

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

<sup>\*1,2</sup>Foroumadi A., <sup>2</sup>Sheibani V., <sup>1</sup>Sakhteman A., <sup>2</sup>Rameshk M., <sup>1</sup>Abbasi M., <sup>2</sup>Farazifard R., Tabatabai S.A., <sup>1</sup>Shafiee A.

<sup>1</sup>Faculty of Pharmacy and Pharmaceutical Sciences Research Center, Tehran University of Medical Sciences, Tehran, <sup>2</sup>Kerman Neuroscience Research Center, Kerman University of Medical Sciences, Kerman, <sup>3</sup>Department of Medicinal Chemistry, Faculty of Pharmacy, Shaheed Beheshti University of Medical Sciences, Tehran, Iran.

Received: 7 Aug 2006; Revised: 1 Nov 2006 Accepted: 11 Nov 2006

### 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 these compounds to protect mice against convulsion induced by a lethal doses of pentylenetetrazole (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<sub>50</sub> of 20.11 and 35.33 mg/kg, respectively.

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

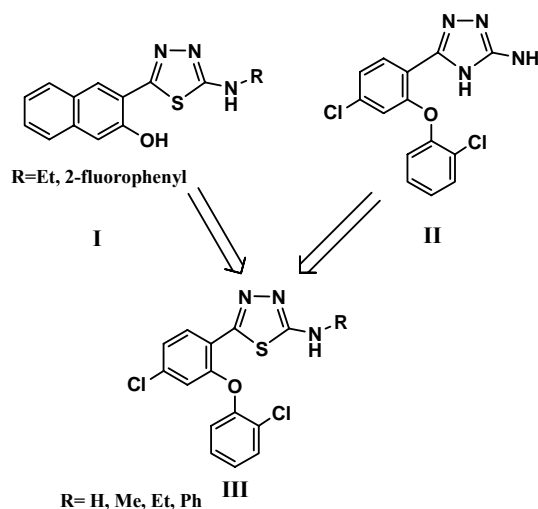
### INTRODUCTION

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 anticonvulsant 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 heterocycle (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 convulsion (8). Anticonvulsant activity for 4H-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 combining 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**

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<sub>2</sub>SO<sub>4</sub> 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, <sup>1</sup>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. <sup>1</sup>H NMR (80 MHz) spectra were taken using CDCl<sub>3</sub> 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 *vacuo* 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):  $\nu$  (cm<sup>-1</sup>) 3333,3207(NH), 1644(C=O); <sup>1</sup>HNMR ( $\delta$ , 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<sub>3</sub>); mass: m/z (%): 371(M<sup>+</sup>+2, 5), 369(M<sup>+</sup>,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):  $\nu$  (cm<sup>-1</sup>) 3329, 3201(N-H), 1644(C=O); <sup>1</sup>H- NMR ( $\delta$ , 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<sub>2</sub>), 1.15 (t, 3H, CH<sub>3</sub>, J=7.12); mass: m/z (%):387(M<sup>+</sup>+4,7), 385(M<sup>+</sup>+2, 43), 383(M<sup>+</sup>,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-phenylthiosemicarbazide mp 175-176°C. IR(KBr):  $\nu$  (cm<sup>-1</sup>)3300, 3205 (NH), 1633(C=O); <sup>1</sup>HNMR ( $\delta$ , 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<sup>+</sup>, 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):  $\nu$  (cm<sup>-1</sup>) 3330, 3110 (NH<sub>2</sub>); NMR ( $\delta$ , ppm) 5.22(brs, 2H, NH<sub>2</sub>), 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):  $\nu$  ( $\text{cm}^{-1}$ ) 3451 (N-H),  $^1\text{H}$ NMR(ppm,  $\text{CDCl}_3$ ) 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,  $\text{CH}_3$ ); 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):  $\nu$  ( $\text{cm}^{-1}$ ) 3431 (N-H);  $^1\text{H}$ NMR ( $\delta$ , 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,  $\text{CH}_2$ ), 1.34 (t, 3H,  $\text{CH}_3$ ,  $J = 7.1$  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):  $\nu$  ( $\text{cm}^{-1}$ ) 3380 (N-H),  $^1\text{H}$ NMR ( $\delta$ , 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 ( $24 \pm 1^\circ\text{C}$ ) and 12 h light/dark schedule. They had free access to standard mouse diet and tap water except during the experiment. On the day of the experiment, animals were transferred to individual cages 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.

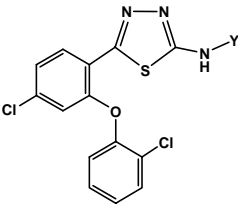
The vehicle had no effect on the test system. Thirty minutes after administration of the test compound, mice were administered the convulsant stimulus: a) maximal electroshock (MES, 60 Hz, 37.2 mA for 0.25 s) b) pentylenetetrazole (PTZ, 100mg/Kg, ip). In each of these tests, mice were considered protected 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  $\text{ED}_{50}$  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  $\text{ED}_{50}$  values were calculated from the result of at least four doses, each administered to at least one group of 10 mice.

*Statistical analyses*

$\text{ED}_{50}$  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  $\text{ED}_{50}$  in logarithmic scale after administration of flumazenil was not significant. All statistical calculations were performed by SPSS for windows.

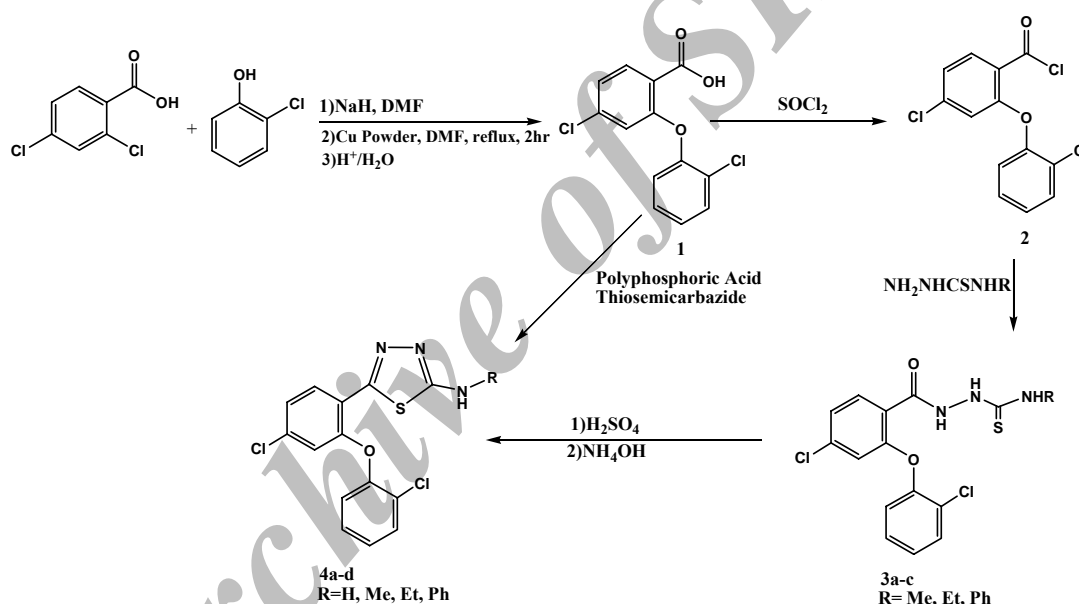
## RESULTS AND DISCUSSION

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 ( $\text{ED}_{50} = 95.52$  mg/kg), the presence of ethyl group in compound **4c** increased the activity in both MES and PTZ tests with an  $\text{ED}_{50}$  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 <sub>50</sub> mg/kg <sup>a</sup>	
		PTZ	MES
<b>4a</b>	H	>100	>100
<b>4b</b>	Me	>100	95.52 (91.23-98.66) <sup>b</sup>
<b>4c</b>	Et	35.33 (31.22-41.75) <sup>b</sup>	20.11 (18.90-23.45) <sup>b</sup>
<b>4d</b>	Ph	>100	>100
Diazepam	-	1.66 (1.28-2.55) <sup>b</sup>	1.62 (1.28-2.03) <sup>b</sup>

<sup>a</sup>n=10, 95% confidence limits in parentheses, LD<sub>50</sub> of all compounds >300 mg/kg.  
<sup>b</sup>ED<sub>50</sub> 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<sub>50</sub>=20.11) and PTZ (ED<sub>50</sub>=35.33) tests.

## ACKNOWLEDGEMENT

This work was supported by a grant from Kerman Neuroscience Research Center.

## REFERENCES

1. Löscher W. New visions in the pharmacology of anticonvulsion. *Eur J Pharm* 1998; 342: 1-13.
2. Gringauz A. *Introduction to Medicinal Chemistry*; 1st ed. Wiley-VCH: New York, 1997; 589-593.
3. Brodie M. Monotherapy trials: prerequisite data. *J Epilepsy Res* 2001; 45:61-64.
4. Mattson. Antiepileptic drug monitoring: a reappraisal *R H Epilepsia (Suppl. 2)* 1995; 36:S-13
5. Chapleo CB, Mayers M, Myers PL, Saville JF, Smith ACB, Stillings MR, Tulloch IF, Walter DS and Welbourn AP. Substituted 1,3,4-thiadiazole with anticonvulsant activity. 1. Hydrazines. *J Med Chem* 1986; 29: 2273-2280.
6. Shafiee A, Rastkari N, Sharifzadeh M. Anticonvulsant Activities of New 1,4-dihydropyridine Derivatives Containing 4-nitroimidazolyl Substituents. *Daru* 2004; 12: 81-86.
7. Bineshmarvasti M, Sharifzadeh M, Jalilian R, Soltaninejad K and Shafiee A. Syntheses and Anticonvulsant Activity of N4-Substituted Triazolylthiazoles. *Daru* 2003; 11: 74-78.
8. Dogan HN, Duran A, Rollas S, Sener G, Uysal MK and Gulen D. Synthesis of new 2,5-disubstituted 1,3,4-thiadiazoles and preliminary evaluation of anticonvulsant and antimicrobial activities. *Bioorg Med Chem* 2002; 10: 2893-2898.
9. Almasirad A, Tabatabai SA, Faizi M, Kebriaeezadeh A, Mehrabi N, Dalvandi A and Shafiee A. Synthesis and anti convulsant activity of new 2-substituted 5-[2-(2-fluorophenoxy)phenyl]-1,3,4-oxadiazole and 1,2,4-triazoles. *Bioorg Med Chem Lett* 2004; 14: 6057-6059.
10. Akbarzadeh T, Tabatabai SA, Khoshnoud MJ, Shafaghi B, Shafiee A. Design and synthesis of 4H-3-(2-Phenoxy) phenyl-1,2,4-triazole derivatives as benzodiazepine receptor agonists. *Bioorg Med Chem* 2003; 11: 769-773.
11. Foroumadi A, Tabatabai SA, Gitinezhad G, Zarrindast MR and Shafiee A. Synthesis and anticonvulsant activity of 5-aryl-1,3,4-thiadiazole derivatives. *Pharm Pharmacol Commun* 2000; 6: 31-33.
12. Woodley HF, Lewis T and Jones I. Method of antagonizing herbicides on soy bean and cotton, US Pat. 4,242,121, 30 Dec 1980; ref. Chem. Abstr 1981; 95: 19713t.
13. Detar DF and Hlynsky A. The thermal decomposition of 2-phenoxybenzoyl peroxide. *J Am Chem Soc* 1955; 77: 4411-4412.
14. Jackson HC, Hansen HC, Kristiansen M, Suzdak PD, Klitgaard H, Judge ME and Swedberg MDB. Anticonvulsant profile of the imidazoquinazolines NNC 14-0185 and NNC 14-0189 in rats and mice. *Eur J Pharmacol* 1996; 306: 21-30.
15. Turner S, Myers M, Gadie B, Nelson AJ, Pape R, Saville JF, Doxey JC and Berridge TL. Antihypertensive Thiadiazoles. 1. Synthesis of some 2-Aryl-5-hydrazino-1,3,4-thiadiazoles with vasodilator activity. *J Med Chem* 1998; 31: 902-905.