SYNTHESIS AND ANTICONVULSANT ACTIVITY OF NOVEL 2-AMINO-5-[4-CHLORO-2-(2-CHLOROPHENOXY) PHENYL]-1,3,4-THIADIAZOLE DERIVATIVES

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ABSTRACT

Several novel 2-amino-5-[4-chloro-2-(2-chlorophenoxy)phenyl]-1,3,4-thiadiazole derivatives **4a-d** were synthesized and their anticonvulsant activity was determined by evaluation of the ability of theses compounds to protect mice against convulsion induced by a lethal doses of pentylentetrazole (PTZ) and maximal electroshock (MES).

The result of anticonvulsant data shows that among the synthesized compounds, 5-[4-chloro-2-(2-chlorophenoxy)phenyl]-*N*-ethyl-1,3,4-thiadiazol-2-amine **4c** was the most active compound in both MES and PTZ tests with an ED_{50} of 20.11 and 35.33 mg/kg, respectively.

Key words: 1,3,4-thiadiazole, Anticonvulsant activity, Maximal electroshock

INTRODUCTION

Epilepsy is a chronic and often progressive disorder characterized by recurrent transient attacks which are caused by an abnormal discharge of cerebral neurons (1, 2). The current therapy of epilepsy with antiepileptic drugs is associated with side effects, dose-related and chronic toxicity, and teratogenic effects (3, 4). Therefore, there is continuing demand for new anticonvulsant agents.

Preparation and anticonvulsant activity of several derivatives of imidazoles, triazoles, oxadiazoles and thiadiazoles have been reported previously (5-7). Among these compounds, potential anti-convulsant activities of 2-aryl-5-hydrazino-1,3,4-thiadiazole derivatives made it a good lead for the synthesis of new compounds (5). The major literally modifications reported for this structure include: substitution of the 5-membered heterocycle with 1,3,4-oxadiazole, replacement of hydrazine with amine or alkyl amines, and using aryls such as phenyl, 2-phenoxyphenyl or 3-hydroxy-2-naphthyl at position 5 of the hetero-cycle (8-11). Among the synthesized compounds, 2 – substituted – 5 -(3-hydroxy-2-naphthyl)-1,3,4-thiadiazole derivatives **I**, (Figure 1) were active in pentylenetetrazole-induced generalized convul-sion (8). Anticonvulsant activity for 4*H*-3-[4-chloro-2-(2-chlorophenoxy) phenyl]-1,2,4-triazole derivative **II**, (Figure 1) has also been reported (10).

In continuation of our studies, for a new anticonvulsant scaffold, the synthesis and anticonvulsant activity of compound **III** is reported. This hybrid structure (**III**) was designed by combinig phenoxyphenyl moiety from compound **II,** and 2-amino-1,3,4-thiadiazole from compound **I** (Figure 1).

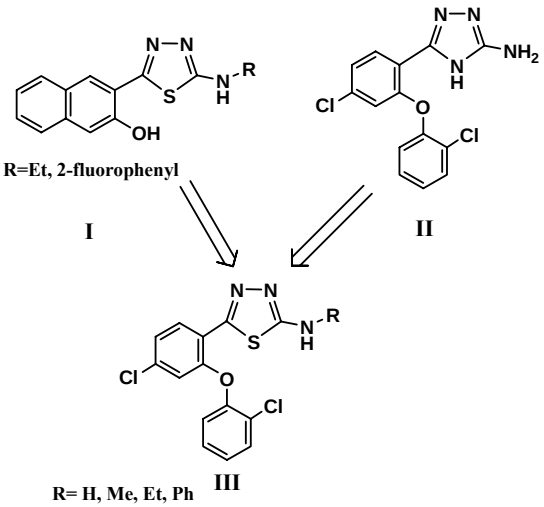

Figure 1. Chemical structure of compounds **I-III**

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The anticonvulsant activity of the synthesized compounds was determined by standard protocols; maximal electro shock (MES) and pentylenetetrazole (PTZ) induced seizure in mice.

4-Chloro-2-(2-chlorophenoxy) benzoic acid **1** was prepared from 2,4-dichlorobenzoic acid and 2-chlorophenol(12). Treatment of **1** with thionyl chloride yielded 4-chloro-2-(2-chlorophenoxy) benzoyl chloride **2** which upon reaction with 4-alkylthiosemicarbazide provided 1-[4-Chloro-2-(2-chlorophenoxy)benzoyl]- 4- alkylthiosemicarbazides **3a-c**. Cyclization of the latter compounds with H_2SO_4 gave the final compounds **4b-d**. Compound **4a** was prepared from the direct reaction of **1** and thiosemicarbazide in polyphosphoric acid (15). The assignments of the structure of the prepared compounds were based on spectral data (IR, Mass, ^{1}H NMR).

MATERIALS AND METHODS

Chemistry

All chemicals were obtained from Sigma and Merck chemical companies. TLC analyses were performed on a 3-10 cm aluminium sheet precoated with silica gel 60-254 (Merck).

Melting points were taken on a Kofler hot stage apparatus and are uncorrected. IR spectra (KBr) were recorded on Shimadzu IR spectrophotometer. The mass spectra were run on a Finigan TSQ-70 spectrometer (Finigan, USA) at 70 eV. ^{1}H NMR (80 MHz) spectra were taken using $CDCl_3$ as solvent and tetramethylsilane (TMS) as internal standard.

4-Chloro-2-(2-chlorophenoxy) benzoic acid **1** was prepared according to the published method (12).

4-Chloro-2-(2-chlorophenoxy)benzoyl chloride **2**

To a stirring solution of thionyl chloride (4mL) was added 4-chloro-2-(2-chlorophenoxy) benzoic acid (2.83g, 10 mmol) and the resulting mixture was refluxed for 1hr. The excess of thionyl chloride was evaporated *in vaccue* and the resulted solid (2.71 g, 90%) was used for the next step without further purification (13).

General procedure for preparation of 1-[4-Chloro-2-(2-chlorophenoxy)benzoyl]-4-substituted thiosemicarbazide (**3a-c)**

To a stirring solution of thiosemicarbazide(9.1g, 0.1 mol) in dry pyridine(30 mL) at -5°C , was added a solution of 4-chloro-2-(2-Chlorophenoxy)benzoyl chloride **2** (30.1g, 0.1mol) in dry benzene (30 mL). The stirring was continued for half an hour at -5°C and then over night at room temperature. After evaporation of the solvents the residue was treated with water and the precipitate was filtered washed with water and crystallized from ethanol.

1-[4-Chloro-2-(2-chlorophenoxy)benzoyl] methylthiosemicarbazide **3a**

This compound (88%)was prepared from the reaction of **2** and 4- methylthiosemicarbazide ; mp 202-204°C . IR(KBr): υ (cm $^{-1}$) 3333,3207(NH), 1644(C=O); ^{1}HNMR (δ, ppm) 10.80-10.65 (m,2H, NH), 10.10-9.80 (m, 1H, NH), 8.11 (d,1H, aromatic, J=8.4 Hz), 7.75-7.10 (m, 5H, aromatic), 6.59 (s, 1H, aromatic), 3.05 (s, 3H, CH_3)); mass: m/z (%): 371(M^++2 , 5) , 369(M^+,12), 269(10), 267(62), 265(100), 230(21), 202(24),173(15).

1-[4-Chloro-2-(2-chlorophenoxy)benzoyl]-4-ethylthiosemicarbazide **3b**

This compound (79%)was prepared from the reaction of **2** and 4-ethylthiosemicarbazide mp 195-197°C. IR (KBr): υ (cm $^{-1}$) 3329, 3201(N-H), 1644(C=O); ^{1}H- NMR (δ, ppm) 10.69 (br s,2H, NH), 10.05-9.78 (m, 1H, NH), 8.11 (d,1H, aromatic, J=8.5 Hz), 7.68-7.00 (m, 5H, aromatic), 6.57 (br s, 1H, aromatic), 3.80-3.30 (m, 2H, CH_2), 1.15 (t, 3H, CH_3, J=7.12); mass: m/z (%):387(M^++4,7), 385(M^++2 , 43) , 383(M^+,64),298(22), 296(28), 269 (28), 267(67), 265(100), 234(12), 230(12), 202(21).

1-[4-Chloro-2-(2-chlorophenoxy)benzoyl]-4-phenylthiosemicarbazide **3c**

This compound (74%)was prepared from the reaction of **2** and 4-phenyllthiosemicarbazide mp 175-176°C. IR(KBr): υ (cm $^{-1}$)3300, 3205 (NH), 1633(C=O); ^{1}HNMR (δ, ppm) 11.4 (br s, 2H, NH), 10.30-9.90 (m, 1H, NH), 8.10 (d,1H, aromatic, J=8.4 Hz), 7.71-6.98 (m, 10H, aromatic), 6.61 (s, 1H, aromatic); mass: m/z (%): 432(M^+, 5), 298(36), 296(54), 269 (10), 267(65), 265(100).

2-Amino-5-[4-chloro-2-(2-chlorophenoxy)phenyl]-1,3,4-thiadiazole **4a**

A finely ground mixture of the 4-chloro-2-(2-Chlorophenoxy) benzoic acid (2.83 g, 10 mmol) and thiosemicarbazide(0.91g, 10 mmol) was added portionwise over 0.5 h to polyphosphoric acid (25g) at 80-90 °C and the mixture was stirred at this temperature for 2-4 hrs. The reaction mixture was then cooled to room temperature and basified by addition of aqueous ammonia. The solids were filtered, washed with water and air-dried to give 2-Amino-5-[4-chloro-2-(2-chlorophenoxy)phenyl]-1,3,4-thiadiazol **4a** (63%). IR (KBr): υ (cm $^{-1}$) 3330, 3110 (NH_2); NMR (δ, ppm) 5.22(brs, 2H, NH_2), 8.35 (d,1H, aromatic,

J= 8.6 Hz), 7.73- 6.65 (m, 4H, aromatic), 6.71(d, 1H, aromatic, *J*=1.6 Hz); mass: m/z (%):337(M^+ ,8) , 304(100), 302 (60), 267 (63).

General procedure for the preparation of 5-[4-Chloro-2-(2-chlorophenoxy) phenyl]-N-substituted -1,3,4-thiadiazol-2-amine **(4b-d)**

Solutions of **3a-c** (0.003 mol, 1.07g) in 3ML of concentrated sulfuric acid were stirred at room temperature for 25 min and the resulting mixture were poured into ice cold water. The solution were made alkaline to pH 8 with aqueous ammonia and the precipitated product were filtered. The final cake were washed with water and crystallized from ethanol.

5-[4-Chloro-2-(2-chlorophenoxy) phenyl]-N-methyl -1,3,4-thiadiazol-2-amine **4b**

(yield 65%); mp 225-228°C. IR(KBr): υ (cm^{-1}) 3451 (N-H), ^{1}HNMR(ppm, $CDCl_3$) 10.70 (br, 1H, NH) , 8.30 (d,1H, aromatic, *J*= 8.6 Hz), 7.70- 6.68 (m, 4H, aromatic), 6.70(d, 1H, aromatic, *J*=1.6 Hz), 3.07 (s, 3H, CH_3); mass: m/z (%):355(M^++ 4, 8), 353 (M^++2, 35), 351(M^+ ,57) , 318 (84), 316 (100), 267 (63), 265(85).

5-[4-Chloro-2-(2-chlorophenoxy)phenyl]-N-ethyl-1,3,4-thiadiazol-2-amine **4c**

(yield 63%); mp 190-192°C. IR(KBr): υ (cm^{-1}) 3431 (N-H); ^{1}HNMR (δ, ppm):10.29 (brs, 1H, NH) 8.33(d,1H, aromatic, *J* = 8.7 Hz), 7.60-7.00 (m, 5H, aromatic), 6.71 (d,1H, aromatic, *J*= 1.6 Hz), 3.65-3.22 (m, 2H, CH_2), 1.34 (t, 3H, CH_3, *J*= 7.1 Hz); mass: m/z (%):367 (M^++ 4, 10), 367 (M^++ 2, 40), 365 (M^+, 81), 332 (39) , 330 (100).

5-[4-Chloro-2-(2-chlorophenoxy)phenyl]-N-phenyl-1,3,4-thiadiazol-2-amine **4d**

(yield 61%); mp 258-262°C. IR(KBr): υ (cm^{-1}) 3380 (N-H), ^{1}HNMR (δ, ppm) 8.29 (d, 1H, aromatic, J= 8.6 Hz), 7.70-7.00 (m, 10 H, aromatic), 6.71 (br, 1H, aromatic), 10.41 (s,1H, NH); mass: m/z (%):413 (M^+ ,5) , 380 (11), 378 (24), 157 (22), 155 (73), 80 (100).

Pharmacological evaluation

Male NMRI mice (Pasteur Institute, Iran) weighting 20–25 g (n=10) were used in these experiments. The animals were kept in the groups of ten in cages under constant temperature (24±1 °C) and 12 h light/dark schedule. They had free access to standard mouse diet and tab water except during the experiment. On the day of the experiment, animals were transferred to individual cases randomly and allowed to acclimatize for 30 min before injection of drug or vehicle. Diazepam (Sigma) was considered as positive control drug with anticonvulsant effect in both models. Test compounds, flumazenil and diazepam (Hoffmann La Roche) were given IP as a freshly prepared solution in 50% DMSO and 50% sterile normal saline.

The vehicle had no effect on the test system. Thirty minutes after administration of the test compound, mice were administered the convulsant stimulus: a) maximal electroshock (MES, 60 Hz, 37.2 mA for 0.25 s) b) pentylenetetrazole (PTZ, 100mg/Kg, ip). In each of these tests, mice were considered protected according to the following criteria: the number of dead animals in PTZ tests and occurrence of HLTE (hind limb tonic extension) in MES model. The ED_{50} was defined as the dose causing significant protection from seizures in 50% of mice and it was calculated when appropriate, with a computer program for the analysis of quantal data. All ED_{50} values were calculated from the result of at least four doses, each administrated to at least one group of 10 mice.

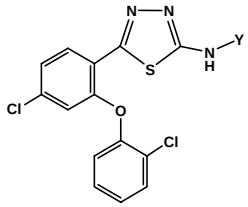
Statistical analyses

ED_{50} values and 95% confidence limits were determined using probit-log (dose) model with flumazenil and the test compounds as a categorical covariate and forcing through parallel dose response. Rightward shift of the ED_{50} in logarithmic scale after administration of flumazenil was not significant. All statistical calculations were performed by SPSS for windows.

RESULTS AND DISCUSSION

The anticonvulsant activities of **4a-d** by two models are reported in table 1.The result shows that compound **4a,** having unsubstituted amino group, was inactive in both PTZ and MES tests. This is in contrast with the good anticonvulsant activity of 2-amino-5-phenyl-1,3,4-thiadiazole of our previous investigation (11). The reason for this observation could have been due to different conformations of the target compound caused by various substituents at phenyl moiety. While substitution of the amino group in **4a** with a methyl group **(4b)** led to slight increase in the activity of the compound in MES method (ED_{50}= 95.52 mg/kg), the presence of ethyl group in compound **4c** increased the activity in both MES and PTZ tests with an ED_{50} of 20.11 and 35.33 mg/Kg, respectively. On the other hand, substitution of the amino group by a phenyl moiety **(4d)** yielded a compound which was inactive in both tests. These findings are similar to the results observed in 2-amino-1,3,4-thiadiazoles substituted by a hydroxynaphthyl group at position 5, in which the highest protection was related to the ethyl derivative (8).

Table1. Structure and anticonvulsant activity of target compounds.



Compound.	Y	ED_{50} mg/kg[a]	
		PTZ	MES
4a	H	>100	>100
4b	Me	>100	95.52 (91.23-98.66)[b]
4c	Et	35.33 (31.22-41.75)[b]	20.11 (18.90-23.45)[b]
4d	Ph	>100	>100
Diazepam	-	1.66 (1.28-2.55)[b]	1.62 (1.28-2.03)[b]

[a] n=10, 95% confidence limits in parentheses, LD_{50} of all compounds >300 mg/kg.
[b] ED_{50} not significantly increased in the presence of flumazenil 10 mg/kg ($p<0.05$).

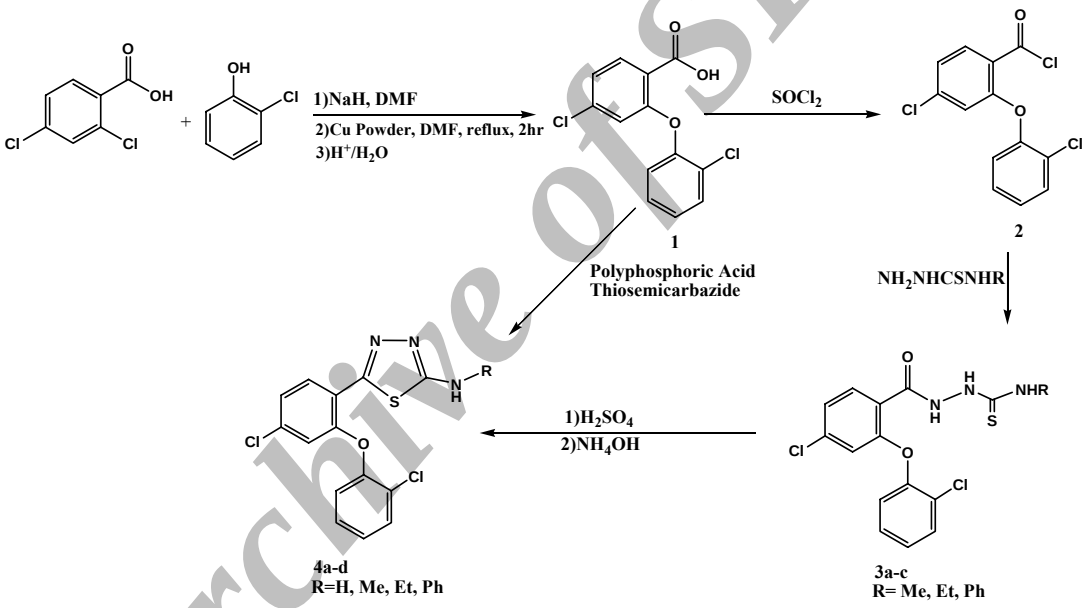


Figure 2. Synthesis of target compounds

To determine whether the anticonvulsant activity of compound **4c** was mediated by benzodiazepine receptors, flumazenil, a benzodiazepine receptor antagonist, was injected 5 min before administration of **4c**. No change in the anticonvulsant activity of compound **4c** was observed.

A comparison of results at this study with those literally reported (10), for action of compound **II** (Figure 1) through benzodiazepine receptors, indicates that the presence of different heterocyclic ring systems could lead to different modes of action for anticonvulsant activity of the synthesized compounds.

CONCLUSION

A number of 2-amino-5-(4-chloro-2-(2-chlorophenoxy)phenyl-1,3,4-thiadiazole derivatives **4a-d** were synthesized and tested for anticonvulsant activity. Compound 5-(4-chloro-2-(2-chlorophenoxy) phenyl)-N-ethyl-1,3,4-thiadiazol-2-amine **4a** was the most active compound in both MES (ED_{50}=20.11) and PTZ (ED_{50}=35.33) tests.

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