Design, synthesis, pharmacological evaluation, and docking study of new acridone-based 1,2,4-oxadiazoles as potential anticonvulsant agents

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Abstract: A number of acridone-based oxadiazoles **11a-n** have been synthesized and evaluated for their anticonvulsant activity against pentylenetetrazole (PTZ)- and maximal electroshock (MES)-induced seizures in mice. Also, their neurotoxicity was evaluated by the rotarod test. Most of the compounds exhibited better anticonvulsant activity and higher safety respect to the standard drug, phenobarbital. Among the tested derivatives, compounds **11l** with ED₅₀ value of 2.08 mg/kg was the most potent compound in the PTZ test. The anticonvulsant effect of compound **11l** was blocked by flumazenil, suggesting the involvement of benzodiazepine (BZD) receptors in the anticonvulsant activity of prototype compound **11l**. Also, docking study of compound **11l** in the BZD-binding site of GABA_A receptor confirms possible binding of compound **11l** with BZD receptors.

Keywords: Anticonvulsant agents; Acridone; Benzodiazepine (BZD) receptors; Docking study; 1,2,4-Oxadiazole

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1. Introduction

Epilepsy is a common neurological disorder that affects approximately 60 million people of the world's population [1]. Although there are many anticonvulsant drugs on the market, the use of most drugs is associated with many side effects and extended drug interactions [2]. On the other hand, anticonvulsant drugs in about 70% of patients with epilepsy can be effective and the treatment of epilepsy remains still inadequate [3,4]. Thus, the development of more effective and safer antiepileptic drugs is an important goal for many of the medicinal chemists.

Various derivatives bearing 5-membered heterocycles such as oxadiazoles, triazoles, and thiadiazoles have been synthesized and evaluated for their anticonvulsant activities [5,6]. Particularly, Lankau et al. reported the anticonvulsant activity of 3-aryl-1,2,4-oxadiazole **I** (Fig. 1) in a variety of experimental models. They found that these compounds act as selective GABA potentiating compounds with no interaction to the benzodiazepine (BZD) binding site [6]. On the other hand, 10-(2-dimethylaminopropyl)-9-acridinone **II** (Fig. 1) has been reported as a potent convulsant agent [7]. In continuation of our efforts to find new bioactive acridones [8], we have designed a series of acridone-based 1,2,4-oxadiazoles **11a-n** (Fig. 1) as new anticonvulsant agents. Presumably, the designed compounds can act as anticonvulsant agents (such as diazepam and estazolam) through benzodiazepine (BZD) receptors; they have the requirements for binding to these receptors: an aromatic ring (A), a coplanar proton-accepting group in a suitable distance (B), and second out-of-plane aromatic ring (C) that the last case could potentiate binding to the receptor especially for agonists (Fig. 2) [9]. Thus we report here, the synthesis, pharmacological evaluation, and docking study of 10-(3-aryl-[1,2,4]oxadiazol-5-ylmethyl)-10*H*-acridin-9-one derivatives **11a-n** as potential anticonvulsant agents.

2. Chemistry

The designed compounds **11a-n** were synthesized according to Scheme 1. The synthetic pathway was started from the Ullmann condensation reaction of 2-bromobenzoic acid **1** and different anilines **2** using potassium carbonate and copper in refluxing EtOH, to afford 2-aryl-amino benzoic acids **3** [10]. Compounds **3** easily participated in the cyclization reaction in the presence of PPA at 100 °C and afforded acridones **4** [11]. On the other hand, 3-aryl-5-(chloromethyl)-1,2,4-oxadiazole derivatives **10** were prepared starting from appropriate benzonitriles **5** [12]. Accordingly, benzonitrile derivatives **5** and hydroxylamine hydrochloride (**6**) reacted in refluxing EtOH in the presence of sodium hydroxide to obtain hydroxybenzamidines **7**. The reaction of compounds **7** and chloroacetyl chloride (**8**) in the presence of potassium carbonate in dry acetone gave *N*-(2-chloroacetoxy)-4-substituted benzimidamide **9** which underwent the further cyclization reaction in refluxing toluene to give 3-aryl-5-(chloromethyl)-1,2,4-oxadiazole derivatives **10**. Finally, the acridone derivatives **4** were reacted with 1,2,4-oxadiazole derivatives **10** in the presence of potassium *tert*-butoxide in DMSO at room temperature to afford the corresponding products **11** in good yields (55-70%).

3. Results and discussion

3.1. Anticonvulsant activity

All the synthesized compounds **11a-n** were screened for their anticonvulsant activity by using pentylenetetrazole (PTZ) and maximal electroshock (MES) tests in mice [13]. The results were compared with phenobarbital and diazepam as standard anticonvulsant drugs. Structurally, the designed compounds **11a-n** could be categorized into three groups considering the type of

substituent on the attached phenyl on 3-position of 1,2,4-oxadiazole ring: 4-methyl, 4-chloro, and 4-methoxy derivatives (**11a-f**, **11g-k** and **11l-n**). In each series, substituents on the 2-position of acridone ring were altered to optimize the anticonvulsant activity (Table 1).

3.1.1. Anticonvulsant activity against PTZ-induced seizures

As can be seen in Table 1, all compounds with exception of **11d** and **11e** had potent anticonvulsant activity in PTZ-induced seizures test, comparable or more potent than the standard drug phenobarbital. Among the tested compounds, 4-methoxyphenyl derivatives **11l** and **11m** with ED₅₀ values of ≈ 2.1 mg/kg were the most potent compounds against PTZ-induced seizures. Although these compounds were more active than phenobarbital, but their activity was less than that of diazepam.

In the 4-methyl derivatives **11a-f** ($R_2 = \text{Me}$), compounds **11a-c** showed almost same anticonvulsant activities (ED₅₀ values of ≈ 6.3 mg/kg). The comparison of 2-substituted acridones **11b-f** with unsubstituted derivative **11a** ($R_1 = \text{H}$) revealed that the chloro or methoxy substituents were tolerated, while the bromo, methyl and ethyl substituents diminished the activity. The obtained ED₅₀ values of compounds **11g-k** ($R_2 = \text{Cl}$) were less than those of their corresponding 4-methyl analogs **11a-e** ($R_2 = \text{Me}$). Thus, the replacement of methyl with chlorine group at R_2 increases the anticonvulsant activity. In the 4-chloro series **11g-k** ($R_2 = \text{Cl}$), better results were obtained with 2-chloro and 2-methoxy substitution on the acridone ring. The obtained results for limited series of 4-methoxy derivatives **11l-n** revealed that the 4-methoxy substituent on the phenyl-oxadiazole moiety improved the anticonvulsant activity. Notably, 4-methoxyphenyl-oxadiazole derivatives **11l** and **11m** bearing a 2-Cl or 2-MeO on the acridone ring showed the highest activity among the all series.

3.1.2. Anticonvulsant activity against MES-induced seizures

The obtained results from MES test revealed that all compounds had potent anticonvulsant activity (ED_{50} s= 2.24-8.71 mg/kg). Compound **11b** with ED_{50} value of 2.24 mg/kg found to be the most potent derivative against MES-induced seizures in mice. Furthermore, compounds **11a**, **11e**, **11h**, **11j** and **11m** displayed ED_{50} values ≤ 2.87 mg/kg, being slightly less potent than compound **11b**. While all compounds were more active than phenobarbital, but their activity was less than that of diazepam. The comparison of obtained results from PTZ and MES tests revealed that most compounds had lower ED_{50} s in the MES test respect to PTZ model of epilepsy. Even compounds **11d** and **11e** which were inactive in the PTZ test, showed significant activity against MES-induced seizures. The alteration of substituent on the acridone ring and on the 3-phenyl-1,2,4-oxadiazole moiety (R_1 and R_2 , respectively) had slight effect on the potency of compounds against MES-induced seizures.

3.2. Neurotoxicity

All compounds were screened for their neurotoxicity in mice by using rotarod test. The obtained results were listed in Table 1 as TD_{50} values (mg/kg). As seen from data, all compounds displayed neurotoxicity at very high doses (TD_{50} s ≥ 438.3 mg/kg). The TD_{50} value for the standard drug phenobarbital was 68.9 mg/kg being significantly less than those of title compounds. Based on the obtained results from PTZ, MES and rotarod tests, the protective index ($PI = TD_{50}/ED_{50}$) was calculated for all compounds and presented in Table 1. The PI values of compounds revealed that the designed compounds enable to display good anticonvulsant activity with high safety profile.

3.3. Study on possible mechanism of action

To gain insight into the mechanism of action of the most potent compound **11l**, flumazenil test were performed. In PTZ test, flumazenil, a well-known benzodiazepine antagonist, antagonized anticonvulsant effects of active compound **11l**, which confirms the involvement of BZD receptors in the anticonvulsant activity of prototype compound **11l**.

3.4. Docking study

To confirm whether the anticonvulsant activity of the synthesized compounds mediates through BZD receptors, the docking of active compound **11l** in the BZD-binding site of GABA_A receptor ($\alpha 1\beta 2\gamma 2$) examined by Autodock Tools (1.5.6). Interactions of the diazepam and target compound **11l** with amino acids of BZD-binding pocket of GABA_A receptor were constructed by Discovery Studio 4.0 Client and showed in Figures 3a and 3b, respectively. As can be seen in Fig. 3a, the most important residues in the binding mode of diazepam are $\alpha 1$ Thr206, $\alpha 1$ Tyr 209, $\alpha 1$ His101, $\alpha 1$ Tyr159, and $\gamma 2$ Phe77 [14]. The acridone ring of compound **11l** is responsible for two important interactions with BZD-binding pocket of GABA_A: π -cation interaction with the positively charged nitrogen of $\alpha 1$ His101 and π - π interaction with $\gamma 2$ Phe77 (Fig. 3b). It is obvious that there is an hydrogen bond between N-2 of 1,2,4-oxadiazole ring and $\alpha 1$ Thr206. Also, $\alpha 1$ Tyr209 possessing aromatic moiety is involved in a π - π interaction with 3-phenyl moiety. Therefore, the results showed that binding mode of compound **11l** in the BZD-binding pocket of GABA_A receptor resembled to that of diazepam.

3.5. Cytotoxicity

Potentially, the acridone/acridine derivatives might intercalate with the double-stranded DNA structure thus inducing toxic effects [15]. So we studied the potential cytotoxic effects of this class of compounds. All derivatives **11a-n** were evaluated for their cytotoxicity against MCF-7,

T-47D, and MDA-MB-231 cell lines. The tested compounds showed no in vitro cytotoxic activity against cancer cell lines (IC_{50} values $>50\text{ }\mu\text{M}$). The obtained IC_{50} s of the standard drug etoposide were 12.4, 11.8 and $15.7\text{ }\mu\text{M}$ against MCF-7, T-47D, and MDA-MB-231, respectively.

3.6. Prediction of BBB permeability

Anticonvulsant agents are CNS-active compounds, so they should have good BBB permeability. Several computational methods have been reported for the prediction of BBB penetration [16]. One of them is the measurement of logBB, which is the ratio of the steady-state concentrations of the compound between the brain and the blood [i.e., $\log (C_{\text{brain}}/C_{\text{blood}})$]. Accordingly, compounds with logBB <-1.0 will have poor distributed to the brain [17]. Clark has reported an equation for measuring the logBB as follow: $\log\text{BB} = -0.0148 \times \text{PSA} + 0.152 \times c\log P + 0.139$ [18]. The logBB values of compounds **11a-n** were calculated by the equation and presented in Table S1 (Supplementary Material). The measured logBB values for compounds **11a-n** were ranging from -0.688 to 0.249 . As seen from data, compounds **11d** and **11i** had more favorable logBB values. Also, the prototype compound **11l** had logBB= 0.185 , being acceptable value for a CNS-active compound.

4. Conclusion

We designed and synthesized a series of 10*H*-acridin-9-one derivatives bearing 10-(3-aryl-[1,2,4]oxadiazol-5-ylmethyl)- moiety as novel anticonvulsant agents. Most of compounds showed better anticonvulsant activities in comparison to phenobarbital as the standard drug. The 2-chloro-acridinone derivative **11l** was the most potent compound in the PTZ test (ED_{50} = 2.08 mg/kg). Furthermore, compounds **11a**, **11b**, **11e**, **11h**, **11j**, and **11m** with ED_{50} values ≤ 2.87

mg/kg were more promising compounds in the MES test, being at least 7 times more potent than phenobarbital. The safety profile of all compounds was better than that of phenobarbital. The experimental investigation of mechanism with flumazenil test and in silico studies by using molecular docking simulations showed that the prototype compound **11l** can act as BZD-receptor agonist.

5. Experimental

Melting points were measured with a Kofler hot stage apparatus and are uncorrected. ^1H and ^{13}C NMR spectra were recorded by a Bruker FT-500, using TMS as an internal standard. IR spectra were obtained by a Nicolet Magna FTIR 550 spectrophotometer (KBr disks). MS were recorded with an Agilent Technology (HP) mass spectrometer operating at an ionization potential of 70 eV. Elemental analysis was performed with an Elementar Analysensystem GmbH VarioEL CHNS mode.

5.1. General procedure for the synthesis of 2-arylaminobenzoic acids **3**

A solution of 2-bromobenzoic acid (**1**, 8.5 mmol), aniline derivative **2** (159.0 mmol), copper powder (4.7 mmol), and potassium carbonate (119.0 mmol) in ethanol (80 ml) was heated under reflux for 7 h. After completion of reaction (checked by TLC), the reaction mixture was cooled down to room temperature, poured into hot water and boiled in the presence of activated charcoal for 15 min. Then, it was filtered through celite and the filtrate was acidified with HCl to obtain precipitated product which was purified using recrystallization from ethanol to obtain pure products (80-85%).

5.2. General procedure for the synthesis of acridone derivatives **4**

A mixture of appropriate 2-arylamino benzoic acid **3** (1 mmol) and polyphosphoric acid (10 mmol) was heated at 100 °C for 3 h. Upon completion, the reaction mixture was poured into hot water (50 ml). After cooling to room temperature, the mixture was made alkaline by ammonia solution. The obtained yellow precipitates were filtered off, washed with hot water, and purified using recrystallization from acetic acid to obtain the pure products (80-85%).

5.3. General procedure for the synthesis of 3-aryl-5-(chloromethyl)-1,2,4-oxadiazole derivatives

10

An aqueous solution of sodium hydroxide (20 mmol in 10 ml) was added to a solution of benzonitrile **5** (10 mmol) and hydroxylamine hydrochloride **6** (20 mmol) in EtOH (50 ml) in a dropwise manner at room temperature. Then, the mixture was heated under reflux for 18 h. After completion of the reaction (checked by TLC), the solvent was evaporated under reduced pressure, water (50 ml) was added to the residue, and the pH of solution was set at pH = 2 using HCl (1 N). The mixture was extracted using ethyl acetate (2 × 25 ml). The aqueous solution was cooled to 0 °C and neutralized using sodium carbonate solution. The obtained precipitate was filtered off, washed with water, and dried at 60 °C to obtain compound **7**. In the next step, chloroacetyl chloride **8** (12 mmol) was added to a stirring solution of compound **7** (11.2 mmol) and K₂CO₃ (11 mmol) in dry acetone (25 ml) in a dropwise manner during 10 min. The reaction mixture was stirred at room temperature for 24 h. After reaction completion, the solvent was evaporated under reduced pressure, the obtained white precipitate was dissolved in ethyl acetate (50 ml), and the organic phase was washed with water (4 × 20 ml) and brine (20 ml). The organic phase was dried over Na₂SO₄ and the solvent was evaporated under reduced pressure. The residue was recrystallized from EtOH to obtain pure *N'*-(2-chloroacetoxy)-benzimidamide **9**. A suspension of compound **9** (8 mmol) in toluene (50 ml) was heated under reflux for 5 h. Then,

the solvent was evaporated under reduced pressure and the obtained yellow oily residue was recrystallized from petroleum ether to give pure yellow 3-aryl-5-(chloromethyl)-1,2,4-oxadiazole derivative **10** (65-70%).

5.4. General procedure for the synthesis of 10-(3-aryl-[1,2,4]oxadiazol-5-ylmethyl)-10H-acridin-9-one derivatives **11a-n**

A suspension of acridone derivative **4** (0.5 mmol) and potassium *tert*-butoxide (0.07 mmol) in DMSO (0.5 ml) was stirred at room temperature for 30 min. Then, the mixture was added to a solution of 3-aryl-5-(chloromethyl)-1,2,4-oxadiazole derivative **10** (1 mmol) in DMSO (2 ml) and the reaction mixture was stirred at room temperature for 3 h. After that, it was poured into crushed ice and the aqueous solution was extracted with dichloromethane (3×10 ml), washed with water, and dried over Na₂SO₄. The solvent was evaporated under reduced pressure and the residue was purified by flash chromatography on silica gel eluting with petroleum ether/ethyl acetate (9:1) to obtain pure products (55-70%).

5.4.1. 10-(3-*p*-Tolyl-[1,2,4]oxadiazol-5-ylmethyl)-10H-acridin-9-one (**11a**). White crystals; yield: 76%, mp 239-241 °C. IR (KBr): 3069, 2904, 1647, 1604, 1486, 1460 cm⁻¹. ¹H NMR (400 MHz, DMSO-*d*₆): 8.39 (d, *J* = 8.0 Hz, 2H, H₁, H₈), 7.84 (m, 4H, H₃, H₄, H₅, H₆), 7.79 (d, *J* = 8.2 Hz, 2H, H₂, H₆), 7.42-7.38 (m, 2H, H₂, H₇), 7.31 (d, *J* = 8.2 Hz, 2H, H₃, H₅), 6.25 (s, 2H, CH₂), 2.34 (s, 3H, CH₃). ¹³C NMR (100 MHz, DMSO-*d*₆): 177.2, 176.4, 168.3, 142.3, 142.2, 135.0, 127.5, 127.3, 123.3, 122.6, 122.3, 116.4, 42.9, 21.5. MS *m/z* (%) 367 (M⁺, 70), 253 (20), 208 (100), 180 (31), 125 (99), 91 (33), 65 (9), 43 (8). Anal. Calcd for C₂₃H₁₇N₃O₂: C, 75.19; H, 4.66; N, 11.44. Found: C, 75.34; H, 4.21; N, 11.68.

5.4.2. *2-Chloro-10-(3-p-tolyl-[1,2,4]oxadiazol-5-ylmethyl)-10H-acridin-9-one (IIb)*. White crystals; yield: 69%, mp >250 °C. IR (KBr): 3093, 2919, 1635, 1597, 1487, 1457 cm⁻¹. ¹H NMR (500 MHz, CDCl₃): 8.61 (dd, *J* = 8.1, 1.4 Hz, 1H, H₈), 8.57 (d, *J* = 2.5 Hz, 1H, H₁), 7.96 (d, *J* = 8.1 Hz, 2H, H₄, H₅), 7.82 (td, *J* = 7.8, 1.5 Hz, 1H, H₃), 7.73 (dd, *J* = 9, 2.5 Hz, 1H, H₆), 7.65 (d, *J* = 8.2 Hz, 2H, H₂, H₆), 7.41 (t, *J* = 7.5 Hz, 1H, H₇), 7.31 (d, *J* = 8.2 Hz, 2H, H₃, H₅), 5.79 (s, 2H, CH₂), 2.45 (s, 3H, CH₃). ¹³C NMR (125 MHz, CDCl₃): 175.6, 174.1, 167.5, 142.1, 141.0, 138.4, 135.0, 134.3, 132.2, 128.6, 128.3, 127.1, 126.6, 125.7, 124.0, 123.0, 122.0, 119.6, 117.0, 42.8, 21.0. MS *m/z* (%) 403 ([M⁺ + 2], 22), 401 (M⁺, 68), 242 (100), 228 (24), 214 (29), 200 (26), 179 (21), 152 (25), 91 (20), 63 (10), 43 (9). Anal. Calcd for C₂₃H₁₆ClN₃O₂: C, 68.74; H, 4.01; N, 10.46. Found: C, 68.39; H, 3.94; N, 10.32.

5.4.3. *2-Methoxy-10-(3-p-tolyl-[1,2,4]oxadiazol-5-ylmethyl)-10H-acridin-9-one (IIc)*. Yellow crystals; yield: 73%, mp 226-228 °C. IR (KBr): 3064, 2922, 1616, 1591, 1488, 1461 cm⁻¹. ¹H NMR (500 MHz, CDCl₃): 8.63 (d, *J* = 7.9 Hz, 1H, H₈), 8.03 (d, *J* = 2.2 Hz, 1H, H₁), 7.97 (d, *J* = 7.8 Hz, 2H, H₂, H₆), 7.79 (t, *J* = 7.8 Hz, 1H, H₃), 7.66 (t, *J* = 8.6 Hz, 2H, H₄, H₅), 7.43 (dd, *J* = 7.9, 2.2 Hz, 1H, H₆), 7.39 (t, *J* = 7.4 Hz, 1H, H₇), 7.31 (d, *J* = 7.8 Hz, 2H, H₃, H₅), 5.81 (s, 2H, CH₂), 3.9 (s, 3H, OCH₃), 2.38 (s, 3H, CH₃). ¹³C NMR (125 MHz, CDCl₃): 176.5, 175.9, 167.7, 154.6, 143.3, 141.9, 137.0, 136.9, 134.6, 128.6, 128.3, 127.1, 124.4, 123.9, 121.6, 120.4, 118.2, 116.6, 106.6, 55.9, 42.9, 20.8. Anal. Calcd for C₂₄H₁₉N₃O₃: C, 72.53; H, 4.82; N, 10.57. Found: C, 72.41; H, 5.03; N, 10.34.

5.4.4. *2-Bromo-10-(3-p-tolyl-[1,2,4]oxadiazol-5-ylmethyl)-10H-acridin-9-one (IId)*. White crystals; yield: 71%, mp >250 °C. IR (KBr): 3111, 2923, 1635, 1598, 1486, 1463 cm⁻¹. ¹H NMR (500 MHz, CDCl₃): 8.72 (d, *J* = 2.4 Hz, 1H, H₁), 8.60 (dd, *J* = 8, 1.3 Hz, 1H, H₈), 7.96 (d, *J* = 8.1 Hz, 2H, H₄, H₅), 7.81 (dd, *J* = 9, 2.4 Hz, 1H, H₆), 7.82 (td, *J* = 7.9, 1.4 Hz, 1H, H₃), 7.65 (d, *J* =

8.6 Hz, 2H, H₂, H₆), 7.42 (t, J = 7.5 Hz, 1H, H₇), 7.31 (d, J = 8.6 Hz, 2H, H₃, H₅), 5.82 (s, 2H, CH₂), 2.44 (s, 3H, CH₃). ¹³C NMR (125 MHz, CDCl₃): 176.0, 174.7, 165.2, 142.9, 142.1, 141.3, 137.2, 136.7, 135.4, 128.9, 128.6, 128.3, 127.1, 124.0, 123.5, 121.1, 120.0, 117.0, 114.4, 42.9, 20.3. Anal. Calcd for C₂₃H₁₆BrN₃O₂: C, 61.90; H, 3.61; N, 9.42. Found: C, 61.71; H, 3.83; N, 9.63.

5.4.5. *2-Methyl-10-(3-p-tolyl-[1,2,4]oxadiazol-5-ylmethyl)-10H-acridin-9-one (Ile)*. White crystals; yield: 73%, mp >250 °C. IR (KBr): 3078, 2919, 1638, 1596, 1483, 1457 cm⁻¹. ¹H NMR (500 MHz, CDCl₃): 8.63 (d, J = 8.0 Hz, 1H, H₈), 8.42 (s, 1H, H₁), 7.97 (d, J = 8.0 Hz, 2H, H₄, H₅), 7.80-7.77 (m, 1H, H₃), 7.64-7.61 (m, 1H, H₆), 7.56 (d, J = 8.7 Hz, 2H, H₂, H₆), 7.38 (t, J = 7.4 Hz, 1H, H₇), 7.31 (d, J = 8.7 Hz, 2H, H₃, H₅), 5.80 (s, 2H, CH₂), 2.52 (s, 3H, CH₃), 2.44 (s, 3H, CH₃). ¹³C NMR (125 MHz, CDCl₃): 176.6, 176.4, 166.0, 143.3, 142.1, 140.4, 136.4, 136.0, 134.5, 131.2, 129.2, 128.6, 128.3, 127.1, 126.3, 123.9, 121.7, 116.8, 116.6, 43.0, 20.9, 20.6. Anal. Calcd for C₂₄H₁₉N₃O₂: C, 75.57; H, 5.02; N, 11.02. Found: C, 75.72; H, 4.89; N, 11.26.

5.4.6. *2-Ethyl-10-(3-p-tolyl-[1,2,4]oxadiazol-5-ylmethyl)-10H-acridin-9-one (If)*. Yellow crystals; yield: 64%, mp >250 °C. IR (KBr): 3065, 2922, 1635, 1593, 1480, 1450 cm⁻¹. ¹H NMR (500 MHz, CDCl₃): 8.63 (dd, J = 7.8, 1.4 Hz, 1H, H₈), 8.43 (d, J = 1.7 Hz, 1H, H₁), 7.97 (d, J = 8.1 Hz, 2H, H₄, H₅), 7.78 (m, 1H, H₃), 7.64 (m, 1H, H₆), 7.58 (d, J = 8.7 Hz, 2H, H₂, H₆), 7.38 (t, J = 7.4 Hz, 1H, H₇), 7.31 (d, J = 8.7 Hz, 2H, H₃, H₅), 5.80 (s, 2H, CH₂), 2.83 (q, J = 7.5 Hz, 2H, CH₂), 2.44 (s, 3H, CH₃), 1.35 (t, J = 7.5 Hz, 3H, CH₃). ¹³C NMR (125 MHz, DMSO-*d*₆): 175.5, 174.1, 167.1, 142.7, 141.6, 140.1, 136.9, 134.4, 133.9, 129.5, 127.8, 126.6, 124.5, 123.0, 121.6, 121.2, 116.4, 116.1, 114.0, 55.1, 41.5, 27.3, 15.5. Anal. Calcd for C₂₅H₂₁N₃O₂: C, 75.93; H, 5.35; N, 10.63. Found: C, 75.71; H, 5.53; N, 10.24.

5.4.7. 10-[3-(4-Chlorophenyl)-[1,2,4]oxadiazol-5-ylmethyl]-10H-acridin-9-one (**11g**). White crystals; yield: 68%, mp 244-245 °C. IR (KBr): 3114, 2922, 1646, 1603, 1488, 1464 cm⁻¹. ¹H NMR (500 MHz, CDCl₃): 8.64 (d, *J* = 7.9 Hz, 2H, H₁, H₈), 8.03 (d, *J* = 8.4 Hz, 2H, H₄, H₅), 7.83-7.80 (m, 2H, H₃, H₆), 7.63 (d, *J* = 8.5 Hz, 2H, H₃, H₅), 7.48 (d, *J* = 8.5 Hz, 2H, H₂, H₆), 5.84 (s, 2H, CH₂). ¹³C NMR (125 MHz, CDCl₃): 177.1, 174.3, 167.2, 142.3, 141.3, 136.4, 134.7, 133.3, 129.2, 127.1, 123.0, 122.0, 117.0, 42.9. MS *m/z* (%) 389 ([M + 2], 21), 387 (M⁺, 67), 208 (100), 228 (24), 193 (29), 174 (23), 152 (26), 91 (22), 65 (13), 43 (10). Anal. Calcd for C₂₂H₁₄ClN₃O₂: C, 68.13; H, 3.64; N, 10.83. Found: C, 67.88; H, 3.91; N, 10.65.

5.4.8. 2-Chloro-10-((3-(4-chlorophenyl)-1,2,4-oxadiazol-5-yl)methyl)acridin-9(10H)-one (**11h**). Yellow crystals; yield: 64%, mp > 250 °C. IR (KBr): 3058, 2924, 1638, 1600, 1487, 1463 cm⁻¹. ¹H NMR (500 MHz, CDCl₃): 8.62-8.58 (m, 2H, H₁, H₈), 8.02 (d, *J* = 8.5 Hz, 2H, H₄, H₅), 7.85-7.81 (m, 1H, H₃), 7.75 (dd, *J* = 9, 2.6 Hz, 1H, H₆), 7.62 (d, *J* = 8.6 Hz, 2H, H₃, H₅), 7.49 (d, *J* = 8.6 Hz, 2H, H₂, H₆), 7.43 (t, *J* = 7.5 Hz, 1H, H₇), 5.86 (s, 2H, CH₂). ¹³C NMR (125 MHz, CDCl₃): 176.1, 175.2, 163.6, 143.5, 142.2, 141.0, 138.3, 136.1, 135.3, 134.3, 131.3, 127.1, 126.6, 125.7, 123.1, 122.8, 121.6, 119.9, 116.3, 42.9. MS *m/z* (%) 423 ([M + 2], 24), 421 (M⁺, 68), 242 (100), 213 (26), 210 (27), 191 (25), 183 (20), 152 (25), 91 (18), 65 (11), 43 (9). Anal. Calcd for C₂₂H₁₃Cl₂N₃O₂: C, 62.58; H, 3.10; N, 9.95. Found: C, 62.31; H, 3.42; N, 9.63.

5.4.9. 10-[3-(4-Chlorophenyl)-[1,2,4]oxadiazol-5-ylmethyl]-2-methoxy-10H-acridin-9-one (**11i**). Yellow crystals; yield: 62%, mp 225-227 °C. IR (KBr): 3086, 2927, 1614, 1592, 1494, 1463 cm⁻¹. ¹H NMR (500 MHz, CDCl₃): 8.64 (d, *J* = 8.0 Hz, 1H, H₈), 8.04-8.01 (m, 3H, H₁, H₄, H₅), 7.81-7.78 (m, 1H, H₃), 7.62 (d, *J* = 8.6 Hz, 2H, H₃, H₅), 7.48 (d, *J* = 8.6 Hz, 2H, H₂, H₆), 7.44 (dd, *J* = 9.2, 3.0 Hz, 1H, H₆), 7.39 (t, *J* = 7.5 Hz, 1H, H₇), 5.83 (s, 2H, CH₂), 3.98 (s, 3H, OCH₃). ¹³C NMR (125 MHz, CDCl₃): 176.4, 173.9, 164.5, 154.3, 143.5, 135.5, 134.3, 131.3, 130.0,

129.1, 127.2, 124.4, 123.9, 122.9, 121.9, 121.5, 118.8, 106.6, 106.1, 56.3, 42.9. Anal. Calcd for $C_{23}H_{16}ClN_3O_3$: C, 66.11; H, 3.86; N, 10.06. Found: C, 65.97; H, 4.03; N, 10.37.

5.4.10. 2-Bromo-10-[3-(4-chlorophenyl)-[1,2,4]oxadiazol-5-ylmethyl]-10H-acridin-9-one (IIj).

Yellow crystals; yield: 61%, mp >250 °C. IR (KBr): 3073, 2923, 1637, 1600, 1486, 1462 cm^{-1} .

1H NMR (500 MHz, $CDCl_3$): 8.73 (d, $J = 2.4$ Hz, 1H, H_1), 8.62 (dd, $J = 8.0, 1.3$ Hz, 1H, H_8), 8.02 (d, $J = 8.5$ Hz, 2H, H_4, H_5), 7.88 (dd, $J = 9.0, 2.5$ Hz, 1H, H_6), 7.84-7.81 (m, 1H, H_3), 7.63 (d, $J = 8.8$ Hz, 2H, H_3', H_5'), 7.55 (d, $J = 8.8$ Hz, 2H, H_2', H_6'), 7.40 (t, $J = 8.1$ Hz, 1H, H_7), 5.70 (s, 2H, CH_2). ^{13}C NMR (125 MHz, $CDCl_3$): 177.1, 173.3, 164.1, 143.0, 142.2, 141.3, 138.0, 136.6, 135.1, 134.2, 129.2, 128.9, 127.2, 124.1, 123.5, 122.5, 119.8, 117.0, 114.4, 42.8. Anal. Calcd for $C_{22}H_{13}BrClN_3O_2$: C, 56.62; H, 2.81; N, 9.00. Found: C, 56.38; H, 3.02; N, 9.24.

5.4.11. 10-[3-(4-Chlorophenyl)-[1,2,4]oxadiazol-5-ylmethyl]-2-methyl-10H-acridin-9-one (IIk).

White crystals; yield: 68%, mp 247-249 °C. IR (KBr): 3110, 2922, 1637, 1597, 1486, 1462 cm^{-1} .

1H NMR (500 MHz, $CDCl_3$): 8.63 (d, $J = 7.9$ Hz, 1H, H_8), 8.42 (s, 1H, H_1), 8.02 (d, $J = 8.4$ Hz, 2H, H_4, H_5), 7.81-7.77 (m, 1H, H_3), 7.63-7.60 (m, 1H, H_6), 7.54 (d, $J = 8.4$ Hz, 2H, H_3', H_5'), 7.48 (d, $J = 8.4$ Hz, 2H, H_2', H_6'), 7.39 (t, $J = 7.4$ Hz, 1H, H_7), 5.92 (s, 2H, CH_2), 2.53 (s, 3H, CH_3). ^{13}C NMR (125 MHz, $CDCl_3$): 176.0, 174.1, 164.9, 143.4, 142.1, 140.4, 136.0, 135.4, 134.5, 133.3, 131.2, 130.2, 129.2, 127.1, 126.3, 124.0, 121.7, 116.8, 116.6, 43.0, 21.0. Anal. Calcd for $C_{23}H_{16}ClN_3O_2$: C, 68.74; H, 4.01; N, 10.46. Found: C, 68.48; H, 4.28; N, 10.61.

5.4.12. 2-Chloro-10-[3-(4-methoxyphenyl)-[1,2,4]oxadiazol-5-ylmethyl]-10H-acridin-9-one (III).

White crystals; yield: 65%, mp > 250 °C. IR (KBr): 3068, 2924, 1636, 1597, 1485, 1458

cm^{-1} . 1H NMR (500 MHz, $CDCl_3$): 8.61-8.57 (m, 2H, H_1, H_8), 8.01 (d, $J = 8.8$ Hz, 2H, H_4, H_5), 7.83-7.80 (m, 1H, H_3), 7.74 (dd, $J = 9.3, 2.5$ Hz, 1H, H_6), 7.65 (d, $J = 8.7$ Hz, 2H, H_2', H_6'), 7.00 (d, $J = 8.7$ Hz, 2H, H_3', H_5'), 5.79 (s, 2H, CH_2), 3.97 (s, 3H, OCH_3). ^{13}C NMR (125 MHz,

CDCl₃): 176.1, 175.1, 165.7, 159.0, 143.5, 142.3, 141.4, 135.1, 134.3, 129.5, 127.2, 126.7, 125.7, 124.5, 123.1, 122.5, 119.6, 116.9, 114.7, 55.4, 43.0. MS *m/z* (%) 419 ([M + 2], 21), 417 (M⁺, 69), 242 (100), 228 (24), 211 (25), 208 (30), 152 (28), 91 (24), 65 (12), 43 (12). Anal. Calcd for C₂₃H₁₆ClN₃O₃: C, 66.11; H, 3.86; N, 10.06. Found: C, 65.98; H, 4.03; N, 10.29.

5.4.13. *2-Methoxy-10-[3-(4-methoxyphenyl)-[1,2,4]oxadiazol-5-ylmethyl]-10H-acridin-9-one (11m)*. Yellow crystals; yield: 73%, mp 232-233 °C. IR (KBr): 3140, 2927, 1635, 1593, 1485, 1464 cm⁻¹. ¹H NMR (500 MHz, CDCl₃): 8.63 (dd, *J* = 8.0, 1.3 Hz, 1H, H₈), 8.03-8.01 (m, 3H, H₁, H₄, H₅), 7.80-7.77 (m, 1H, H₃), 7.64 (d, *J* = 8.3 Hz, 2H, H₂, H₆), 7.43 (dd, *J* = 9.2, 3.1 Hz, 1H, H₆), 7.39 (t, *J* = 7.4 Hz, 1H, H₇), 7.00 (d, *J* = 8.3 Hz, 2H, H₃, H₅), 5.70 (s, 2H, CH₂), 3.99 (s, 3H, OCH₃), 3.90 (s, 3H, OCH₃). ¹³C NMR (125 MHz, CDCl₃): 176.0, 175.0, 165.7, 159.0, 154.3, 142.3, 141.4, 136.8, 133.3, 127.5, 126.7, 125.0, 123.0, 122.3, 120.4, 118.6, 116.7, 114.1, 106.0, 55.8, 55.3, 43.0. MS *m/z* (%) 413 (M⁺, 70), 238 (100), 205 (22), 187 (31), 152 (31), 129 (61), 91 (35), 65 (8), 43 (14). Anal. Calcd for C₂₄H₁₉N₃O₄: C, 69.72; H, 4.63; N, 10.16. Found: C, 69.56; H, 4.84; N, 10.33.

5.4.14. *2-Bromo-10-[3-(4-methoxyphenyl)-[1,2,4]oxadiazol-5-ylmethyl]-10H-acridin-9-one (11n)*. White crystals; yield: 64%, mp > 250 °C. IR (KBr): 3054, 2924, 1635, 1595, 1484, 1458 cm⁻¹. ¹H NMR (500 MHz, CDCl₃): 8.73 (d, *J* = 2.1 Hz, 1H, H₁), 8.61 (d, *J* = 7.9 Hz, 1H, H₈), 8.01 (d, *J* = 8.5 Hz, 2H, H₄, H₅), 7.87 (dd, *J* = 9.10, 2.5 Hz, 1H, H₆), 7.84-7.81 (m, 1H, H₃), 7.65 (d, *J* = 8.5 Hz, 2H, H₂, H₆), 7.41 (t, *J* = 7.5 Hz, 1H, H₇), 7.00 (d, *J* = 8.5 Hz, 2H, H₃, H₅), 5.76 (s, 2H, CH₂), 3.90 (s, 3H, OCH₃). ¹³C NMR (125 MHz, DMSO-*d*₆): 175.6, 174.2, 162.5, 159.1, 145.8, 142.1, 141.3, 136.3, 134.4, 128.9, 128.4, 127.0, 126.6, 123.0, 122.0, 121.6, 119.4, 116.5, 114.0, 55.1, 42.8. Anal. Calcd for C₂₃H₁₆BrN₃O₃: C, 59.76; H, 3.49; N, 9.09. Found: C, 59.37; H, 3.81; N, 9.32.

5.5. Anticonvulsant activity

5.5.1. Animals and drugs

Male mice (Pasteur Institute, Iran), weighing 20–25 g, were used as experimental animals. Mice were housed in a temperature-controlled condition (25–30 °C) and 12 h light/dark cycle. They were allowed to acclimatize with free access to food and water for a 24 h period before testing except during the experiment. Animals were arranged to experimental groups randomly and each mouse was used only one time for the experiments. Phenobarbital (Sigma) and diazepam (Sigma) were used as a reference drug for comparison. Pentylenetetrazole (PTZ, Sigma) was used to induce convulsions in the experimental animals.

5.5.2. *Pentylenetetrazole (PTZ)-induced seizures test*

The anticonvulsant activity of the novel compounds was determined with PTZ-induced seizure test. In this model of induced seizure, the ability of the novel compounds to protect mice against lethal dose of PTZ (80 mg/kg) was evaluated. The test compounds were dissolved in 5% DMSO (in saline) and injected intraperitoneally (i.p.) in the doses of 10, 5, 2.5, 1 mg/kg animal weight using the same dosing volume of 0.2 ml per 20 g. PTZ was dissolved in normal saline in 2% concentration and was given i.p. in a dose of 80 mg/kg body weight. Phenobarbital and diazepam were dissolved in normal saline in 2% concentration and they were i.p. given in the doses of 10, 5, 2.5, and 1 mg/kg for phenobarbital and 1, 0.5, and 0.25 mg/kg for diazepam using the same dosing volume. All drugs were freshly prepared to the desired concentration just before use. Control animals received an equal volume of vehicle (5% DMSO in saline). After 30 min, PTZ was injected i.p. to animals. Observations were made at least 30 min after the administration of PTZ and the number of dead mice was recorded. The median effective dose (ED₅₀) was

calculated by probit-regression method and SPSS software (Chicago, IL; version 13). The ED₅₀ values were presented as mean with 95% confidence intervals calculated by probit analysis. $P < 0.05$ was considered as statistically significant.

In addition, the selected compound **11l**, vehicle, or diazepam, as reference BZD agonist, were administered 30 min and flumazenil, as antagonist of BZD receptors, 15 min before the experiments. Then PTZ (80 mg/kg; i.p.) was injected. Mice were under observation for 30 min after the injection of PTZ and the dead mice were counted.

5.5.3. Maximal electroshock (MES) induced seizures test

The ability of the novel compounds **11a-n** to prevent MES-induced seizures was evaluated in mice. The test compounds were dissolved in 5% DMSO (in saline) and injected intraperitoneally (i.p.) in the doses of 10, 5, 2.5, 1 mg/kg animal weight using the same dosing volume of 0.2 ml per 20 g. Phenobarbital and diazepam (i.p.) were used as standard drugs. Phenobarbital was given at the doses of 1, 2.5, 5, and 10 mg/kg. Diazepam was administered at the doses of 0.25, 0.5, and 1 mg/kg. Control animals received an equal volume of vehicle (5% DMSO in saline). The occurrence of hind limb tonic extension (HLTE) in mice following applying MES (60 Hz, 37.2 mA and 0.25 s, 30 min after intraperitoneal administration of the tested compounds) was assessed. The electrical current was applied through ear electrodes and mice were observed for 30 s for incidence of HLTE. The results were recorded as number of animal protected/number of animals tested. The ED₅₀ was calculated by probit-regression method and SPSS software (Chicago, IL; version 13). The ED₅₀ values were presented as mean with 95% confidence intervals calculated by probit analysis. $P < 0.05$ was considered as statistically significant.

5.6. Neurotoxicity screening

The neurotoxicity of compounds **11a-n** was studied by evaluating minimal motor impairment in mice using rotarod test. The animals were placed on a 1 inch diameter knurled plastic rod rotating at 6 rpm. Trained animals were treated with the test compounds (i.p. injection) at the doses of 30, 100, and 300 mg/kg. Neurotoxicity was indicated by the inability of the animal to maintain equilibrium on the rod for at least 1 min in each of the four trials [19]. The neurotoxicity was expressed as the median toxic dose (TD₅₀ in mg/kg) eliciting minimal neurological toxicity in 50% of animals.

5.7. Cytotoxicity screening

The human cancer cell lines MCF-7, T-47D and MDA-MB-231 were purchased from the National Cell Bank of Iran (NCBI). The cells were cultured in RPMI 1640 medium supplemented with 10% heat-inactivated fetal calf serum and 100 µg/mL streptomycin and 100 U/mL penicillin at 37 °C in 5% CO₂-humidified atmosphere. The cells (5×10⁴ cells/mL in 96-well culture plates) were incubated for 48 h with different concentrations of compounds **11a-n** dissolved in DMSO. Etoposide and DMSO were used as positive and negative controls, respectively. The viability of cells was determined by using MTT method [20]. All experiments were performed three times and the IC₅₀ values for all compounds were calculated by nonlinear regression analysis.

5.8. Molecular modeling

The homology model of the diazepam-bound GABA_A receptor developed by Ernst et al. was retrieved from the Supplementary material of their published paper [14]. The 3D structure of compound **11l** was provided using MarvinSketch 5.8.3, 2012, ChemAxon (<http://www.chemaxon.com>) and converted to pdbqt format file using AutoDockTools version 1.5.6.

(<http://mgltools.scripps.edu>). Also, the autodock format of protein was provided using the same software. Polar hydrogens and Kollman united atom partial charges were assigned to the individual protein atoms. A docking grid box was built with 40, 40 and 40 points in $x = 43.640$, $y = 43.866$ and $z = 9.3290$ directions and each docked system was carried out by 100 runs of the AUTODOCK search by the Lamarckian genetic algorithm (LGA). The lowest energy conformation of ligand-enzyme complex was evaluated by Discovery Studio 4.0 Client.

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Captions:

Figure 1. The structures of lead compounds **I** and **II**, and designed compounds **11a-n**.

Figure 2. Pharmacophoric features of benzodiazepine agonists (Diazepam and Estazolam), and the new designed compounds **11a-n**.

Figure 3. (a) Diazepam and (b) the most active compound **11l** in the BZD-binding pocket of GABA_A.

Scheme 1. Synthesis of compounds **11a-n**.

Table 1Anticonvulsant activity of compounds **11a-n** in PTZ-induced convulsion test.

Compound	R ₁	R ₂	ED ₅₀ ^a (mg/kg)		TD ₅₀ (mg/kg)	PI ^c	
			PTZ	MES		PTZ	MES
11a	H	Me	6.37 (4.43-10.24) ^b	2.52 (1.88-3.06)	538.3 (488.4-594.7)	84.5	213.6
11b	Cl	Me	6.25 (4.68-9.52)	2.24 (1.74-2.93)	483.2 (437.1-532.6)	77.3	215.7
11c	OMe	Me	6.32 (4.98-11.02)	3.25 (2.35-4.00)	521.8 (473.3-572.5)	82.4	160.5
11d	Br	Me	>50	5.12 (4.16-7.01)	594.5 (538.1-649.3)	<11.9	116.1
11e	Me	Me	>50	2.77 (2.18-3.28)	542.3 (488.7-601.2)	<10.8	195.8
11f	Et	Me	18.93 (10.62-25.31)	8.31 (6.21-10.51)	495.7 (447.6-546.8)	26.1	59.6
11g	H	Cl	5.78 (3.53-8.12)	3.57 (1.61-4.38)	528.2 (475.2-580.4)	91.3	147.9
11h	Cl	Cl	4.16 (2.51-7.33)	2.64 (1.75-3.24)	454.5 (401.5-513.3)	109.1	172.1
11i	OMe	Cl	5.22 (3.47-9.42)	3.68 (3.18-4.19)	552.6 (498.9-611.2)	105.7	150.2
11j	Br	Cl	7.57 (5.73-13.56)	2.87 (2.30-3.40)	512.1 (461.3-563.6)	67.6	178.4
11k	Me	Cl	15.15 (7.71-19.19)	6.53 (4.41-7.52)	474.8 (427.7-526.2)	31.3	72.7
11l	Cl	OMe	2.08 (1.35-3.41)	3.71 (2.76-4.34)	438.3 (392.9-490.5)	210.6	118.1
11m	OMe	OMe	2.12 (1.81-3.74)	2.42 (1.82-2.90)	461.7 (419.1-507.3)	217.4	190.8
11n	Br	OMe	6.21 (4.61-11.53)	8.71 (6.16-10.13)	471.2 (426.5-522.8)	75.8	54.1
Phenobarbital	-	-	12.45 (8.73-16.11)	20.6 (18.8-24.3)	68.9 (64.4-73.1)	5.5	3.3
Diazepam	-	-	0.68 (0.41-0.83)	0.98 (0.69-1.22)	nd ^d	nd	nd

^a ED₅₀ median effective dose affording anticonvulsant protection in 50 % of animals^b 95 % confidence intervals given in parentheses^c PI= Protective Index (TD₅₀/ED₅₀)^d Not determind

Figure 1. The structures of lead compounds **I** and **II**, and designed compounds **11a-n**.

- A) aromatic ring
- B) coplanar proton-accepting group
- C) out-of-plane aromatic ring

Figure 2. Pharmacophoric features of benzodiazepine agonists (Diazepam and Estazolam), and the new designed compounds **11a-n**.

Figure 3. (a) Diazepam and (b) the most active compound **111** in the BZD-binding pocket of GABA_A.

Scheme 1. Synthesis of compounds **11a-n**.

Graphical Abstract

Design, synthesis, pharmacological evaluation, and docking study of new acridone-based 1,2,4-oxadiazoles as potential anticonvulsant agents

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A series of acridone-based oxadiazoles were synthesized and evaluated as new anticonvulsant agents. The 2-chloro-acridinone derivative **11l** was the most potent compound in the PTZ test.