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Design, synthesis, and pharmacological evaluation of novel 1,2,4-triazol-3-amine derivatives as potential agonists of $GABA_A$ subtype receptors with anticonvulsant and hypnotic effects

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ABSTRACT

In the current study, a series of novel 1,2,4-triazol-3-amine derivatives were designed, synthesized, and biologically evaluated *in vivo* for their anticonvulsant and hypnotic effects in the pentylenetetrazole (PTZ)-induced seizures, maximal electroshock (MES)-induced seizures, and pentobarbital-induced sleeping tests. Furthermore, the possible side effects of the most potent compounds on the memory, motor coordination, and muscle strength were evaluated in passive avoidance, rotarod, and grip strength tests, respectively. The designed compounds with the main benzodiazepine pharmacophores including aromatic ring and proton accepting group completely mimiced the structure of zolpidem as an α1-selective agonist of $GABA_A$ receptor. Compounds 5c (ED_{50} ≈ 52.5 mg/kg) and 5 g (ED_{50} ≈ 16.5 mg/kg) in the PTZ test were the most potent compounds among the designed compounds. In the MES test, the observed ED_{50s} for compounds 5c and 5 g were reduced to around 11.8 mg/kg and 10.5 mg/kg, respectively. The considerable hypnotic effect in a dose-dependent manner was observed following the administration of newly synthesized compounds. In all experiments administration of flumazenil as an antagonist of benzodiazepines receptor fully antagonized observed effects which indicated the involvement of $GABA_A$ receptors. Since there was no negative effect on memory, motor coordination, and muscle strength following the administration of compounds 5c and 5g as the most potent compounds, it could be concluded that the novel compounds most likely act through α1-containing $GABA_A$ receptors and possess no affinity for α5-containing receptors. The newly designed compounds could be considered as leading compounds in synthesizing novel $GABA_A$ receptor agonists with minimum side effects.

1. Introduction

Epilepsy is characterized by a lasting predisposition to generate spontaneous epileptic seizures which affects over 65 million individuals worldwide [1] and has numerous neurobiological, cognitive, and psychosocial consequences [2]. The presence of comorbidities such as depression, anxiety disorder, psychosis, autism, and insomnia is the norm in more than 50% of patients who suffer from seizure [3]. Antiseizure medicines with the aim of stopping seizures at the earliest opportunity and reducing morbidity are the main treatment modality in epilepsy [4,5]. Despite a large number of available drugs in different categories for the treatment of epilepsy, only 66% of individuals are treated with the current drugs [6]. Unfortunately, antiepileptic drugs that are used to achieve or maintain seizure control are frequently associated with unwanted side effects such as memory impairment, loss of coordination, muscle relaxation, fatigue, dizziness, loss of libido, and drug dependency [7]. Therefore, compliance with medication is an important issue in anticonvulsant therapy [8].

Benzodiazepines (BZDs) as a class of agents that work in the central nervous system are widely used in various conditions such as epilepsy, anxiety, and sleep disorders [9]. They act by binding to a specific binding site known as the BZD receptor on the gamma-aminobutyric acid-A ($GABA_A$) receptors, opening of a selective chloride ion channel, and hyperpolarization of the postsynaptic membrane [10]. Since like other antiepileptic agents, BZDs have their own unwanted side effects, the design and synthesis of novel compounds with BZDs structure are a

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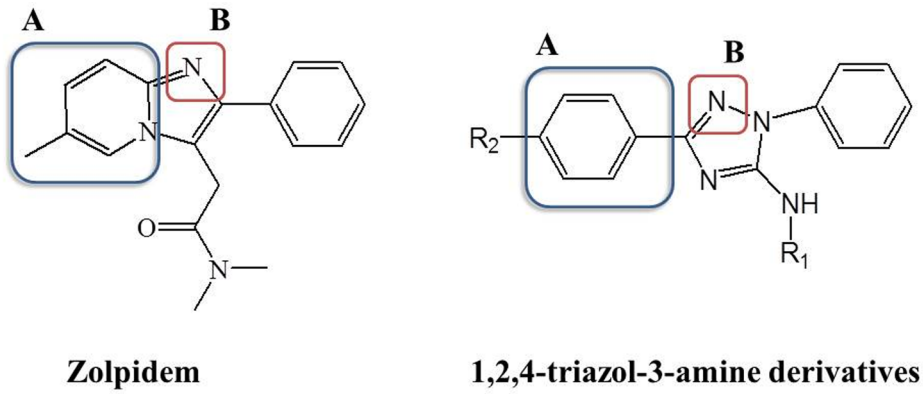


Fig. 1. The structure of zolpidem and novel synthesized 1,2,4-triazol-3-amine derivatives. The aromatic ring (A) and coplanar proton accepting group (B) are highlighted.

great deal of attention [11,12]. Based on the structure–activity relationship of BZDs, for any high-affinity benzodiazepine ligand, some pharmacophore groups such as an aromatic ring that is located in a suitable distance (5 Angstrom) from a coplanar proton accepting functional group in the same plane is required. The presence of a second out-of-plane aromatic ring potentiates binding to the $GABA_A$ receptors [13].

In this research, based on the previous studies conducted on the triazole nucleus [14] we designed novel 1,2,4-triazol-3-amine derivatives according to the structure–activity relationship (SAR) of benzodiazepines with all suggested requirements for binding to $GABA_A$ receptors [15,16] (Fig. 1). To confirm whether the designed compounds could mimic the structure of a BZD agonist, conformational analysis and superimposition of energy minima conformers of them on zolpidem as a known BZD agonist were performed.

The potential anticonvulsant activity of the synthesized compounds was evaluated in the pentylenetetrazole (PTZ)-induced lethal convulsion and maximal electroshock (MES) tests. Pentobarbital-induced sleep test was employed to evaluate the hypnotic effect of novel compounds. Step-through passive avoidance, grip strength, and rotarod tests were carried out in order to assess whether the novel compounds induce anterograde memory impairment, muscle relaxation, and impairment in motor coordination, respectively. Finally, the effect of flumazenil as a BZD receptor antagonist was determined to confirm the possible mechanism of action of the synthesized compounds.

2. Materials and methods

2.1. Conformational analysis

MMX force field method followed by AM1 calculation implemented in HyperChem 8 software (Hypercube, Inc.) was used for performing conformational analysis of novel compounds along with zolpidem as a well-known $GABA_A$ receptor agonist to find out whether the designed molecules are able to mimic the proper conformation for binding to the benzodiazepine receptor or not. For this purpose, the superimposition of energy minima conformers of the novel compounds on the corresponding conformer of zolpidem was carried out.

2.2. Materials and instrumentation

All chemicals and reagents used in this study were purchased from Sigma/Aldrich (St. Louis, MO, USA) or Merck (Darmstadt, Germany) Company and were used without any further purification. An electrothermal 9300 apparatus (Ontario, Canada) was used for melting points measuring. To obtain infrared spectra and elemental analysis for the synthesized compounds, Shimadzu FT-IR 8400S spectrographs (Shimadzu, Japan) using the potassium bromide (KBr) disc method and elemental analyzer (Costech, Italy) were used, respectively. $_1$H and $_{13}$C NMR spectra were recorded in $CDCl_3$ by a Bruker FT-500 MHz instrument (Bruker Biosciences, USA). MS was recorded using an Agilent Technology (HP) mass instrument operating at 70 eV.

2.3. General procedure for the synthesis of novel 1,2,4-triazol-3-amine derivatives

A mixture of aniline derivatives (2.0 mmol) and trichloroacetonitrile (3.0 mmol) was stirred in dimethylformamide solvent (3.0 mL) for 20 min at room temperature (RT) to afford trichloroacetamidine analogs. Then the obtained derivatives were reacted with different hydrazonoyl chlorides analogs (2.0 mmol) in dimethylformamide solvent (2.0 mL) in the presence of trimethylamine (1.0 mmol) and CuI (0.10 mmol) as a catalyst for 2 h at room temperature to obtain target compounds. After the end of the reaction, the mixture washed with water (4 mL), ammonium chloride solution (4 mL), and dichloromethane (4 mL). Finally, the non-aqueous layer was separated and evaporated under reduced pressure and the precipitate washed with *n*-hexane solvent. Further purification was obtained by recrystallization from absolute ethanol.

2.3.1. N-benzyl-2-phenyl-5-p-totyl-2H-1,2,4-triazol-3-amine (5a)

White powder; **Yield**: 0.91 g (65%); **Mp**: 189 °C; **FT-IR spectrum**, ν, cm^{-1}: 3496, 2996, 1755, 1483; 500 MHz **^{1}H NMR** ($CDCl_3$): δ ppm: 2.41(s, 3H, CH_3), 4.65(s, 2H, CH2), 6.69(s, 1H, NH), 6.92(t, J = 7.3 Hz, 1H, Ar), 7.15(d, J = 7.6 Hz, 2H, Ar), 7.21(d, J = 8.0 Hz, 2H, Ar), 7.32(t, J = 7.3 Hz, 2H, Ar), 7.38–7.41(m, 3H, Ar), 7.56–7.60(m, 4H, Ar). 125 MHz **^{13}C NMR** ($CDCl_3$) δ_C:18.88, 45.58, 124.64, 126.68, 128.84, 129.31, 129.84, 132.64, 133.17, 138.12, 138.88, 142.20, 143.86, 145.40, 160.53, 164.21. **MS**: *m/z* (%) = 340(M^+, 20), 263(90), 234(40), 143(30), 106(100), 91(65). Anal calcd for $C_{22}H_{20}N_4$: C, 77.62; H, 5.92; N, 16.46; Found: C, 77.66; H, 5.96; N, 16.39.

2.3.2. 3-(4-bromophenyl)-N-(4-methylbenzyl)-1-phenyl-1H-1,2,4-triazol-5-amine (5b)

White powder; **Yield**: 0.97 g (63%); **Mp**: 191 °C; **FT-IR spectrum**, ν, cm^{-1}: 3406, 3078, 1666, 1591, 1457; 500 MHz **^{1}H NMR** ($CDCl_3$): δ ppm: 2.45(s, 3H, CH3), 4.32(s, 2H, CH2), 5.53(s, 1H, NH), 6.88(t, J = 7.3 Hz, 1H, Ar), 7.11(d, J = 7.7 Hz, 2H, Ar), 7.18(d, J = 8.1 Hz, 2H, Ar), 7.27–7.30(m, 6H, Ar), 7.55(d, J = 8.1 Hz, 2H, Ar). 125 MHz **^{13}C NMR** ($CDCl_3$) δ_C: 20.81, 45.88, 122.11, 124.27, 126.65, 129.73, 129.79, 130.36, 132.98, 133.04, 138.27, 138.97, 145.23, 155.53, 164.22, 167.12. **MS**: *m/z* (%) = 419(M^+, 20), 341(55), 327(50), 263(60), 154(50), 120(100). Anal calcd for $C_{22}H_{19}BrN_4$: C, 63.02; H, 4.57; N, 13.36; Found: C, 63.09; H, 4.62; N, 13.31.

2.3.3. N-(4-chlorobenzyl)-2-phenyl-5-p-tolyl-2H-1,2,4-triazol-3-amine (5c)

White powder; **Yield**: 0.85 g (60%); **Mp**: 174 °C; **FT-IR spectrum**, ν, cm^{-1}: 3320, 3066, 1818, 1449; 500 MHz **^{1}H NMR** ($CDCl_3$): δ ppm: 2.45(s, 3H, CH3), 4.52(s, 2H, CH2), 6.16(s, 1H, NH), 7.31–7.34(m, 5H, Ar), 7.40(d, *J* = 7.9 Hz, 2H, Ar), 7.49(d, *J* = 8.1 Hz, 2H, Ar), 7.78(d, *J* = 8.1 Hz, 2H, Ar), 7.92(d, *J* = 8.5 Hz, 2H, Ar). 125 MHz **^{13}C NMR** ($CDCl_3$) δ_C: 19.99, 45.20, 127.44, 128.74, 129.21, 130.15, 130.27, 130.68, 132.53, 133.14, 134.65, 142.11, 145.97, 147.28, 161.02, 163.66. **MS**: *m/z* (%) = 297(80), 283(100), 263(50), 234(55), 140(90), 134(80). Anal calcd for $C_{22}H_{19}ClN_4$: C, 70.49; H, 5.11; N, 14.95; Found: C, 70.43; H, 5.07; N, 14.90.

2.3.4. N-benzyl-5-(4-chlorophenyl)-2-phenyl-2H-1,2,4-triazol-3-amine (5d)

Cream powder; **Yield**: 0.88 g (70%); **Mp**: 169 °C; **FT-IR spectrum**, ν, cm^{-1}: 3496, 1621, 1476, 1397; 500 MHz **^{1}H NMR** ($CDCl_3$): δ ppm: 4.45(s, 2H, CH2), 6.01(s, 1H, NH), 7.38(d, *J* = 8.4 Hz, 2H, Ar), 7.47–7.50(m, 7H, Ar), 7.59(t, *J* = 7.4 Hz, 1H, Ar), 7.91(d, *J* = 8.4 Hz, 2H, Ar), 7.97(d, *J* = 8.5 Hz, 2H, Ar). 125 MHz **^{13}C NMR** ($CDCl_3$) δ_C: 46.64, 127.48, 128.75, 129.03, 129.30, 130.67, 131.62, 131.90, 132.30, 134.42, 140.34, 142.14, 147.23, 162.13, 164.64. **MS**: *m/z* (%) = 360(M^+, 20), 283(30), 250(50), 235(30), 138(100), 107(30), 77(70). Anal calcd for $C_{21}H_{17}ClN_4$: C, 69.90; H, 4.75; N, 15.53; Found: C, 69.95; H, 4.68; N, 15.59.

2.3.5. 5-(4-chlorophenyl)-2-phenyl-N-p-tolyl-2H-1,2,4-triazol-3-amine (5e)

White powder; **Yield**: 0.81 g (65%); **Mp**: 211 °C; **FT-IR spectrum**, ν, cm^{-1}: 3444, 2963, 1606, 1472; 500 MHz **^{1}H NMR** ($CDCl_3$): δ ppm: 2.60(s, 3H, CH3), 6.12(s, 1H, NH), 7.29–7.34(m, 5H, Ar), 7.47–7.50(m, 5H, Ar), 7.60(t, *J* = 7.4 Hz, 1H, Ar), 7.96(d, *J* = 8.5 Hz, 2H, Ar). 125 MHz **^{13}C NMR** ($CDCl_3$) δ_C: 19.49, 128.77, 129.25, 129.32, 129.35, 133.22, 133.35, 134.11, 134.41, 136.11, 137.61, 141.56, 145.21, 162.15, 165.72. **MS**: *m/z* (%) = 360(M^+, 50), 282(60), 267(30), 248(65), 119(65), 106(75), 91(100). Anal calcd for $C_{21}H_{17}ClN_4$: C, 69.90; H, 4.75; N, 15.53; Found: C, 69.94; H, 4.80; N, 15.48.

2.3.6. 5-(4-bromophenyl)-N,2-diphenyl-2H-1,2,4-triazol-3-amine (5f)

Cream powder; **Yield**: 0.79 g (61%); **Mp**: 183 °C; **FT-IR spectrum**, ν, cm^{-1}: 3507, 1621, 1382, 1319; 500 MHz **^{1}H NMR** ($CDCl_3$): δ ppm: 6.40(s, 1H, NH), 7.30–7.35(m, 7H, Ar), 7.48(d, *J* = 8.5 Hz, 2H, Ar), 7.60(t, *J* = 7.7 Hz, 1H, Ar), 7.45(t, *J* = 7.4 Hz, 2H, Ar), 8.04(d, *J* = 8.5 Hz, 2H, Ar). 125 MHz **^{13}C NMR** ($CDCl_3$) δ_C: 126.17, 127.42, 127.76, 128.75, 129.23, 130.00, 130.14, 132.56, 133.35, 135.68, 142.24, 144.86, 155.10, 162.31, 164.55. **MS**: *m/z* (%) = 391(M^+, 10), 313(20), 298(35), 235(25), 153(25), 91(100), 77(50). Anal calcd for $C_{20}H_{15}BrN_4$: C, 61.39; H, 3.86; N, 14.32; Found: C, 61.45; H, 3.81; N, 14.37.

2.3.7. N-(4-chlorophenyl)-2,5-diphenyl-2H-1,2,4-triazol-3-amine (5 g)

Cream powder; **Yield**: 0.86 g (68%); Mp: 180 °C; **FT-IR spectrum**, ν, cm^{-1}: 3216, 1941, 1442, 1364; 500 MHz **^{1}H NMR** ($CDCl_3$): δ ppm: 6.35(s, 1H, NH), 7.38(t, *J* = 8.4 Hz, 1H, Ar), 7.47–7.50(m, 8H, Ar), 7.60(t, *J* = 7.4 Hz, 1H, Ar), 7.91(d, *J* = 8.4 Hz, 2H, Ar), 7.97(d, *J* = 7.4 Hz, 2H, Ar). 125 MHz **^{13}C NMR** ($CDCl_3$) δ_C: 127.48, 128.75, 129.03, 129.23, 129.30, 130.67, 131.62, 131.90, 132.30, 134.39, 134.42, 156.34, 163.12, 165.67. **MS**: *m/z* (%) = 235(50), 220(85), 111(100), 77(75). Anal calcd for $C_{20}H_{15}ClN_4$: C, 69.26; H, 4.36; N, 16.15; Found: C, 69.20; H, 4.42; N, 16.09.

2.3.8. N-(4-bromophenyl)-5-(4-chlorophenyl)-2-phenyl-2H-1,2,4-triazol-3-amine (5 h)

White powder; **Yield**: 0.94 g (59%); Mp: 171 °C; **FT-IR spectrum**, ν, cm^{-1}: 3328, 3089, 1643, 1494; 500 MHz **^{1}H NMR** ($CDCl_3$): δ ppm: 6.63(s, 1H, NH), 7.29–7.34(m, 7H, Ar), 7.38(d, *J* = 8.1 Hz, 2H, Ar), 7.48(d, *J* = 7.3 Hz, 2H, Ar), 7.91(d, *J* = 7.3 Hz, 2H, Ar). 125 MHz **^{13}C NMR** ($CDCl_3$) δ_C: 126.57, 127.46, 128.76, 129.24, 130.70, 131.62, 132.55, 133.42, 136.89, 142.11, 144.51, 154.16, 163.21, 164.39. **MS**: *m/z* (%) = 424(M^+, 30), 345(25), 313(25), 154(38), 111(38), 77(100). Anal calcd for $C_{20}H_{14}BrClN_4$: C, 56.43; H, 3.31; N, 13.16; Found: C, 56.49; H, 3.27; N, 13.11.

2.4. Docking study

Molecular docking using the Lamarckian genetic algorithm search method implemented in Auto Dock 4.2.3. Software (http://autodock.scripps.edu) was performed, using the previously described method [11]. Docking study was performed in order to find out the mode of corresponding interactions of designed compounds with amino acids of the BZD-binding pocket of the $GABA_A$ receptor (α1β2γ2).

2.5. Pharmacology

2.5.1. Animals and drugs

All experiments were performed with male NMRI mice (weighing 18–25 g). Animals were housed in plexiglass cages under the same ambient temperature of 22 ± 2 °C, 50% ± 10% humidity, and a 12 h: 12 h light–dark cycle. They had free access to food and water. Experimental animals were randomly allocated to groups of ten and were transferred to the animal laboratory at least 1 h prior to the beginning of each experiment to get acclimatized to the laboratory condition. Novel synthesized compounds, diazepam, and flumazenil were dissolved in dimethyl sulfoxide and administrated in the volume of 5 mL/kg by intraperitoneal route (i.p.). Pentobarbital and pentylenetetrazole (PTZ) were prepared in normal saline. The injection volume for pentobarbital and pentylenetetrazole was 10 mL/kg. All experiments were carried out based on the National Institutes of Health (NIH) guidelines for the Care and Use of Laboratory Animals. Each mouse was used once and possible efforts were made to decrease animal numbers and distress.

2.5.2. The anticonvulsant activity

The anticonvulsant activity of the novel compounds was evaluated in PTZ-induced seizures and MES-induced seizures tests as described before [17]. To summarize, mice were treated with different doses of novel compounds, diazepam, or vehicle 30 min before the induction of seizure in each model. In the PTZ and MES models, the seizure was induced by the administration of pentylenetetrazole (100 mg/kg) and application of electroshock (10 Hz, 37.2 mA, and 0.3 s) through the ear electrodes, respectively. Protection of mice against the lethal dose of pentylenetetrazole was considered as the potential anti-seizure activity in the PTZ model while in the MES model the potential activity of compounds was determined by the reduction in the number of hind limb tonic extension (HLTE). In both models of seizure, flumazenil as a $GABA_A$ receptor antagonism was used for the evaluation of the possible mechanism of action.

2.5.3. Potentiation of pentobarbital sleeping time

The experiment was performed with a slight modification of the previously described method [18]. Mice were administered a single dose of novel compounds with different concentrations 30 min prior to the administration of pentobarbital (50 mg/kg). Pentobarbital was used to induce the loss of the righting reflex (LRR). Each mouse was observed and the LRR duration (the time between the loss and the recovery of the righting reflex) was recorded for each mouse. To examine the possible mechanism of action of novel compounds in the pentobarbital-induced sleeping model, flumazenil was administrated at 10 mg/kg and 15 min prior to injection of pentobarbital.

2.5.4. Step-through passive avoidance test

The Step-through passive avoidance test was carried out using a

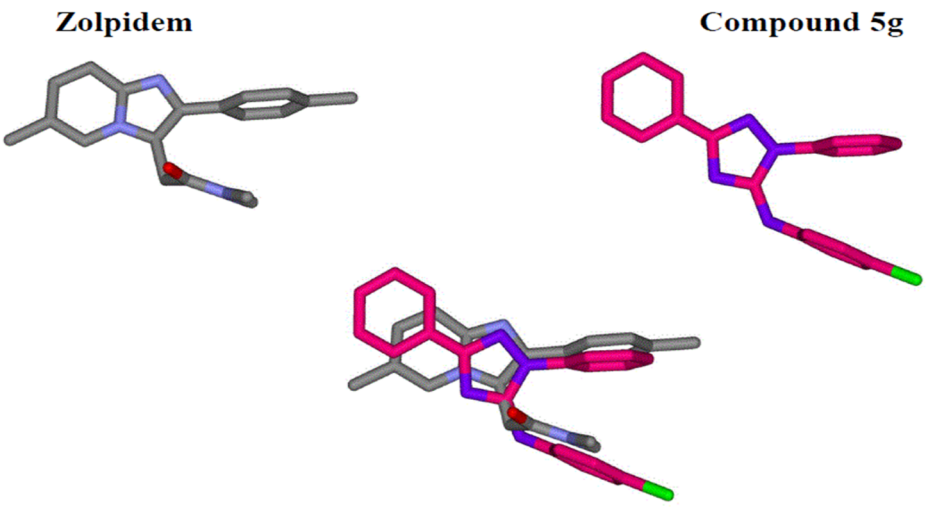


Fig. 2. Superimposition of compound 5g and zolpidem as a well-known $GABA_A$ receptor agonist.

common procedure with a slight modification [19]. On the training day, mice received the novel compounds at doses corresponding to their ED_{50} and twice ED_{50} values from the PTZ test, diazepam (2 mg/kg), or vehicle. After 30 min, mice were placed individually in the light chamber of apparatus and the gate between the two compartments was opened after 30 s. Animals were allowed to explore the light compartment for 8 min and they got punishment by an adequate electric footshock (0.2 mA, 50 Hz, 2 s) when they entered the dark compartment. On the test day (24 h later), the experiment was repeated on the pre-trained animals while the gate between light and dark compartments was open. The time that the mice took to enter the dark box was recorded and considered as an index for retention of the learned experience.

2.5.5. Open field test

Thirty minutes and 24 h after administration of novel compounds at doses corresponding to their ED_{50} and twice ED_{50} values, vehicle, and diazepam (2 mg/kg), the locomotor activity of animals was examined in a Plexiglas box (42 cm × 42 cm × 42 cm). The apparatus was equipped with a video-based Ethovision System (Noldus, Wageningen, Netherlands). The camera recorded the animals' movement while they were exploring the open filed arena for ten minutes. The total locomotor activity of animals was expressed as a total distance movement in centimeter [20]. Ethanol 70% was used to clean the open field arena between the two subjects.

2.5.6. Rotarod test

The possible unwanted effect of novel compounds on motor coordination of animals was assessed using the rotarod test [21]. Mice were trained for 3 days on an accelerating rotarod (rod diameter: 2 cm) that rotated at 6 revolutions /min. During each training session, mice were trained for 3 min with an unlimited number of trials. On the test day (24 h after the last training), mice were intraperitoneally pretreated with the test compounds, vehicle, and diazepam (2 mg/kg). Following 30 min, the animals were tested on the rotarod apparatus. Mice were observed with a cut-off time of 3 min and the latency to the first fall from the rod was recorded. Motor impairment was defined as the inability to remain on the rotating rod compared to the control group.

2.5.7. Grip strength test

The effect novel compounds at doses corresponding to their ED_{50} and twice ED_{50} values from the PTZ test, vehicle, and diazepam (2 mg/kg) on skeletal muscular strength in mice were quantified in the grip-strength test [22]. Thirty minutes after each treatment, mice were placed on the tension pad (8 cm × 8 cm) of the apparatus which was connected to an isometric force transducer. Animals were lifted by their tail so that they grasp the tension pad and then pulled back until the pad was released. This procedure was repeated in triplicate and the averaged force (gram) was recorded automatically by the apparatus.

2.5.8. Preliminary evaluation of toxicity

The toxicity of the most potent compounds was evaluated in 10 mice. Mice were treated with 105 mg/kg of compound 5c and 70 mg/kg of compound 5 g which were 10 times more than ED_{50s} of hypnotic doses and were observed for 24 h for any possible toxicity.

2.6. Data analysis

GraphPad Prism Software (Graph Pad Prism software, San Diego, CA; version 5.0) was used for data analysis. ED_{50s} with their 95% confidence limits were estimated using the log-probit method. The results were statistically compared using one-way analysis of variance (ANOVA), followed by Tukey post-test, when appropriate. The numerical results of the experiment were expressed as mean ± standard error of the mean (SEM). Statistical significance was considered when p-values were < 0.05.

3. Results and discussion

3.1. Conformational analysis and molecular modeling (docking) studies

As shown in Fig. 2, following superimposing energy minima conformers of novel compounds on zolpidem, aromatic ring and proton accepting group as two main benzodiazepine pharmacophores of compound 5 g and zolpidem are completely matched. The protein–ligand interaction of zolpidem and the most potent designed compound (5 g) with amino acids of the BZD-binding site of the $GABA_A$ receptor is illustrated in Fig. 3. As Fig. 3 depicts, zolpidem and compound 5 g establish the same hydrogen binding with Thr109 and Thr206. Furthermore, compound 5 g interacts with Tyr159 and His101 through π-π stacking and hydrophobic interactions, respectively.

3.2. Chemistry

The newly designed compounds (**5a-5 h**) were synthesized according to the following synthetic pathway (Scheme 1). Briefly, the synthetic pathway was started with the reaction of different aniline derivatives **1** and trichloroacetonitrile **2** in dimethylformamide solvent for 20 min at room temperature to afford required compound **3**. Compound 3 then participated in the reaction with different

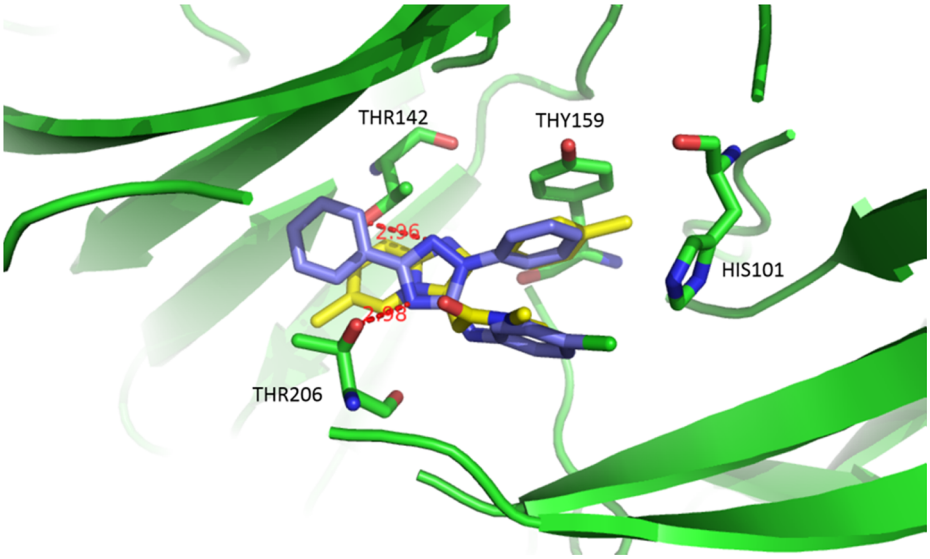


Fig. 3. The binding modes of zolpidem (yellow color) and the most potent novel agent 5 g (blue color) with amino acids in the BZD-binding pocket of the $GABA_A$ receptor. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

hydrazonoyl chlorides analogs **4** in dimethylformamide solvent in the presence of trimethylamine and CuI catalyzer for 2 h at room temperature to obtain target compounds in good yields (59–70%). The structures of novel synthesized compounds were characterized by the aid of elemental analysis, $_1$H NMR, $_{13}$C NMR, FT-IR, and mass spectrometry analysis.

3.3. Pharmacology

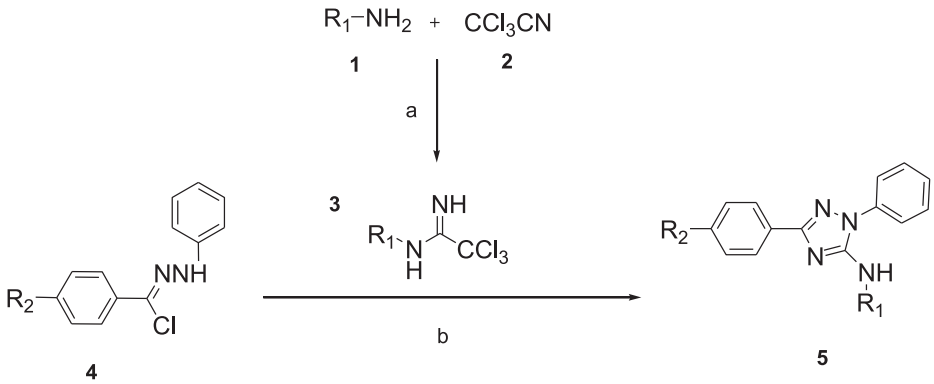
3.3.1. The anticonvulsant activity

The novel synthesized compounds were screened for their possible anticonvulsant activity in the PTZ and MES models of seizure. Diazepam was used as a standard anticonvulsant agent. The results are presented as ED_{50} values with 95% confidence intervals. ED_{50} refers to the median effective dose which protects 50% of animals against induced seizure. As illustrated in Table 1, compound 5c (ED_{50} ≈ 52.5 mg/kg) and 5 g (ED_{50} ≈ 16.5 mg/kg) had potent anticonvulsant activity in the PTZ-induced seizures model while other compounds were almost inactive. In the MES-induced seizures model, all compounds were found to have anticonvulsant activity but compounds 5c and 5 g with ED_{50} values of 11.8 mg/kg and 10.50 mg/kg, respectively were the most potent compounds among the novel 1,2,4-triazol-3-amine derivatives. The higher potency of the tested agents in the MES test compared to the PTZ test could be related to the type of induced epilepsy. The MES induces epileptic seizure represents the grand mal type of epilepsy while PTZ induces epileptic seizure produces petit mal or tonic-clonic epileptic seizures [23]. In both models of seizure, flumazenil as an antagonist of benzodiazepines receptor significantly antagonized anticonvulsant effects of the novel compounds which indicate the involvement of $GABA_A$ receptors in the observed effects.

3.3.2. Potentiation of pentobarbital sleeping time

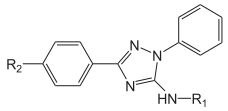
The hypnotic effect of all compounds was evaluated in the pentobarbital induced loss of righting test. The results were compared to diazepam as a standard hypnotic agent. ED_{50} of each tested compound which refers to the dose that doubles the sleeping time compared to the control group with the calculated 95% confidence interval is reported in Table 1. In all compounds, the sleeping time was increased in a dose-dependent manner. Compounds 5 g (ED_{50} ≈ 7.1 mg/kg) and compound 5c (ED_{50} ≈ 10.5 mg/kg) were found more potent than other agents. The observed pharmacological effect was completely blocked following the administration of flumazenil which reconfirms the responsibility of benzodiazepines receptors.

Considering the obtained ED_{50s} in the PTZ, MES and anticonvulsant and hypnotic activities of the novel compounds, the presence of a halogen substituent at the para position of phenyl ring at R_2 (especially Cl) increases the observed anticonvulsant and hypnotic effects while a methyl substituent diminishes the activity. Replacement of phenyl ring by benzyl ring increases activity probably due to better hydrophobic interaction. Furthermore, the presence of an electron-donating group like a methyl substituent at R_2 position produces more potent compounds compared to electron-withdrawing groups such as halogens.

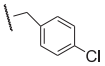
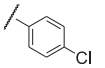
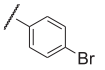


Scheme 1. Reagents and conditions: (a) Dimethylformamide, RT, 20 min; (b) Triethylamine, CuI, Dimethylformamide, RT, 2 h.

Table 1
The anticonvulsant and hypnotic activities of novel synthesized compounds and diazepam.



ED_{50} mg/kg(95% confidence intervals)

Pentobarbital test	MES test	PTZ test	R2	R1	Compound
80.54 (68.66–109.70)	> 100	> 100	CH_3		5a
95.24 (79.32–139.50)	> 100	> 100	Br		5b
10.53 (8.92–14.93)	11.80 (8.26–15.92)	52.48 (36.14–72.27)	CH_3		5c
31.30 (25.06–41.04)	27.92 (16.21–42.53)	> 100	Cl		5d
96.98 (88.10–160.30)	> 100	> 100	Cl		5e
50.52 (32.28–73.90)	47.42 (31.26–67.76)	> 100	Br		5f
7.11 (5.93–8.70)	10.54 (7.74–14.32)	16.55 (13.03–19.54)	–		5 g
32.29 (24.53–43.85)	> 100	> 100	Cl		5 h
1.60 (0.98–2.01)	1.02 (0.73–1.26)	0.96 (0.66–1.20)			diazepam

3.3.3. Passive avoidance test

Passive avoidance test which is a well-known model in the assessment of anterograde amnesia was carried out to determine whether the novel compounds cause memory impairment in mice or not. Since compounds 5c and 5g were more potent than other agents in the previous experiments, the possible unwanted effect of these agents on anterograde memory were evaluated. Following the administration of compounds 5c and 5g, the latency in the entrance to the dark box did not change compared to the control group while the administration of diazepam significantly reduced the latency time (Fig. 4). This finding reveals that the novel agents most probably act through α1-containing $GABA_A$ receptors that are responsible for the sedative effect and anticonvulsant action of benzodiazepine-like agents and rules out the involvement of α5-containing $GABA_A$ receptors which affect cognitive functions [24,25].

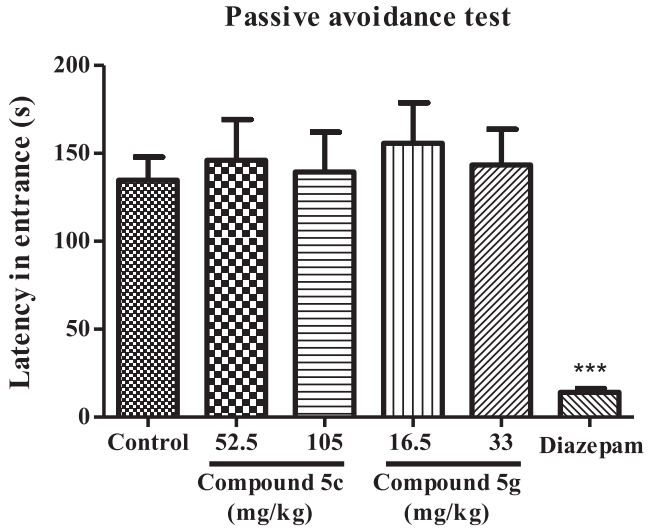


Fig. 4. The effect of novel compounds and diazepam (2 mg/kg) on avoidance latency in the passive avoidance test. Values are reported as mean ± SEM. *** indicates $p < 0.001$ compared to the control group; n = 10 in all groups.

3.3.4. Open field test

The assessment of locomotor activity in the open field test represented a significant reduction in the spontaneous motor activities of animals 30 min after administration of compounds 5c, 5g, and diazepam (Fig. 5A). No obvious reduction in the locomotor activities was observed on the next day of treatment (Fig. 5B). Therefore, it should be pinpointed that the latency time in the entrance to the dark box in the passive avoidance test is not due to reduced locomotor activity caused by tested compounds.

3.3.5. Rotarod test

The effect of compounds 5c and 5g on the motor coordination of animals in the rotarod test is shown in Fig. 6. The evaluation of mice in this test revealed that the treatment of animals with the novel compounds was unable to produce alteration in the motor coordination of animals. Finally, the administration of diazepam (2 mg/kg) as a reference drug significantly reduced the time mice stayed on the rod, with respect to the control group.

3.3.6. Grip strength test

The results of the grip strength test revealed that compounds 5c and 5g do not cause significant muscle tone reduction compared to the control group while administration of diazepam as a standard muscle relaxant meaningfully affects the skeletal muscular strength in mice (Fig. 7). Since α5-containing $GABA_A$ receptors significantly contribute to the benzodiazepine induced muscle relaxation [26], it could be concluded that the novel compounds do not affect this type of receptors.

3.3.7. Preliminary evaluation of toxicity

No significant toxicity reaction except sedation and hypnosis was observed following injection of 105 mg/kg of compound 5c and 70 mg/kg of compound 5g, which were 10 times more than the estimated

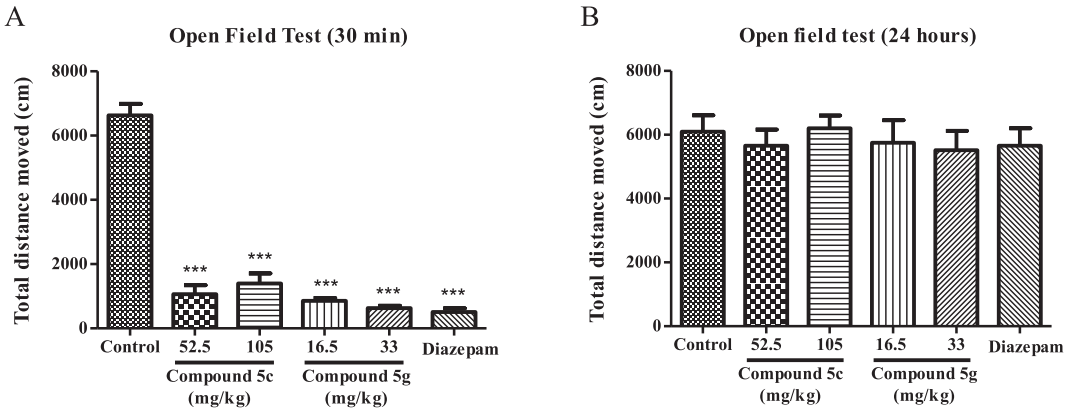


Fig. 5. The effect of novel compounds and diazepam (2 mg/kg) on the locomotor activity of animals in the open field test 30 min (A) and 24 h (B) after treatment. Values are reported as mean ± SEM. *** indicates $p < 0.001$ compared to the control group; n = 10 in all groups.

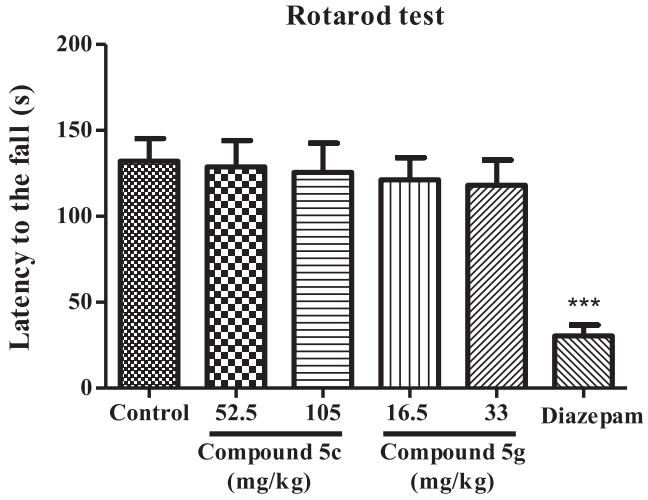


Fig. 6. The effect of novel compounds and diazepam (2 mg/kg) on the motor coordination of animals in the rotarod test. Values are reported as mean ± SEM. *** indicates $p < 0.001$ compared to the control group; n = 10 in all groups.

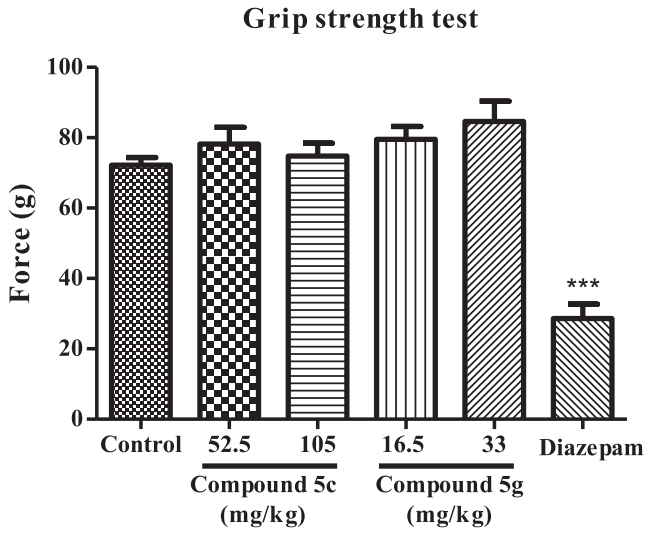


Fig. 7. The effect of novel compounds and diazepam (2 mg/kg) on the skeletal muscular strength of animals in the grip strength test. Values are reported as mean ± SEM. *** indicates $p < 0.001$ compared to the control group; n = 10 in all groups.

hypnotic doses. Probably the synthesized compounds are safe enough for future studies but more comprehensive toxicity studies need to be carried out.

4. Conclusion

In summary, a series of novel 1,2,4-triazol-3-amine derivatives were designed and synthesized as potential agonists of $GABA_A$ subtype receptors. The biological effects of synthesized compounds were investigated using animal models. Compounds 5c and 5g in the PTZ test and compounds 5c, 5d, 5f, and 5g in the MES test revealed significant anticonvulsant activity while all the designed compounds showed a considerable hypnotic effect. Furthermore, compounds 5g and 5c were the most potent compounds in all experiments. The observed effects were completely antagonized by flumazenil which confirms the involvement of $GABA_A$ receptors in the observed effects. No negative effects on the memory, motor coordination, and muscle strength were observed in the passive avoidance test, rotarod test, and grip strength test, respectively. Therefore, we can conclude that the novel compounds most likely act similar to α1-selective agonist zolpidem which possesses no affinity for α5-containing $GABA_A$ receptors. The promising compounds (especially compound 5g) could be used in designing and synthesizing novel benzodiazepine receptor agonists with minimum side effects.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bioorg.2020.104212.

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