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Research article

Anticonvulsant activity of some semicarbazone and thiosemicarbazone derivatives of isatin on PTZ induced seizure

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Abstract

Epilepsy is a syndrome characterized by excessive discharge of many neurons. In spite of the optimal use of available antiepileptic drugs; seizures in lots of patients fail to be controlled. On the other hand many patients experience side effects which can limit the use of these drugs. Hence, investigation on chemicals with anticonvulsant properties and lower side effects are a valuable task. Isatin is a heterocyclic compound with different biological properties. We synthesized 14 isatinsemicarbazone and thiosemicarbazone derivatives ***(a-n)*** in our previous study. Here we are going to evaluate these compounds for their anticonvulsant effects.

We performed Pentylenetetrazole (PTZ) model (acute) in male mice (85mg/kg) and chemical kindling model (chronic) in male rats (37.5 mg/kg each 48 h for 14-20 days). Intraperitoneal injection was used to induce seizures in rodents.

In the acute test, compounds ***b, d, f, I, j, k, l***, ***m*** and ***n***, with 3 doses of 10, 20 and30 mg/kg showed significant anticonvulsant effects in comparison to the control group and increase the seizure onsets and the time of death ($p<0.05$). In the kindling model, derivatives ***k*** and ***n*** were selected as effective compounds with doses of 10, 30 mg/kg. These derivatives significantly prevented the epileptogenesis and improved motor coordination in Rotarod test. In conclusion, these compounds seem to be potential anticonvulsant agents with low side effects.

Introduction

Epilepsy is characterized by recurrent impulsive seizures of cerebral origin, donating with episodes of sensory, motor or autonomic phenomenon with or without loss of awareness. About 1%of the world populace has epilepsy, with nearly 90% of these individuals being in developing countries [1]. Epilepsy is a variety of disorders reflecting underlying brain dysfunction that may result from many diverse reasons [2]. Glutamate and γ-aminobutyric acid (GABA) are two important excitatory and inhibitory neurotransmitters in epilepsy [3]. Epilepsy may occur at any age, but the maximum occurrence is during the first years of life and after the age of 65 years. It has different symptoms such as staring, muscle stiffness (tonic movements), muscle spasms (clonic movements), and impaired consciousness[4]. The cellular and neurocircuit base of epilepsy is not well understood [5]. Treatment of epilepsy was improved by several third generation of anticonvulsant drugs duringthe past decades. But, resistance to antiepileptic drugs and intolerability in 20-30%of the patients led to the fact that currently available antiepileptic drugs (AEDs) are symptomatically ineffective in25-35% patients. However there are serious demands for developing new drugs or strategies for epilepsy treatment [6,7]. Therefore search and investigation for new antiepileptic drugs (AED) with better efficacy and lesser side effects remains an essential goal.

Isatin and its derivatives have different biological effects, including anti-inflammatory, antibacterial, antifungal, antiviral, antituberculosis, anticancer, anti-HIV and anticonvulsant [8-13]. During initial screening of isatin products, they have shown good activity in the maximum electroshock seizure (MES) test [14, 15]. In the semicarbazone analogues of isatin it has been proposed a binding site hypothesis for these compounds eliciting anticonvulsant activity. A revision for certain selected structures as active anticonvulsant agents has been shown to possess hydrophobic units, an electron donor group and hydrogen donor acceptor unit as shown in Figure1[14].

There are several reports on the anti-convulsant activity of some isatin derivatives. In one report 3-substituted isatin compounds bearing electron donating groups exhibited good anticonvulsant activity while those with electron withdrawing substituent showed less activity in both the models maximum electroshock (MES) and subcutaneous metrazole (ScMet) (Figure 2A) [16].

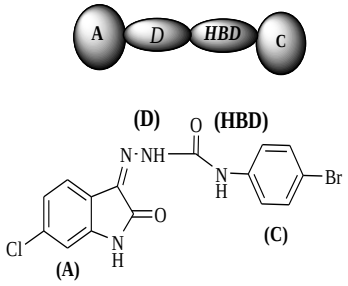
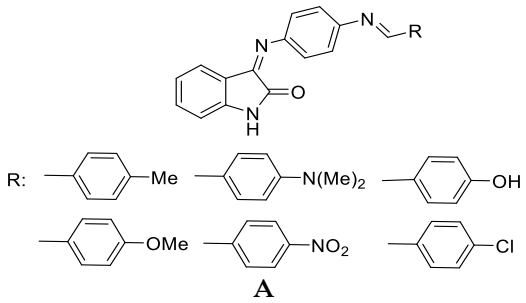


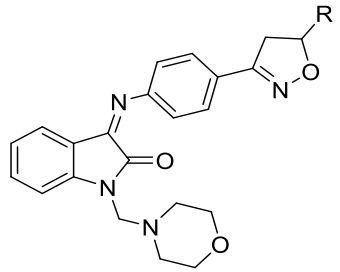
Figure 1. Pharmacophore model suggested having anti-convulsant activity. A and C are hydrophobic binding site, D is an electron donor group, HBD is hydrogen-binding domain site.

In another study synthesis, characterization and anticonvulsant activity of 1-(morpholinomethyl)-3-substituted isatin derivatives was reported. The synthesized compounds were investigated for antiepileptic activity using MES and scPTZ seizures tests. Neurotoxicity study was performed by the rotarod test. The relationship between the functional group variation and the biological activity of the evaluated compounds shows the isoxazole ring with electron donating groups exhibited higher antiepileptic activity than the electron withdrawing groups (Figure 2B).

As part of our ongoing research towards the development of anticonvulsant agents, we synthesized a variety of isatin β-thiosemicarbazone, and isatin semicarbazone derivatives in our previous study [17]. Herein, we are going to evaluate their anticonvulsant activity in a PTZ model induced seizure.



A



R: electron donatig groups

B

Figure 2. Some isatin scaffold as anticonvulsant agents

Experimental

Material and Methods

All solvents and chemicals were of analytical grade and were obtained from Merck, Germany. PTZ and diazepam were purchased from Sigma-Aldrich Co., USA. All compounds were suspended in water and Tween 80 (3% W/V) and administered intraperitoneally (i.p.). Solutions were prepared on a weight/volume basis on the day of use. Isatin derivatives and diazepam were dissolved in DMSO and saline (1:1 v/v) and were administered in volume of 0.1 ml/10 g of mice and 1 ml/kg of rat body weight. PTZ was dissolved in physiologic saline and administered i.p.

A series of isatin derivatives including semicarbazone ***(a-i)*** and thiosemicarbazone ***(j-n)*** analogues which were synthesized previously were choose (Table 1). Anticonvulsant evaluations of the compounds were done by PTZ induced seizure test and diazepam was used as positive control. PTZ-induced kindling models were also used to elucidate the possible mechanism of action of these compounds. Finally a rotarod, motor coordination test was likewise complete to assess sedation and strength / stamina.

Male NMRI mice, weighting about 20-25g at the beginning of the experiment were used in the PTZ-induced seizure test and adult male Wistar rats (150-170 g) were used in the PTZ-induced kindling model. The animals were housed in standard Plexiglas cages in a temperature-controlled room (23±2°C) on a 12-h light/dark cycle with free access to food and water and acclimated at least 2 days before the experiments. The protocol for this project was approved by the ethics committee of the university. Wholly experiments were performed according to the institutional guidelines for animal care and use. The experiments took place between 10 AM and 3 PM, and totally possible measures were taken to minimize animals' discomfort during the procedures. Each treatment group consisted of at least six animals.

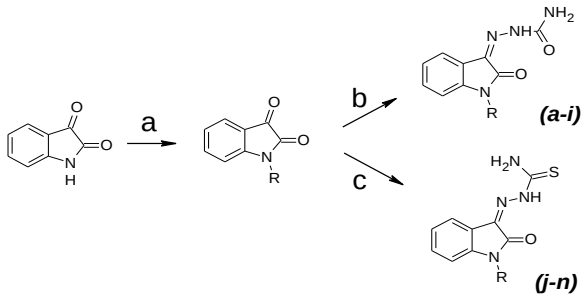
Synthesis

N-alkylated isatins were prepared by using of calcium hydride in DMF. Then in the next step, prerequisite N-substituted isatin intermediates were used to synthesize the desired semicarbazone and thiosemicarbazone analogs of isatin.

N-substituted isatinswere reacted with semicarbazide in ethanol containing a catalytic amount of glacial acetic acid under reflux conditions to give the corresponding isatin semicarbazones ***(a-i)***.

Thiosemicarbazone derivatives were synthesized from N-substituted isatin derivatives. For this purpose, analogs of the synthesized N-substituted isatins were reacted with thiosemicarbazide in ethanol containing a catalytic

amount of glacial acetic acid under reflux conditions to give the corresponding isatin thiosemicarbazones ***(j-n)***.



a: CaH_2, DMF, b: semicarbazide, ethanol, acetic acid, c: thiosemicarbazide, ethanol, acetic acid

Scheme 1. General procedure for synthesis of isatin derivatives

Data for (Z)-1-(1-sec-butyl-2-oxoindolin-3-ylidene) semicarbazide (a)

Pure yellow crystals (2.3 g, 89%),mp 178-179º C, R_f(EtOAc/*n*-hexane, 1:3) 0.41, ^{1}H-NMR (250 MHz, DMSO-d_6) δ 10.24 (1H, b.s), 7.02-8.09 (4H, m), 6.86 (2H, s), 4.27-4.33 (1H, m), 1.93-1.99 (2H, m) 1.35 (3H, d, *J*=7.5 Hz), 0.75 (3H, t, *J*=5 Hz),^{13}C-NMR (62.5 MHz, DMSO-d_6). δ 163.27, 155.87, 142.91, 132.26, 131.37, 125.10, 121.60, 115.19, 110.03, MS (EI, 70 eV) *m*/*z* (%): 260.31 $[M+H]^+$ (260.30 calcd for $C_{13}H_{16}N_4O_2$), *Anal.Calcd*. for $C_{13}H_{16}N_4O_2$: C, 59.99, H, 6.20, N, 21.79. *Found*: C, 59.79, H, 6.27, N, 21.95, IR (KBr) $\nu_{max.}$: 3372, 3342, 1676, 1598, 1453, 650.

Data for (E)-1-(1-cyclopentyl-2-oxindolin-3-yeliden) semicarbazide (b)

In a double-neck round bottomed flask (100 ml,) a mixture of 1-cyclopentylindoline-2,3-dione (***10a***) (2.15 g, 0.01 mol), semicarbazide(1.12 g, 0.015 mol) and catalytic amount of acetic acid (2-3 drops) was dissolved in ethanol (15 ml). The solution was refluxed for 7 hours. After this time the reaction was completed as indicated by TLC. The reaction mixture was kept in a refrigerator overnight. Filtration of the reaction mixture followed by washing with cool ethanol (2×5 ml) and recrystalization from MeOH/H_2O gave a pure, yellow crystalline solid. (2.3 g, 85%), mp 215-216º C, R_f(EtOAc/*n*-hexane, 1:8) 0.53, ^{1}H-NMR (250 MHz, DMSO-d_6) δ 10.16 (1H, br. s), 7.30-8.10 (4H, m), 6.84 (2H, br. s), 4.04 (1H, m), 1.60-2.06 (8H, m), ^{13}C-NMR (62.5 MHz,DMSO-d_6) δ 163.14, 155.88, 154.84, 142.63, 131.32, 130.10, 121.69, 115.31, 109.86, 51.87, 27.43, 24.55, MS (EI, 70 eV) *m*/*z* (%): 272.33 $[M+H]^+$ (272.31 calcd for $C_{14}H_{16}N_4O_2$), *Anal. Calcd*. For $C_{14}H_{16}N_4O_2$: C, 58.31, H, 5.59, N, 19.43. *Found*: C, 58.70,H, 6.01, N, 19.20, IR (KBr) ν_{max}: 3455, 3238, 3165, 2957, 1666, 1638, 2985, 1203, 692.

Data for (E)-1-(1-allyl-2-oxoindolin-3-yeliden) semicarbazide (c)

Pure yellow crystalline (2.2 g, 91%),m.p 218-220º C, R_f(EtOAc/n-hexane, 1:5) , ^{1}H-NMR (250 MHz, DMSO-d_6): δ 10.17,(broad singlet, 1H.=N-NH) 6.98-8.12, (complex, 4H, isatin), 6.87, (broad singlet, 2H, NH2) 5.75-5.88 (m, 1H, =CH) 5.08-5.15(dd, 2H, =CH2, *J*=7.5-10) 4.33-4.35 (d, 2H, N-CH2, *J*=5),^{13}C-NMR (62.5MHz, DMSO-d_6):δ 64.89, 109.64, 114.30, 120.76, 121.99, 124.84, 129.43, 131.33, 143.37, 155.82, 157.98, 163.5,Anal.Calcd.for $C_{12}H_{12}N_4O_2$: C, 59.01, H, 4.95, N, 22.94,O,13.10. Found: C, 58.98, H, 4.93, N, 22.89, O, 13.7, IR (KBr) ν_{max}:3352, 3330, 3170, 1676, 1598, 1453, 630.

Data for (E)-1-(2-oxo-1-(2-phenoxy ethyl) indolin-3- ylidene) semicarbazide (d)

Recrystalization from MeOH/H_2O gave(2.9, 89%) gave pure, yellow crystalline solid of corresponding semicarbzide, mp 212-213º C, R_f(EtOAc/n-hexane (0.55), 1:1) 0.72, ^{1}H-NMR (250 MHz, $CDCl_3$): δ 11.51 (bs, 1H, NH), (complex, 11H, aryl, isatin, NH_2), (m, 4H, CH_2-CH_2), ^{13}C-NMR (62.5 MHz, DMSO, d_6):δ 39.75, 64.89, 109.66, 114.30, 115.02, 120.76, 122.00, 124.85, 129.44, 131.34, 132.09, 143.37, 155.82, 157.97, 163.53, Anal. Calcd.for $C_{17}H_{16}N_4O_3$: C, 62.95, H, 4.97, N, 17.27, Found: C, 62.71, H, 5.04, N, 17.80, IR (KBr) ν_{max} : 3396, 3340, 3120, 1696, 1548, 1475, 590.

Data for (Z)-2-(1-(2-methylbenzyl)-2-oxoindolin-3-ylidene) hydrazine-1-carboxamide (e)

Pure yellow crystals (2.6 g, 87%), mp 224-225º C, R_f(EtOAc/*n*-hexane, 1:3) 0.85, ^{1}H-NMR (250 MHz, DMSO- d_6) δ 11.65 (1H, bs), 6.84-8.12 (10H, m), 4.92 (2H, s), 2.35 (3H, s), ^{13}C-NMR (62.5 MHz,, DMSO- d_6) δ 163.5, 157.43, 147.43, 140.72, 134.82, 132.83, 129.43, 128.93, 126.93, 126.73, 125.65, 121.70, 117.87, 40.96, 17.85, MS (EI, 70 eV) *m*/*z* (%): 308.35 $[M+H]^+$(308.34 calcd for $C_{17}H_{16}N_4O_2$),*Anal. Calcd*.for $C_{17}H_{16}N_4O_2$: C, 66.22, H, 5.23, N, 18.17. *Found:* C, 66.12.0, H, 5.8, N, 18.09, IR (KBr)ν_{max}: 3475, 3289, 3240, 1687, 1642, 1443, 740.

Data for (Z)-1-(1-(but-3-en-2-yl)-2oxindolin-3 ylidene) semicarbazide (f)

Pure yellow crystals (2.4 g, 95%), mp 202-203º C, R_f(EtOAc/*n*-hexane, 1:3) 0.50, ^{1}H-NMR (250 MHz, DMSO-d_6) δ 12.26 (1H, s), 7.50-8.51 (4H, m), 7.39 (2H, b.s) 5.75-5.88 (1H, m) 5.08-5.15(2H, dd, *J*=7.5-10 Hz) 4.33-4.35 (1H, m, *J*=5Hz) 3.15 (3H, d),^{13}C-NMR (62.5 MHz, DMSO-d_6) δ 178.64, 160.82, 142.78, 135.59, 132.98, 131.00, 130.86, 127.27, 125.87, 122.98, 120.75, 119.50, 110.38, 18.83, MS (EI, 70 eV) *m*/*z* (%): 258.29 $[M+H]^+$ (258.28 calcd for $C_{13}H_{14}N_4O_2$), *Anal. Calcd*.for $C_{13}H_{14}N_4O_2$: C, 60.45, H, 5.46, N, 21.69. *Found*: C,

60.61, H, 5.92, N, 21.85, IR (KBr) $\nu_{max.:}$3352, 3330, 3170, 1676, 1598, 1453, 630.

Data for (Z)-1-(2-oxo-1(2-oxo-3-phenoxypropyl) indolin- ylidene) semicarbazide (g)

Pure yellow crystals (3.0 g, 86%), mp 196-197º C, *R*f(EtOAc/*n*-hexane, 3:1) 0.63, ^{1}H-NMR (250 MHz, DMSO-d_6): δ 10.29 (1H , b.s), 7.80 -8.11 (9H, m), 6.88 (2H, b.s) 5.21 (2H, s) 4.71 (2H, s), ^{13}C-NMR (62.5 MHz, DMSO-d_6) δ 167.81, 163.52, 155.67, 142.78, 135.50, 131.75, 131.38, 128.38, 128.13, 127.89, 124.96, 122.36, 115.06, 109.33, 66.47, 40.97, MS (EI, 70 eV) *m*/*z* (%): 352.37 [*M*+H]$^+$ (352.35 calcd for $C_{18}H_{16}N_4O_4$), *Anal. Calcd*.for $C_{18}H_{16}N_4O_4$: C, 61.36, H, 4.58, N, 15.90. *Found*: C, 61.30, H, 4.47, N, 15.72, IR (KBr) ν_{max}: 3751, 3438, 3291, 3180, 2364, 1752, 1693, 1504, 1468, 699

Datafor(E)-1-(1-butyl-2-oxindolin-3ylidene) semicarbazide(h)

Pure yellow crystals (2.5 g, 88%), mp 197-198º C, *R*f(EtOAc/n-hexane, 1:3) 0.87, ^{1}H-NMR (250 MHz, DMSO-d_6):δ 11.57 (bs, 1H, NH), 7.02-8.13 (complex, 4H, isatin), 6.88 (bs, 2H, NH_2), 5.12 (m, 2H, N-CH_2), 4.05 (m, 2H, N-CH_2-CH_2) 1.48 (m, 2H, CH_2-CH_3) 1.07(t, 3H, terminal CH_3), ^{13}C-NMR (62.5MHz, DMSO-d_6):δ 13.9, 14.11, 48.54, 61.11, 109.35, 115.28, 122.18, 125.18, 131.38, 142.02, 155.77, 162.91,169.71. Anal.Calcd.for $C_9H_{12}N_4O_2$: C, 51.92, H, 5.81, N, 26.91. Found: C, 51.66, H, 5.20, N, 26.70, IR ν_{max}: 34921, 3380, 3167, 1660, 1548, 1472, 730.

Data for (Z)-ethyl 2-(3-(2-carbamoylhydrazono)-2-oxoindolin-1-yl) propanoate (i)

Pure yellow crystals (2.8 g, 93%), mp 194-195º C, *R*f(EtOAc/*n*-hexane, 1:3) 0.85, ^{1}H-NMR (250 MHz, DMSO-d_6) δ 7.03-7.6 (4H, m), 6.87 (1H, b.s), 3.67 (1H, q, *J*=7.5 Hz), 3.33 (2H, b.s) 1.49 (2H, q, *J*=7.5 Hz) 1.23 (3H, d, *J*=7.5Hz), ^{13}C-NMR (62.5 MHz, DMSO-d_6), δ 163.38, 160.70, 156.03, 154.84, 143.11, 130.25, 124.95, 121.85, 115.07, 109.05, 29.05, 19.42, 13.53, 13.49 , MS (EI, 70 eV) *m*/*z* (%): 304.32 [*M*+H]$^+$ (304.31calcd for $C_{14}H_{16}N_4O_4$), *Anal. Calcd*.for $C_{14}H_{16}N_4O_4$: C, 88.26, H, 5.30, N, 18.41. *Found*: C, 88.56, H, 5.50, N, 18.10, IR (KBr) ν_{max}: 3869, 3345, 3263, 3170, 2936, 1725, 1670, 1648, 1078, 683

Data for Synthesis of (Z)-1-(1-allyl-2-oxoindolin-3-yeliden) thiosemicarbazide (j)

Pure yellow crystals (2.4 g, 93%),m.p 216-218º C *R*f(EtOAc/n-hexane, 1:5), 0.79,^{1}H-NMR (250 MHz, DMSO-d_6):δ 12.77,(broad singlet, 1H, =N-NH) 6.83-7.76,(complex,4H ,isatin), 6.80 (broad singlet, 2H, NH2) 5.75-5.88 (m, 1H, =CH) 5.16-5.23(dd, 2H, =CH2,*J*=2.5-15 Hz) 4.31-4.33 (dd, 2H, N-CH2, *J*=2.5, 5Hz),^{13}C-NMR(62.5MHz,DMSO-d_6): δ 61.25, 110.24, 117.33, 119.32, 120.9, 122.85, 130.99, 131.39, 142.60, 160.42, 178.63,Anal.Calcd.for $C_{12}H_{12}N_4OS$: C, 55.37, H, 4.65, N, 21.52. Found: C, 55.98, H, 4.93, N, 21.89, IR (KBr) ν_{max}: 3352, 3330, 3170, 1676, 1598, 1183, 630.

Data for (Z)-1-(1-butyl-2-oxindolin-3ylidene) thiosemicarbazide (k)

Pure yellow crystals (2.3 g, 85%), mp 220-222º C, *R*f(EtOAc/*n*-hexane, 1:3) 0.77, ^{1}H-NMR (250 MHz, DMSO-d_6):δ 12.29 (b.s, 1H, NH), 9.14 (s, 1H, NH (HS-C=NH), 8.83 (s, 1H, SH (HS-C=NH) 7.04-7.76 (complex, 4H, isatin), 5.17 (t, 2H, N-CH_2, *J*=7.1 Hz), 4.09-4.17 (m, 2H, N-CH_2), 1.56-1.67 (m, 2H, N-CH_2-CH_2) 1.097 (t, 3H, terminal CH_3, *J*=7.5), ^{13}C-NMR (62.5MHz, DMSO-d_6):δ 13.88, 48.75, 61.33, 110.23, 119.42, 120.88, 123.00, 130.26, 131.04, 141.70, 160.27, 109.23, 178.68,*Anal. Calcd*.for $C_9H_{12}N_4OS$: C, 56.50, H, 5.84, N, 20.27. *Found*: C, 56.66, H, 5.46, N, 20.70, IR (KBr)ν_{max}: 3452, 3228, 3155, 1664, 1428, 1250, 680

Data for (Z)-2-(1-(2-methylbenzyl)-2-oxoindolin-3-ylidene) hydrazinecarbothioamide (l)

Pure yellow crystals(2.9 g, 90%), mp 231-232º C, *R*f(EtOAc/*n*-hexane, 1:3) 0.75, ^{1}H-NMR (250 MHz, DMSO- d_6) δ 12.38 (1H, br.s), 9.09(1H, s), 8.75 (1H, s) 7.01-7.74 (8H, m), 4.93 (2H, s), 2.35 (3H, s), ^{13}C-NMR (62.5 MHz, DMSO- d_6) δ 178.64, 160.82, 142.78, 135.59, 132.98, 131.00, 130.86, 127.27, 125.87, 122.98, 120.75, 119.50, 110.38, 18.38, MS (EI, 70 eV) *m*/*z* (%): 324.41 [*M*+H]$^+$ (324.40 calcd for $C_{17}H_{16}N_4OS$), *Anal. Calcd*.for $C_{17}H_{16}N_4OS$: C, 62.94, H, 4.97, N, 17.27. *Found:* C, 62.12, H, 5.01, N, 17.09, IR (KBr)ν_{max}: 3452, 3348, 3167, 2995, 2754, 1656, 1468, 1058, 679*3*

Data for (Z)-ethyl 2-(3-(2-carbamo-thioylhydrazono)-2-oxoindolin-1-yl)propanoate (m)

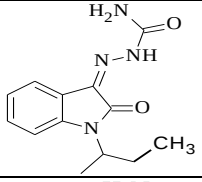
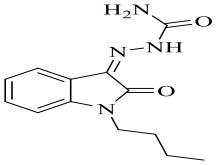
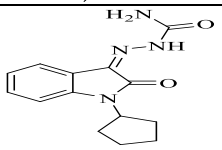
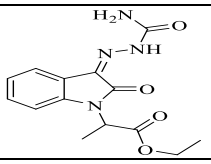
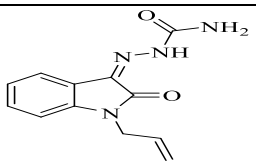
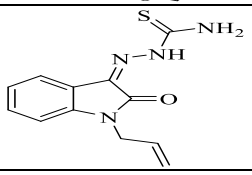
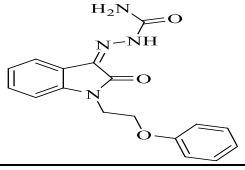
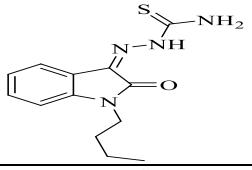
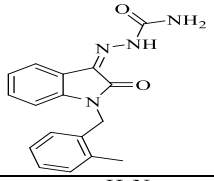
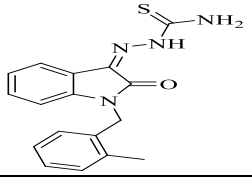
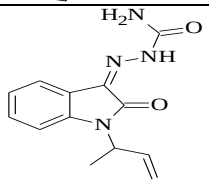
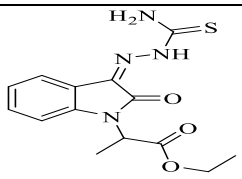
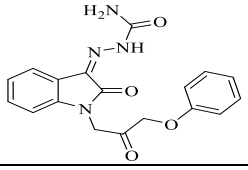
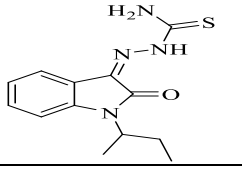
Pure yellow crystals (2.9 g, 92%), mp 98-99º C, *R*f(EtOAc/*n*-hexane, 1:3) 0.75, ^{1}H-NMR (250 MHz, DMSO-d_6) δ 12.46 (1H, br.s), 9.11 (1H, s), 8.76 (1H, s) 7.08-7.74 (4H, m), 5.16-5.25 (1H, q, *J*=7.5 Hz), 4.08-4.17 (2H, q, *J*=7.5 Hz), 1.53 (3H, d, *J*=7.5 Hz) 1.32 (3H, t, *J*=7.5),^{13}C-NMR (62.5 MHz, DMSO-d_6) δ 178.65, 169.26, 160.27, 141.73, 131.07, 130.28, 123.02, 120.90, 119.42, 110.25, 61.32, 48.73, 14.09, 13.94 , MS (EI, 70 eV) *m*/*z* (%): 320.38 [*M*+H]$^+$ (320.37 calcd for $C_{14}H_{16}N_4O_3S$), *Anal. Calcd*.for $C_{14}H_{16}N_4O_3S$: C, 52.49, H, 5.03, N, 17.49. *Found*: C, 52.56, H, 5.50, N, 18.10, IR(KBr)ν_{max}:3889, 3374, 3253, 3166, 2596, 1728, 1696, 1492, 1102, 747.

Data for (Z)-1-(1-sec-butyl-oxoindolin-3-ylidene) thiosemicarbzide (n)

Pure yellow crystals (2.5 g, 92%), mp 178-179º C, *R*f(EtOAc/*n*-hexane, 1:3) 0.51, ^{1}H-NMR (250 MHz, DMSO-d_6) δ 12.69 (1H, br.s), 9.07 (1H, s), 8.88 (1H, s)7.09-8.03 (4H, m), 4.23-4.29 (1H, m), 1.72-1.97 (2H, qd, *J*=7.5, 46 Hz), 1.40 (3H, d, *J*=7.5 Hz), 0.75 (3H, t,

J=7.5),^{13}C-NMR (62.5 MHz, DMSO-d_6) δ 163.27, 155.87, 142.91, 132.26, 131.37, 125.10, 121.60, 115.19, 110.03, 49.31, 25.56, 17.43, 10.98, MS (EI, 70 eV) *m*/*z* (%): 276.38 [*M*+H]$^+$ (276.36 calcd for $C_{13}H_{16}N_4OS$), *Anal.Calcd.*for $C_{13}H_{16}N_4OS$: C, 56.50, H, 5.84, N, 20.27. *Found*: C, 56.79, H, 5.27, N, 20.95, IR (KBr) ν_{max}: 3482, 3320, 3165, 1676, 1498, 1223, 700.

Table 1. Chemical structures and names of the semicarbazone *(a-i)* and thiosemicarbazone *(j-n)* analogues of isatin

a		(Z)-1-(1-sec-butyl-2-oxoindolin-3-ylidene) semicarbazide	***h***		(Z)-1-(1-butyl-2-oxoindolin-3-ylidene)semicarbazide
b		(Z)-1-(1-cyclopentyl-2-oxoindolin-3-ylidene) semicarbazide	***i***		(Z)-ethyl 2-(3-(2-carbamothioylhdrazone)-2-oxoindolin-1-yl)propanoate
c		(Z)-1-(1-allyl-2-oxoindolin-3-ylidene)semicarbazide	***j***		(Z)-1-(1-allyl-2-oxoindolin-3-ylidene)thiosemicarbazide
d		(Z)-1-(2-oxo-1-(2-phenoxyethyl)indolin-3-ylidene)semicarbazide	***k***		(Z)-1-(1-buthyl-2-oxoindolin-3ylidene) thiosemicarbazide
e		(Z)-1-(2-oxo-1-(2-oxo-1(methyl benzyl) indolin-3-ylidene)semicarbazide	***l***		(Z)-2-(1-(2-methylbenzyl)-2-oxoindolin-3-ylidene) hydrazinecarbothioamide
f		(Z)-1-(1-(but-3-en-2-yl)-2-oxoindolin-3-ylidene)semicarbazide	***m***		(Z)-ethyl 2-(3-(2-carbamothioylhdrazono)-2-oxoindolin-1-yl) propanoate
g		(Z)-1-(2-oxo-1-(2-oxo-3-phenoxypropyl) indolin-3-ylidene)semicarbazide	***n***		(Z)-1-(1-sec-butyl-2-oxoindolin-3-ylidene)thiosemicarbazide

Behavioral seizure evaluation

Intraperitoneal PTZ-induced seizure

Male mice 20-25 g were divided into 46 groups of six animals each. The first group received vehicle control (DMSO/saline) whereas Group 2,3,4 received standard drug (Diazepam, 0.5,1 and 2 mg/kg) i.p. Group 5 to 46 received semicarbasone isatin (***a,b,c,d,e,f,g,h,i***) and thiosemicarbazone isatin (***j,k,l,m,n***) (10,20 and 30 mg/kg body weight).Altogether received PTZ (85 mg/kg body weight i.p.) 30 min after administration of the mentioned compounds.

Immediately after the injection of PTZ, animals were transferred to an open field (50 cm in diameter) and monitored for the appearance of convulsion or death for 30 min. Following the administration of 85 mg/kg of PTZ, time latencies for the first myoclonic, clonic, and tonic seizures and death were measured. Latencies were calculated as a time between PTZ injections and the onset of these stages.

Induction of PTZ kindling in normal rats

Chemical kindling in rats was established by administration of sub convulsant doses of PTZ (30 mg/kg, i.p.) 3 times a week on every alternate day. Development of fully kindled stage 5 seizures, i.e. generalized tonic clonic sezures in these rats was observed after 14

injections of PTZ. This kindling was confirmed when the animals were rechallenged on the 3rd and the 10th day after PTZ treatment ended.

PTZ-induced kindling model in rat

Compounds ***k*** and ***n*** were administrated prior to injecting 35 mg/kg PTZ in fully kindled animals for anti-epileptogenic effects. The most suitable procedure for kindling acquisition was selected on the basis of a dose response study showing that 11 injections of 35 mg/kg PTZ every 48h produced fully kindled animals[18]. Subsequently, the animals were used for testing by injection of test compound prior to PTZ injection. After each injection, the rats were placed singly in an open field and were observed for 30 min. Seizure intensity were classified into 6 stages as follows: 0: no response, 1: ear and facial twitching, 2: myoclonic jerks without rearing, 3: myoclonic jerks, rearing, 4: turn over into side position, tonic–clonic seizures, 5: turn over into back position, generalized tonic-clonic convulsions. Incidence of seizure and latency of seizure were also recorded. Only animals displaying at least three consecutive stage 4 or 5 seizures were defined to be kindled rats and include in this study[19].

The kindled rats (n=6) were randomly divided into 7 groups .Group 1 served as the kindled control which received only the vehicle (PTZ in saline), group 2and 3 received diazepam (1 and 2 mg/kg i.p.) as positive control groups, and isatin derivatives ***k*** and ***n*** at doses of (10 and 30 mg/kg, ip) were given to the groups 4 to 7. All groups received PTZ 30 min after their handling. Seizure stage, seizure times and latency of seizure were recorded within 1 h and the onset of seizures (according to 6 stages) was also calculated.

Motor coordination

Rotarod was used to evaluate the muscle coordination in the mice treated with isatin derivatives. 70 BALB/a male mouse20-30 mg were placed on a rotating rod, spinning at 5 and 15 rpm. Once stabilized, each animal underwent 1trial, then the duration of time that the mice managed to remain on the rod, and the speed at which they fell off from the apparatus were recorded [20].

Statistical analysis

Seizure severity scores were compared using one-way Anovaanalysis of variance on ranks followed by multiple comparison tests (tukey). All analyses were made using the SPSS statistical software package and P-values<0.05 was accepted as statistically significant.

Result and Discussion

Results

Here PTZ induced seizure and PTZ-induced kindling models were used to investigate both anticonvulsive and antiepileptogenic properties of the some semicarbazone and thiosemicarbazone derivatives of isatin. The effects of different doses of isatin derivatives ***(a-n)*** (10, 20 and 30 mg/kg, i.p.) 30 min prior to intraperitoneal PTZ-induced myoclonic seizure threshold are showed in Figure 3. One-way ANOVA did not reveal a significant effect for some derivatives. But the results showed a significant effect in the groups which received ***k*** and ***n*** at all doses.

Figure 4 shows the effects of different doses of isatin derivatives ***(a-n)*** (10, 20 and 30 mg/kg, i.p.) 30 min prior to intraperitoneal PTZ-induced clonic seizure threshold. One-way ANOVA did not reveal a significant effect for some derivatives. But the results showed a significant effect in the groups which received ***k, l, m*** and ***n*** at two doses which administered.

Figure 5 shows the effects of different doses of isatin derivatives ***(a-n)*** (10, 20 and 30 mg/kg, i.p.) 30 min prior to intraperitoneal PTZ-induced tonic seizure threshold. One-way ANOVA did not reveal a significant effect for some derivatives. But the results showed a significant effect in the groups which received ***d, k, m*** and ***n*** at two doses which administered.

Figure 6 shows the effects of different doses of isatin derivatives ***(a-n)*** (10, 20 and 30 mg/kg, i.p.) on the latency times to death induced by intraperitoneal injection of PTZ. One-way ANOVA showed a significant effect in the groups which received ***d, I, k, m*** and ***n*** at different doses.

Effect of isatin derivatives on PTZ kindling in rats

Fully kindled rats were randomly divided into 7 groups (6 animals per each group). Antiepileptogenic effects were assessed by administration of the vehicle (group 1), diazepam 1 and 2 mg/kg, i.p. (group 2-3), compounds ***k***10, and 30 mg/kg, i.p. (group 4-5) and compound ***n*** 10, and 30 mg/kg, i.p. (group 6-7) 30 min before injection of PTZ (35 mg/kg, i.p.). All animals which pretreated with vehicle had generalized seizures after PTZ (Figure7). The latency to seizure onset was significantly increased from 0.89±0.1min to more than 3min ($P < 0.05$) in animals pretreated with doses of 10 and 30 mg/kg of compounds ***k*** and ***n***. The seizure indicator was also significantly decreased in rats pretreated with these two doses of compounds ***k*** and ***n*** in comparison to control group. Compound ***k*** and ***n***, exhibited more effective action in delaying seizures as well as decreasing seizure duration in this model of epilepsy.

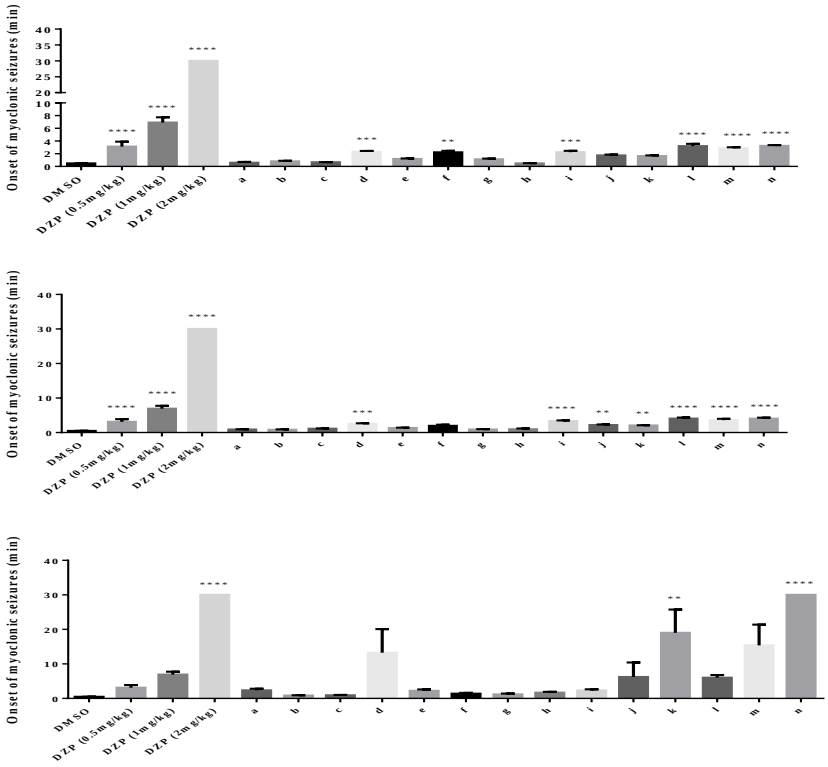


Figure 3. The effects of isatin derivatives (I: 10, II: 20 andIII: 30 mg/kg i.p) on the latency times to myoclonic seizures induced by PTZ when administered 30 min before PTZ. Data are expressed as mean±S.E.M (n=5).*P<0.05, **P<0.01 and ***P<0.001 compared with Solvent-treated animals.

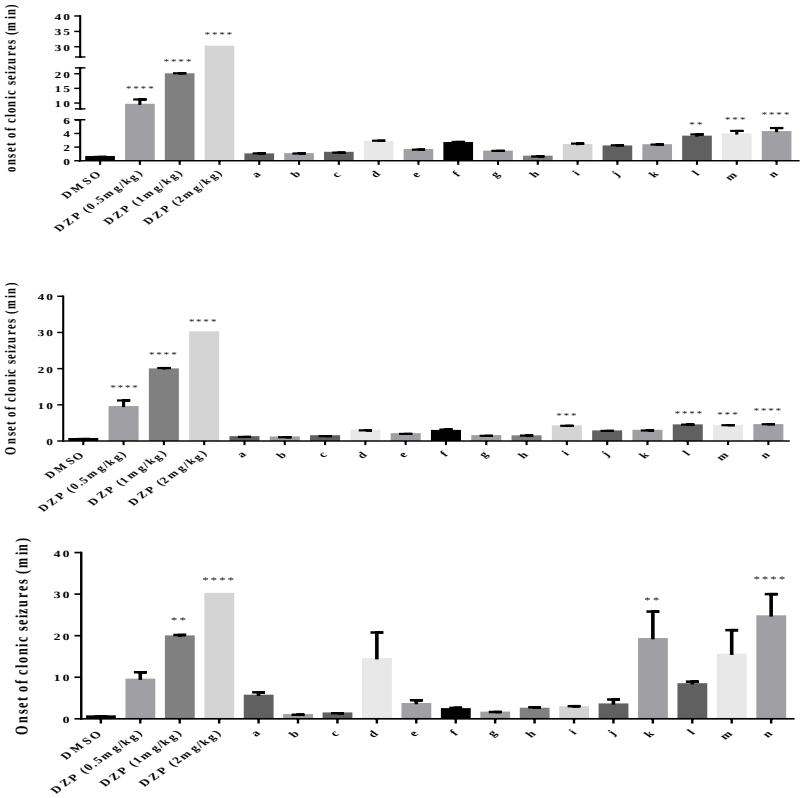


Figure 4. The effects of isatin derivatives (I: 10, II: 20 and III: 30 mg/kg i.p.) on the latency times to clonic seizures induced by PTZ when administered 30 min before PTZ. Data are expressed as mean±S.E.M (n=5). *P<0.05, **P<0.01and ***P<0.001 compared with Solvent-treated animals.

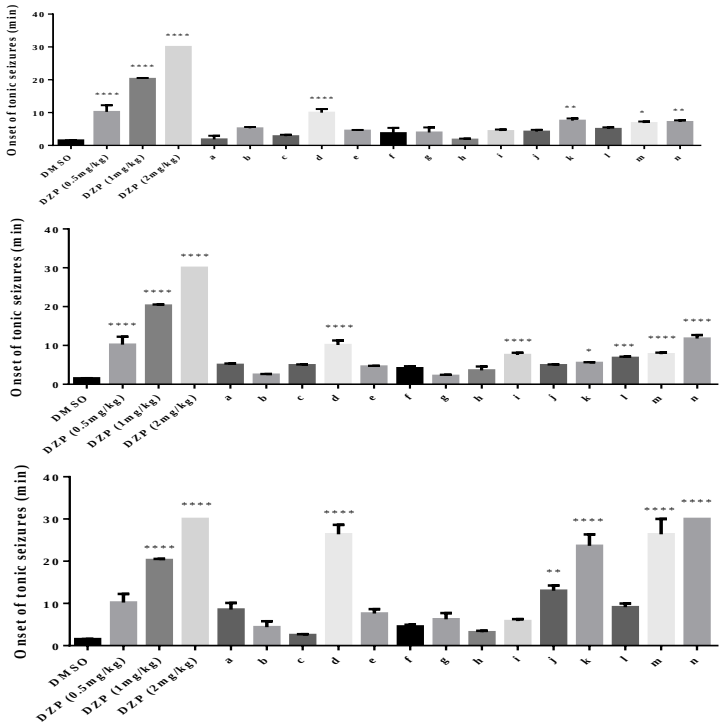


Figure 5. The effects of isatin derivatives (I: 10, II: 20 and III: 30 mg/kg i.p.) on the latency times to tonic seizures induced by PTZ when administered 30 min before PTZ. Data are expressed as mean±S.E.M (n=5). *P<0.05, **P<0.01 and ***P<0.001 compared with Solvent-treated animals.

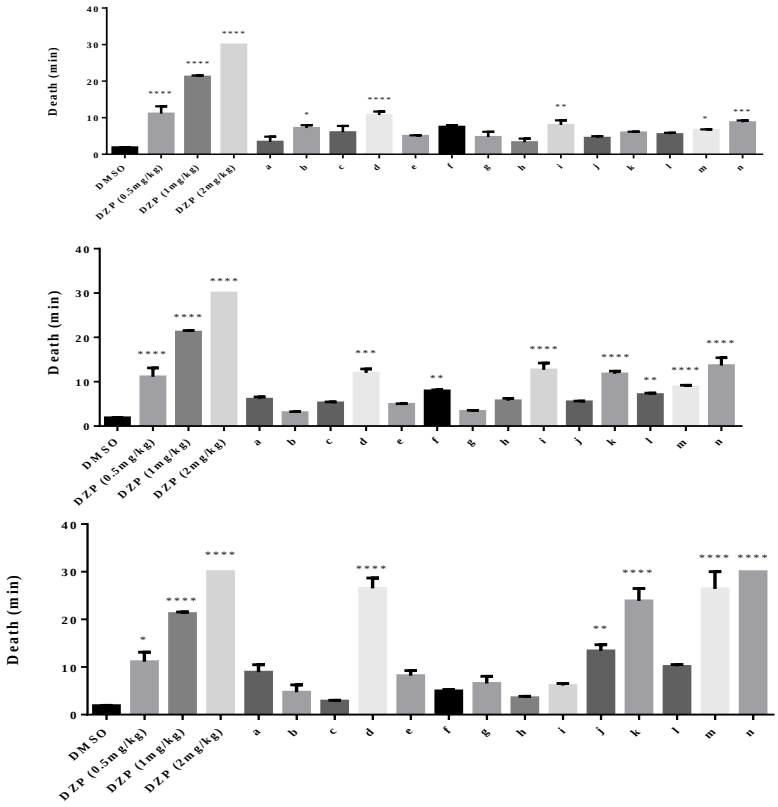


Figure 6. The effects of isatin derivatives (I: 10, II: 20 and III: 30 mg/kg i.p.) on the latency times to death induced by PTZ when administered 30 min before PTZ. Data are expressed as mean±S.E.M (n=5). *P<0.05, **P<0.01 and ***P<0.001 compared with Solvent-treated animals.

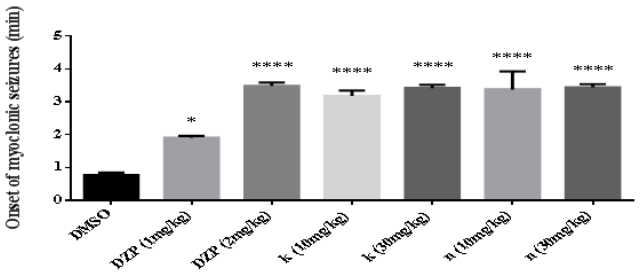


Figure 7. Effects of isatin derivatives *k* and *n* (10 and 30 mg/kg,i.p.) on the onset of myoclonic seizures induced by PTZ in fully kindled rats. Results for latency to first seizure are expressed as mean±S.E.M (n=5). *P<0.05, **P<0.01 and ***P<0.001 compared with Solvent-treated animals.

As shown in Figure 8, analysis of the rotarod data revealed no alteration in motor coordination of rat before and after administration of different doses of compounds ***k*** and ***n*** (no falloff during 90 s on the rod). DMSO and compounds ***k*** and ***n*** (10mg/kg,i.p.) had significantly increase the time on rotarod 5 and 15 rpm in comparison to diazepam.

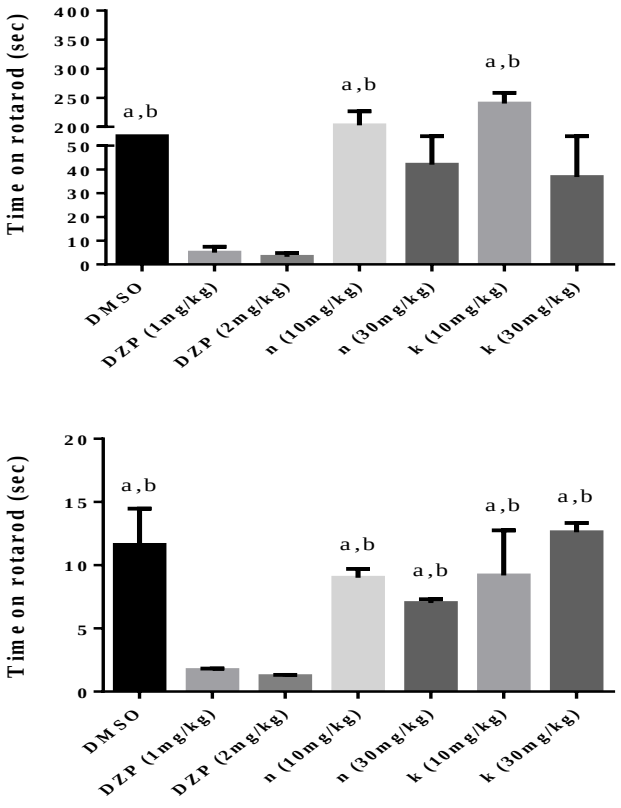


Figure 8.The effects of compounds k and n (10 and 30 mg/kg i.p.) on the rotarod performance test (5 (a) and 15 (b) rpm).Data are expressed as mean ± S.E.M (n=5).a: significant difference in comparison to diazepam 1 mg/kg i.p. (P<0. 05).b: significant difference in comparison to diazepam 2 mg/kg i.p. (P<0.05).

Discussion

Epilepsy is a major neurological disorder and even there are some synthetic AEDs currently available for use in the management, control and treatment of epilepsy, but they have also many toxic adverse effects. Apart from epilepsy itself causing cognitive impairment, anti-convulsant drug induce cognitive impairment in individuals with epilepsy[21]. So, investigation for effective and safe anticonvulsant agents with multiple actions and no/minimal side effects is a strong need[22].

Choosing a proper animal model is one of the most important and basic steps of developing new AEDs [23]. Prevention of PTZ-induced seizures in animals is the most common preliminary screening test for characterizing potential anti-convulsive compounds [24]. Any agents which can protect against seizures induced by PTZ are suggested to be useful to treat myoclonic and absence seizures in humans [25].

PTZ kindling model is also a very well acknowledged animal model for studying the process of epileptogenesis. It this way repetitive administration of a subconvulsive dose of chemical agents results in growth of convulsive effect, finishing in generalized seizures[26]. Kindling is also a proper model for investigation of partial seizures especially complex partial seizures and drug-resistant partial seizures [27].

Recently, there has been an upsurge in the investigation of isatin derivatives for their biological properties, because of their low toxicity, potent pharmacological effects and economic availability [28].

Usually synthesized compounds screened for anticonvulsant activities against different models of epilepsy such as MES, ScMet and PTZ induced seizure model but there are no any reports related to its anti-epileptogenic effect in kindling model.

Therefore, in the current study, 14 synthetic isatin derivatives***(a-n)***of our previously synthesized compounds based on pharmacophoric model shown in Figure 1evaluated for their anticonvulsant effect on the PTZ model and also the most effective compounds, ***k*** and ***n*** were selected for evaluation in the kindling model.

In our study, diazepam (2mg/kg) had abolished the seizure with injection of PTZ (85mg/kg,i.p.) and offered 100% protection. The percentage of protection was found to be dose dependent.

According to our results compounds ***b, d, f, i, j, k, l, m*** and ***n*** produced significant increase in the onset of PTZ-induced seizures and time of death as compared to control.

In the kindled rats treated with ***k*** and ***n*** significant protection was observed. These compounds, showed behavioral changes only up to stage 1 (myoclonic jerk, like standard group but with a different latencies) on the seizure score as compared to stage 5 in the vehicle

treated. Compounds ***k*** and ***n*** were able to prevent the epileptogenesis process and their percent of protection were 100%.

The rats treated with ***k*** and ***n*** were less sedated and were less muscle relaxed and they acted like normal rats while receiving the PTZ. Therefore, for testing the motor coordination and for better understanding of their mechanism, the effect of them on motor activity was evaluated using rotarod test (5 and 15 rpm).

It was observed that dose of 10 mg/kg,i.p. did not cause any motor impairment as evident by the insignificant change in the rotarod performance test between ***k*** and ***n*** treated and the control values. Moreover, on general observation no eyelid ptosis, weeping eyes were observed in the ***k*** and ***n*** derivatives treated groups.

According to the present study, we concluded the isatin derivatives might have anti-convulsant effect via GABA-ergic neurotransmission. As these compounds delayed the occurrence of PTZ induced convulsion and PTZ has been shown to interact with the GABA neurotransmitter, it is suggested that our compounds interfering with GABAergic mechanism or other PTZ involved mechanisms.

It is also proposed that semicarbazone derivatives act by affecting on sodium voltage gated channels according to the behavioral patterns and rotarod test results [29]. So far isatin derivatives might be exhibiting their effects through the enhancing GABA-mediated synaptic inhibition or preventing seizure spread through the neural tissue by the blockade of voltage-activated Na^{+}channels.

As PTZ induce seizure in both acute and chronic model resulted in increased oxidative stress, the efficacy of isatin derivatives might be ascribed by inhibition of oxidative stress though other mechanisms cannot be ruled out [30-32].

Compounds ***d, i, j, k, l, m, n*** had significantly increased the onset of tonic convulsion and compounds ***i, l, m, n*** had significantly increased the onset of clonic convulsion.

According to this study, thiosemicarbazone isatins had more anticonvulsant effect than semicarbaszone isatins. Accordingly **S** element improve the anticonvulsant effect in compounds ***i*** and ***e*** in comparison to compounds ***m*** and ***j***.

In semicarbazone isatins, N-substituted compounds showed anticonvulsant effect and if this substitution became extended, the anticonvulsant effects eliminated. May be because of the molecular weight will be increase by large group incorporation.

Compounds ***k*** and ***n*** are structurally isomers with same molecular weight. But it seems that solubility of branched-alkyl substitution was better than straight one. It may due to increasing of steric effect in ***k***, and subsequently solubility [33].

Conclusion

In conclusion, current study illustrates the anticonvulsant effect of some isatin derivatives, in acute model of PTZ test and on fully kindled rats which represent a chronic model. The findings of the present study also demonstrate the isatin derivatives don't affect the motor coordination as much as diazepam. The results obtained from these experimental models clearly suggested these effective isatin derivatives can be a good candidate for new AEDs and treatment of epilepsy.

Acknowledgment

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