30	1/1	0/1	0/4	0/2	0/1	0/1
			100	2/3	0/3	0/8	4/4	1/1	0/1
			300	1/1	0/1	0/4	2/2	1/1	0/1
4e	2-F	1	30	0/1	0/1	0/4	2/2	0/1	0/1
			100	0/3	2/3	0/8	4/4	1/1	1/1
			300	1/1	1/1	0/4	2/2	1/1	1/1
4f	3-F	3	30	0/1	0/1	0/4	0/2	0/1	0/1
			100	0/3	0/3	8/8	4/4	0/1	0/1
			300	1/1	1/1	4/4	2/2	1/1	0/1
4g	4-F	1	30	0/1	0/1	0/4	0/2	0/1	0/1
			100	3/3	3/3	0/8	0/4	1/1	1/1
			300	1/1	1/1	0/4	0/2	1/1	1/1
4h	3-NO_2	1	30	1/1	1/1	0/4	0/2	1/1	1/1
			100	3/3	3/3	0/8	0/4	1/1	1/1
			300	1/1	1/1	0/4	0/2	1/1	1/1
4i	4-NO_2	1	30	0/1	0/1	0/4	0/2	0/1	0/1
			100	1/3	0/3	0/8	4/4	0/1	0/1
			300	0/1	0/1	4/4	2/2	0/1	0/1
4j	2-OCH_3	1	30	0/1	0/1	0/4	0/2	0/1	0/1
			100	3/3	3/3	0/8	0/4	0/1	0/1
			300	1/1	1/1	4/4	2/2	1/1	1/1
4k	3-OCH_3	1	30	0/1	0/1	0/4	0/2	1/1	1/1
			100	0/3	3/3	0/8	0/4	1/1	1/1
			300	1/1	1/1	0/4	0/2	1/1	1/1
4l	4-OCH_3	1	30	0/1	0/1	4/4	0/2	0/1	0/1
			100	3/3	3/3	8/8	0/4	1/1	1/1
			300	1/1	1/1	4/4	0/2	1/1	1/1

[a] MES, maximal electroshock seizure test; [b] TOX, rotarod toxicity test; [c] PTZ, subcutaneous pentylenetetrazole seizure threshold test.
[*] Number protected or toxic/number tested.

Table 3. Median effective dose (ED_{50}) and median neurotoxic dose (TD_{50}) of selected compounds.

Compound	R	MES[a] test ED_{50} (µmol/kg)	Rotarod test TD_{50} (µmol/kg)	PI	PTZ[b] test ED_{50} (µmol/kg)
	3-NO_2	53	0% [c] (775) [d]	> 14.6[e]	46.12
	2-Cl	55	230	4.18	50.87
	4-OCH_3	205	1286	6.27	176.8
	4-F	204	0% (833)	> 4.08	194.3
	H	60	0% (879)	> 14.6	60.82
Diazepam	-	7.5	0.52	0.06	1.763

[a] MES, maximal electroshock seizure test; [b] PTZ, subcutaneous pentylenetetrazole seizure threshold test. [c] The figures outside parenthesis refer to the percentage of fallen mice in rotarod test. [d] The figures in the parenthesis refer to the maximum solubility of the agent in laboratory conditions. [e] The results are reported as being greater than the last meaningful PI When there is a solubility limitation.

DISCUSSION

Anti-seizure activities of all synthesized compounds **4a-4l** were explored using MES and PTZ (Table 1). Compounds **4a**, **4b,** and **4h** were chosen in phase I of the pharmacologic studies based on the fact that they could prevent seizure in most animals used in the screening procedure. All tested doses of these derivatives could not prevent the convulsing effects of MES as well as PTZ. It hypothesized that *ortho* positioning of chlorine atom and also *meta* positioning of nitro moiety on the phenyl residue have beneficial impact on anti-seizure activity. Comparison of compound **4b** (2-Cl) with compound **4e** (2-F) imply that electron withdrawing effect of the corresponding moieties is an interrupting factor, because the enhancement of the electron receiving effect in fluorine atom attenuated the activity. Chlorine has larger size than fluorine and therefore more lipophilicity and steric effect. It could be imagined that steric effect and lipophilicity that associated with chlorine are the fortifying factors. But, according to the potent activity of compound **4a** without any moiety on the phenyl residue, it could be concluded that the presence of chlorine for anti-seizure potency is not a requisite at *ortho* position. Compound **4j** (2-OCH_3) with methoxy substituent at *ortho* position also demonstrated a remarkable activity in dose 300 mg/kg. Compound **4c** (3-Cl) exhibited an acceptable onset and duration of action at dose 300 mg/kg after 0.5 and 4 h in both models. Fluorine substituent was effective in some positions of the phenyl residue. The electron withdrawing effect, hydrogen bond capability and also low steric impact are the featured properties of the fluorine moiety. The fluorine caused an acceptable anti-seizure effect especially at *ortho* and *para* positions (**4e**) (**4g**). Compounds **4e** and **4g** exerted their anti-seizure activity at 100 as well as 300 mg/kg doses in both MES and PTZ models. The onset action for compound **4e** was slower than **4g** in MES model. Compound **4f** (3-F) exhibited its anti-seizure activity solely in 300 mg/kg in MES model. Nitro group rendered a better anti-seizure activity while substituted at *meta* position compared to *para* position. Nitro group is a strong electron withdrawing as well as hydrophilic moiety. As observed in compounds **4c** (3-Cl) and **4f** (3-F) electron receiving effect is a beneficial effect at *meta* position. However, it is probable that hydrophilic property of nitro moiety at *para* position reduces the anti-seizure effect as seen in compound **4h**. It is interesting that nitro moiety caused a favorable anti-seizure activity when substituted at *meta* position. Chlorine and fluorine groups with lipophilic property showed a superior activity than nitro at *para* position. More hydrophilic characteristic of the nitro group compared to chlorine and fluorine substituents maybe a logical reason for abolishment of the anti-seizure effect. All methoxylated derivatives (**4j**, **4k**, **4l**) showed a significant anti-seizure activity in MES model especially at 100 and 300 mg/kg doses. Compound **4k** (3-OCH_3) was the most active methoxylated derivative in PTZ model. This compound displayed its anti-seizure effect in all tested doses in PTZ model. But, compounds **4j** exhibited its anti-seizure activity in 300 mg/kg dose and compound **4l** showed this effect at both 100 and 300 mg/kg. Comparison of the anticonvulsant activity of newly synthesized compounds with our previously reported results (19) demonstrated that using *p*-aminobenzoic acid as the linker in these series did not cause an increase in anticonvulsant activity rather than compounds without this linker. *p*-aminobenzoic acid attenuated the anticonvulsant potency in both of utilized methods namely PTZ and MES.

Neurotoxicity of tested compounds was investigated using rotarod protocol. All tested derivatives demonstrated a high safety in tested dose 30 mg/kg except compounds **4e** (2-F) and **4l** (4-OCH_3) (Table 2). Compound **4e** did not render any toxicity after 0.5 h of injection of tested compounds but the toxicity was observed after 4 h at all tested dose levels. Compound **4l** displayed the most toxicity among the tested derivatives. Namely, 0.5 h after injection of compound **4l** the neurotoxicity was induced in mice. Overall, tested compounds showed a high safety. Neurotoxicity was observed merely at dose levels higher than 30 mg/kg in some cases.

CONCLUSION

A new series of isatin-based anti-seizure derivatives were designed, synthesized and their activity was investigated by MES and PTZ models in mice. These compounds displayed a remarkable protective activity in both utilized convulsing models. A low level of toxicity was also observed for all tested derivatives in rotarod protocol. All methoxylated derivatives (**4j**, **4k**, **4l**) showed a significant anti-seizure activity in MES model. Compounds **4j** (2-OCH_3) and **4l** (4-OCH_3) also demonstrated a potent anti-seizure activity against PTZ. Compound **4k** (*m*-OCH_3) did not cause protection towards PTZ induced convulsion.

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