Sci Pharm. 2008; 76: 185–201 doi:10.3797/scipharm.0803-10

Synthesis and Pharmacological Evaluation of New 2-Substituted-5-{2-[(2-halobenzyl)thio)phenyl}-1,3,4-oxadiazoles as Anticonvulsant Agents

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Abstract

A new series of 2-substituted-5-{2-[(2-halobenzyl)thio)phenyl}-1,3,4-oxadiazoles was designed, synthesized and investigated for anticonvulsant activities. The designed compounds contain the main essential pharmacophore for binding to the benzodiazepine receptors. Conformational analysis and superimposition of energy minima conformers of designed molecules on estazolam, a known benzodiazepine receptor agonist, revealed that the main characteristics of the proposed benzodiazepine pharmacophore were well matched. Electroshock and pentylenetetrazole-induced lethal convulsion tests showed that some of the synthesized compounds had significant anticonvulsant activity. The structure-activity relationship study of these compounds indicated that the introduction of an amino group at position 2 of 1,3,4-oxadiazole ring and a fluoro substituent at the ortho position of the benzylthio moiety had the best anticonvulsant activity. Anticonvulsant effects of active compounds were antagonized by flumazenil, a

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benzodiazepine antagonist, which establishes the involvement of benzodiazepine receptors in these effects.

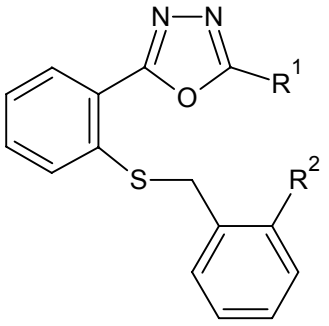
Keywords

Benzodiazepine receptor • 1,3,4-Oxadiazoles • Anticonvulsant • SAR

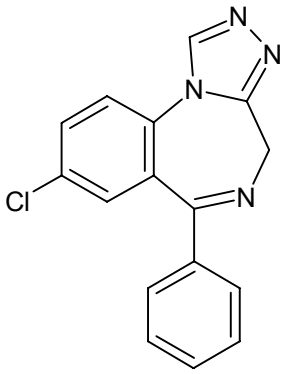
Introduction

Benzodiazepine (BZD) receptor ligands allosterically modulate the action of GABA on neuronal chloride ion flux, thus eliciting a wide variety of pharmacological actions ranging a continuum from full agonists (anxiolytic, sedative/hypnotic, and anticonvulsant activities) to inverse agonists (proconvulsant and anxiogenic activities), and antagonists, which do not exhibit any pharmacological effects but can antagonize the action of both agonists and inverse agonists [1–3]. The BZD binding sites in the brain were identified and described by radioligand receptor binding assays and originally it was found that only 1,4-BZD derivatives bind to these receptors. It has since been shown that many groups of compounds bind to the BZD receptor with high affinity, e. g., triazolopyridazines, cyclopyrrolones, quinolines and β-carbolines [4–7]. Several pharmacophore models have been proposed for BZDs, and amongst all models suggested for binding to the BZD receptor at least two features are common: an aromatic ring and a coplanar proton accepting group in suitable distance (5A^{o}). Also, the presence of a second out-of-plane, aromatic ring could potentiate binding to the receptor [8–12]. On this basis, a wide variety of compounds with a chemical structure different from that of benzodiazepines have been synthesized and tested. We recently started a wide research program aimed to design new BZD receptor ligands characterized by a higher degree of flexibility compared with classic BZD ligands. Accordingly, we reported several 1,3,4-oxadiazole derivatives which showed considerable anticonvulsant activity [13–15]. As part of our ongoing research program to design new anticonvulsant agents, we describe herein the synthesis and biological evaluation of a novel group of 2-substituted-5-[2-(2-halobenzylthio)phenyl]-1,3,4-

oxadiazoles (Fig.1) with a flexible second out-of-plane aromatic ring, benzylthio group which has all the essential pharmacophore groups for binding to the BZD receptors.



R^1 = NH_2, NHPh, SH, SMe, SEt, SBz
R^2 = F, Cl



Estazolam

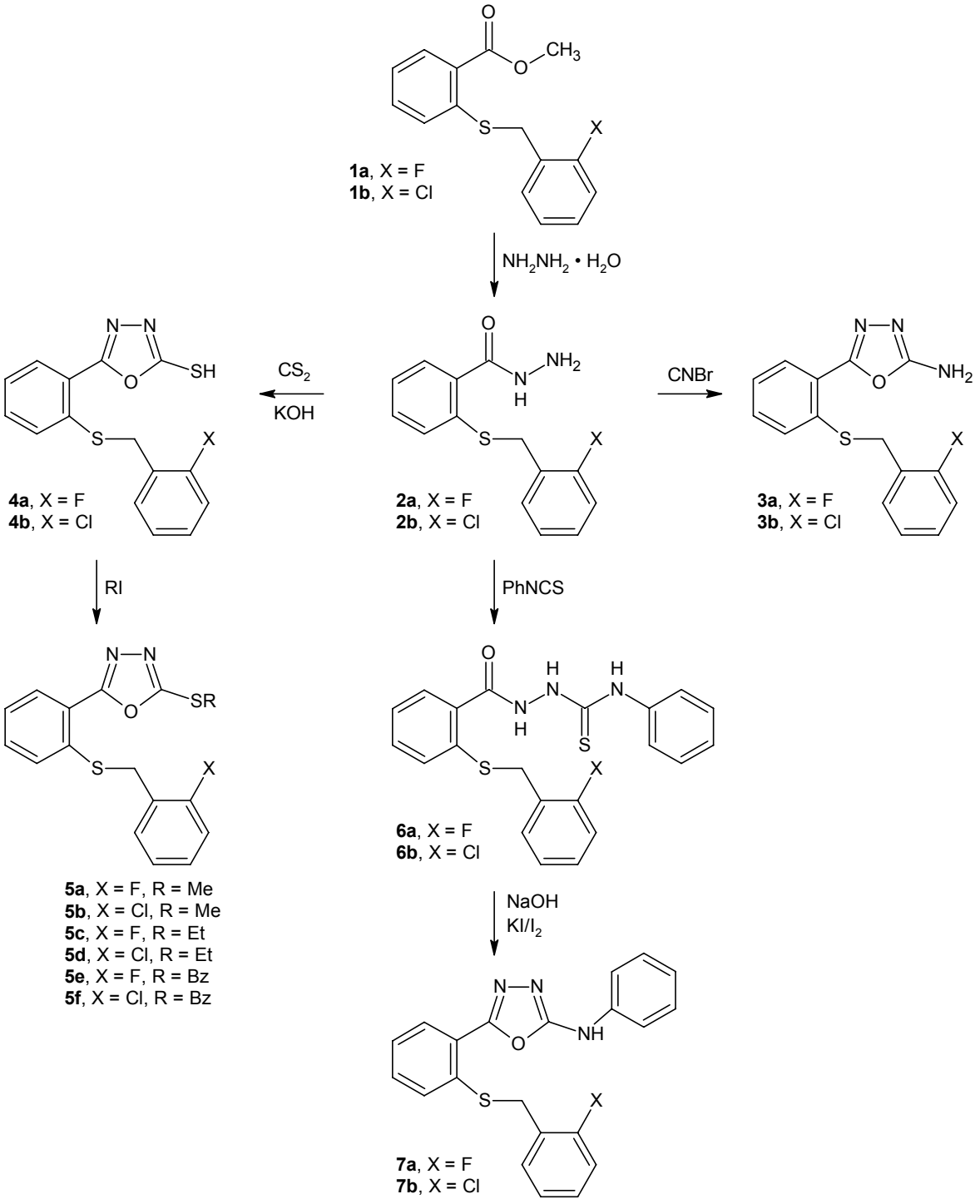
Fig. 1.

Results and Discussion

1. Chemistry

The target 1,3,4-oxadiazole derivatives were synthesized according to Scheme 1. Accordingly, reaction of 2-[(2-halobenzyl)thio]benzoic acid methyl ester **1** [15] with hydrazine hydrate in DMF at room temperature afforded the corresponding 2-[(2-halobenzyl)thio]benzoic acid hydrazide **2** in high yields (87–90%). The hydrazides were converted to 5-{2-[(2-halobenzyl)thio]phenyl}-1,3,4-oxadiazol-2-amines **3** using cyanic bromide in methanol (70–87%). 5-{2-[(2-halobenzyl)thio]phenyl}-1,3,4-oxadiazole-2-thiols **4** were prepared by the reaction of hydrazide **2** with carbon disulfide under basic condition (80–90%). Sonication of compound **4** in the presence of a suitable alkyl halide in alkaline media afforded 5-(alkylthio)-2-{2-[(2-halobenzyl)thio]phenyl}-1,3,4-oxadiazoles **5a–f** (68–92%). Treatment of hydrazide **2** with phenylisothiocyanate followed by reaction with KI/I_2 in alkaline hydro-ethanol (1:1 v/v) gave 5-{2-[(2-halobenzyl)thio]phenyl}-*N*-phenyl-1,3,4-oxadiazol-2-amines **7** (60–70%). The purity of all products was determined by

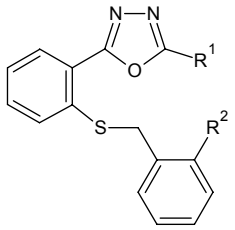
thin layer chromatography using several solvent systems of different polarity. All compounds were pure and stable. The compounds were characterized by ^1H nuclear magnetic resonance, infrared, mass spectrometry and CHN analysis.



Sch. 1.

Physicochemical data of these compounds are summarized in Table 1. Conformational analysis of the synthesized compounds and estazolam were preliminarily performed by MMX force field method implemented in Hyperchem 7.0 software. The conformers were optimized further by AM1 calculation using the MOPAC 6.0 program [21]. Global energy minima conformers of the designed compounds were superimposed on the corresponding conformer of the estazolam molecule which was considered as a reference BZD agonist.

Tab.1. Physical data of the synthesized compounds



Compound	R_1	R_2	mp (^oC)	Yield (%)	Molecular formula	Molecular weight
3a	NH_2	F	146–147	70	$C_{15}H_{12}N_3OSF$	301.33
3b	NH_2	Cl	191–193	75	$C_{15}H_{12}N_3OSCl$	317.79
4a	SH	F	175–177	80	$C_{15}H_{11}N_2OS_2F$	318.38
4b	SH	Cl	170–171	90	$C_{15}H_{11}N_2OS_2Cl$	334.83
5a	SCH_3	F	68	72	$C_{16}H_{13}N_2OS_2F$	332.41
5b	SCH_3	Cl	82–83	92	$C_{16}H_{13}N_2OS_2Cl$	348.86
5c	SC_2H_5	F	64–65	68	$C_{17}H_{15}N_2OS_2F$	346.43
5d	SC_2H_5	Cl	87	70	$C_{17}H_{15}N_2OS_2Cl$	362.89
5e	SBz	F	109–110	79	$C_{22}H_{17}N_2OS_2F$	408.50
5f	SBz	Cl	102–104	75	$C_{22}H_{17}N_2OS_2Cl$	424.96
7a	NHPh	F	176–178	60	$C_{21}H_{16}N_3OSF$	377.43
7b	NHPh	Cl	185–187	70	$C_{21}H_{16}N_3OSCl$	393.89

Satisfactory analysis for C, H, N was obtained for all the compounds within ± 0.4% of the theoretical values.

2. Pharmacology

The BZD activity of the synthesized compounds was determined through the evaluation of the ability of the compounds to protect mice against convulsion induced by a lethal dose of PTZ and electroshock as two routine models. Diazepam was considered as a reference BZD agonist with anticonvulsant effect in both models. As shown in Table 2, compound **3a** with an amino group on position 2 of the oxadiazole ring and a fluoro subtituent at ortho position of the benzylthio moiety has the best anticonvulsant activity in both PTZ and MES models. The activity was antagonized with flumazenil, a benzodiazepine antagonist, which establishes the involvement of benzodiazepine receptors in this effect. Figure 2 shows the superimposition of energy minima conformers of the compound **3a,** the most potent synthesized analogues, and estazolam. Obviously, the main BZD pharmacophores, aromatic rings and proton accepting groups (π1 interaction), nitrogen (N-3) of the 1,3,4-oxadiazole and triazolobenzodiazepine rings, are well matched.

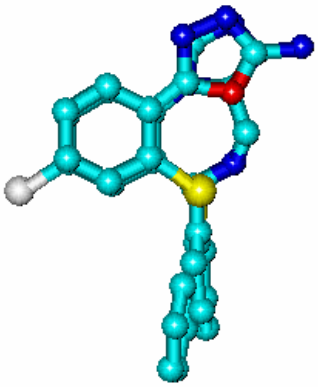
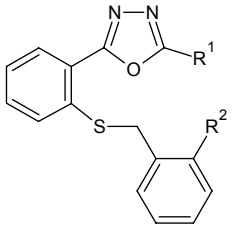


Fig. 2.

Replacement of the fluoro substituent with a larger electron-withdrawing group such as Cl (**3b**) decreases the activity which may be explained by a steric hindrance effect. These results are in good agreement with the classical SAR data of BZDs [22] and our previous studies on 1,3,4-oxadiazoles [14,15]. In the series of 2-alkylthio oxadiazoles, compounds **5a** and **5b** possessing a small alkylthio group at C-2 of the 1,3,4-oxadiazole ring had good anticonvulsant activity in the MES

model but showed mild activity against PTZ induced convulsion. Increasing the size of the alkyl group (**5c** and **5f**) significantly decreases the anticonvulsant activity in both PTZ and MES models. Accordingly, compounds **5e** and **5f** with a bulky benzylthio group did not show any anticonvulsant effects. Similarly, compounds **7a** and **7b** did not have any considerable anticonvulsant activity in both models. Therefore, the size and nature of groups at C-2 position of 1,3,4-oxadiazole ring are very important for anticonvulsant activity in both PTZ and MES models. In addition, the size of electron withdrawing substituents at ortho position of the benzylthio moiety is also important for their anticonvulsant effects.

Tab. 2. Pharmacological evaluation of the synthesized compounds



Compoud	R_1	R_2	ED_{50} mg/kg[a]	
			PTZ	**MES`**
3a	NH_2	F	57.0(43.3–71.8)[b]	20.2 (11.3–32.8)[b]
3b	NH_2	Cl	>100	88.9 (74.7–106.8)[bc]
4a	SH	F	> 100	> 100
4b	SH	Cl	> 100	> 100
5a	SCH_3	F	75.1 (59.8–88.2)[b]	25.1 (14.7–38.5)[b]
5b	SCH_3	Cl	95.1 (80.8–110.2)[bc]	34.4 (23.5–49.4)[b]
5c	SC_2H_5	F	>100	65.3 (52.6–78.7)[b]
5d	SC_2H_5	Cl	> 100	91.4 (75.6–110.5)[bc]
5e	SBz	F	> 100	> 100
5f	SBz	Cl	> 100	> 100
7a	NHPh	F	> 100	> 100
7b	NHPh	Cl	> 100	> 100
Diazepam			0.7 (0.6–0.9) [b]	0.8 (0.5-1.1)[b]

[a] n = 10, 95% Confidence limits in parentheses, LD_{50} of all compounds > 300 mg/kg.
[b] ED_{50} significantly increased in the presence of flumazenil 10 mg/kg ($P<0.05$).
[c] The highest concentration of the compounds tested was 150 mg/kg.

Experimental

1. Chemistry

Melting points (mp) were determined using a Thomas Hoover capillary apparatus (Philadelphia, USA). Infrared spectra were acquired on a Perkin-Elmer 1420 ratio recording spectrometer. A Bruker FT-500 MHz instrument (Bruker Biosciences, USA) was used to acquire ^{1}H-NMR spectra; chloroform-d, DMSO-d_6 and methanol-d_4 were used as solvents. Mass spectra were acquired with a Finnigan TSQ-70 mass spectrometer. Electron-impact ionization was performed at an ionizing energy of 70 eV; the source temperature was 250 ^{o}C. Elemental analyses were carried out with a Perkin Elmer Model 240-C apparatus (Perkin Elmer, Norwalk, CT, USA). The results of the elemental analyses (C,H,N) were within ± 0.4% of the calculated amounts. All chemicals and reagents were obtained from Aldrich (USA) or VWR (Germany) and were used without further purification.

General procedure for preparation of 2-[(2-Halobenzyl)thio]benzoic acid hydrazides 2

To a solution of 2-[(2-halobenzyl)thio]benzoic acid methyl ester **1** (10 mmol) in DMF (10 ml), hydrazine hydrate (50 mmol) was added and stirred at room temperature for 10 hours. After this time, 100 ml water was added and the solid thus separated was filtered, dried and recrystallized from ethanol [16].

2-Fluoro-2-benzylthio benzoic acid hydrazide (2a)

Yield: 87%; mp: 105-106 °C; IR (KBr): ν (cm^{-1}) 3310, 3200 (NH_2), 1680 (C=O) Mass, *m/z* (%): 275.9 (M+, 20), 245 (30), 167 (50), 141.1 (20), 136.1 (30), 109.0 (100), 83.1 (60).

2-Chloro-2-benzylthio benzoic acid hydrazide (2b)

Yield: 90%; mp: 106 °C; IR (KBr): ν (cm^{-1}) 3300, 3200 (NH_2), 1680 (C=O) Mass, *m/z* (%): 292.5 (M+, 30), 261.5 (40), 157.6 (55), 125.1 (100), 89.1 (70).

General procedure for preparation of 5-{2-[(2-Halobenzyl)thio]phenyl}-1,3,4-oxadiazol-2-amines 3

To a methanolic solution of **2** (5.1 mmol), cyanogens bromide (7.5 mmol) was added. The reaction mixture was stirred at room temperature for 3 hours. The resulting solution was neutralized with sodium bicarbonate solution. The solid thus separated out was filtered, washed with water, dried and recrystallized from ethanol [17].

5-{2-[(2-Fluorobenzyl)thio]phenyl}-1,3,4-oxadiazol-2-amine (3a)

Yield: 70%; mp: 146-147 °C; IR (KBr): ν (cm^{-1}) 3380, 3290 (NH_2), 1650 (C=N); ^{1}HNMR (methanol-D4): δ (ppm) 4.23 (s, 2H, CH_2), 7.01-7.31 (m, 4H, 2-fluorobenzyl H_4-H_6, Phenyl H_3), 7.34 (dd, 1H, Phenyl H_5), 7.49 (dd, 1H, Phenyl H_4), 7.56 (dd, 1H, 2-fluorophenyl H_3), 7.76 (d, 1H, Phenyl H_6, J= 7.8 Hz) ; Mass, *m/z* (%): 301.3 (M+, 20), 284.1 (20), 242.1 (25), 228.1 (50), 192.1 (30), 150.1 (50), 109 (100), 89.1 (40). Anal. Calcd. for $C_{15}H_{12}N_3OSF$: C, 59.79; H, 4.01; N, 13.94. Found: C, 59.85; H, 4.12; N, 13.85.

5-{2-[(2-Chlorobenzyl)thio]phenyl}-1,3,4-oxadiazol-2-amine (3b)

Yield: 87%; mp: 191-193 °C; IR (KBr): ν (cm^{-1}) 3350, 3190 (NH_2), 1660 (C=N); ^{1}HNMR (methanol-D4): δ (ppm) 4.31 (s, 2H, CH_2), 7.15-7.26 (m, 4H, 2-chlorobenzyl H_4-H_6, Phenyl H_3), 7.37 (d, 1H, 2-chlorobenzyl H_3, J= 7.8 Hz), 7.41 (dd, 1H, Phenyl H_5), 7.56 (dd, 1H, Phenyl H_4), 7.80 (d, 1H, Phenyl H_6, J= 7.8 Hz); Mass, *m/z* (%): 317.1 (M+,10), 292.1 (20), 260.1 (20), 125.0 (100), 109.1 (50), 89.1 (100). Anal. Calcd. for $C_{15}H_{12}N_3OSCl$: C, 56.69; H, 3.81; N, 13.22. Found: C, 56.80; H, 3.92; N, 13.18.

General procedure for preparation of 5-{2-[(2-Halobenzyl)thio]phenyl}-1,3,4-oxadiazole-2-thiols 4

A mixture of **2** (3.6 mmol), KOH (10 mmol) and carbon disulfide (10 mmol) in ethanol (10 ml) was refluxed on a steam bath for 12 hours. The solution was then concentrated, cooled and acidified with dilute HCl. The solid product that separated was filtered, washed with cold ethanol, dried and recrystallized from ethanol [18].

5-{2-[(2-Fluorobenzyl)thio]phenyl}-1,3,4-oxadiazole-2-thiol (4a)

Yield: 80%; mp: 175-177 °C; IR (KBr): ν (cm^{-1}) 2550 (SH), 1600-1490 (Aromatic); ^{1}HNMR (DMSO-D6): δ (ppm) 4.33 (s, 2H, CH_2), 7.03-7.46 (m, 6H, ArH), 7.58 (dd, 1H, 2-fluorobenzyl H_3), 7.72 (d, Phenyl H_6, J=7.8 Hz); Mass, *m/z* (%): 318.3 (M+, 20), 285.1 (30), 243.1 (20), 228.1 (30), 193.1 (10), 150.1 (60), 136 (90), 109 (100), 89.1 (45). Anal. Calcd. for $C_{15}H_{11}N_2OS_2F$: C, 56.58; H, 3.48; N, 8.80. Found: C, 56.75; H, 3.55; N, 8.74.

5-{2-[(2-Chlorobenzyl)thio]phenyl}-1,3,4-oxadiazole-2-thiol (4b)

Yield: 90%; mp: 170-171 °C; IR (KBr): ν (cm^{-1}) 2580 (SH), 1590-1480 (Aromatic);^{1}HNMR (DMSO-D6): δ (ppm) 4.33 (s, 2H, CH_2), 7.03-7.45 (m, 6H, ArH), 7.56 (d, 1H, 2-chlorobenzyl H_3, J=7.9 Hz), 7.72 (d, Phenyl H_6, J=7.8 Hz); Mass, *m/z* (%): 335.0 (M+, 70), 301.1 (20), 247.1 (30), 209.1 (20), 151.1 (25), 125 (100), 89.1 (40). Anal. Calcd. for $C_{15}H_{11}N_2OS_2Cl$: C, 53.80; H, 3.31; N, 8.37. Found: C, 53.96; H, 3.45; N, 8.24.

General procedurefor preparation of 5-(Alkylthio)-2-{2-[(2-halobenzyl)thio]phenyl}-1,3,4-oxadiazoles 5

An ethanolic solution of appropriate alkyl iodide (3.0 mmol) was added to a solution of compound **4** (2.4 mmol) in 10% aqueous sodium hydroxide (5 ml), and the mixture was sonicated in a sonication bath for 30-60 minutes. The reaction mixture was poured into cold water (50 ml) and the solid thus separated was filtered, washed with water, dried and recrystallized from ethanol [19].

2-{2-[(2-Fluorobenzyl)thio]phenyl}-5-(methylthio)-1,3,4-oxadiazole (5a)

Yield: 72%; mp: 68 °C; IR (KBr): ν (cm^{-1}) 1590-1460 (Aromatic); ^{1}HNMR ($CDCl_3$): δ (ppm) 2.81 (s, 3H, CH_3), 4.27 (s, 2H, CH_2), 7.08-7.48 (m, 6H, ArH), 7.61 (dd, 1H, 2-fluorobenzyl H_3), 7.88 (d, Phenyl H_6, J=7.8 Hz); Mass, *m/z* (%): 332.4 (M+, 20), 285.2 (30), 228.1 (40), 183.1 (20), 150.1 (60), 109.1 (100), 83.1 (60). Anal. Calcd. for $C_{16}H_{13}N_2OS_2F$: C, 57.81; H, 3.94; N, 8.43. Found: C, 57.96; H, 4.02; N, 8.61.

2-{2-[(2-Chlorobenzyl)thio]phenyl}-5-(methylthio)-1,3,4-oxadiazole (5b)

Yield: 92%; mp: 82-83 °C; IR (KBr): ν (cm^{-1}) 1580-1480 (Aromatic); ^{1}HNMR ($CDCl_3$): δ (ppm) 2.77 (s, 3H, CH_3), 4.30 (s, 2H, CH_2), 7.15-7.47 (m, 6H, ArH), 7.57 (d, 1H, 2-chlorobenzyl H_3, J=7.9 Hz), 7.85 (d, Phenyl H_6, J=7.8 Hz); Mass, *m/z* (%): 348.9 (M+, 70), 301.1 (85), 244.1 (50), 223.1 (20), 150.1 (60), 125 (100), 89.1 (40). Anal. Calcd. for $C_{16}H_{13}N_2OS_2Cl$: C, 55.08; H, 3.76; N, 8.03. Found: C, 55.16; H, 3.88; N, 8.01.

2-(Ethylthio)-5-{2-[(2-fluorobenzyl)thio]phenyl}-1,3,4-oxadiazole(5c)

Yield: 68%; mp: 64-65 °C; IR (KBr): ν (cm^{-1}) 1570-1480 (Aromatic); ^{1}HNMR ($CDCl_3$): δ (ppm) 1.57 (t, 3H, CH_3), 3.36 (q, 2H, CH_2), 4.28 (s, 2H, SCH_2), 7.08-7.46 (m, 6H, ArH), 7.60 (dd, 1H, 2-fluorobenzyl H_3), 7.90 (d, Phenyl H_6, J=7.8 Hz); Mass, *m/z* (%): 346.4 (M+, 10), 301.3 (20), 228.1 (20), 150.1 (60), 109.1 (100), 83.1 (50). Anal. Calcd. for $C_{17}H_{15}N_2OS_2F$: C, 58.94; H, 4.36; N, 8.09. Found: C, 58.85; H, 4.12; N, 8.15.

2-{2-[(2-Chlorobenzyl)thio]phenyl}-5-(ethylthio)-1,3,4-oxadiazole (5d)

Yield: 70%; mp: 87 °C; IR (KBr): ν (cm^{-1}) 1580-1470 (Aromatic); ^{1}HNMR ($CDCl_3$): δ (ppm) 1.52 (t, 3H, CH_3), 3.31 (q, 2H, CH_2), 4.31 (s, 2H, SCH_2), 7.16-7.44 (m, 8H, ArH), 7.55 (d, 1H, 2-chlorobenzyl H_3, J=7.9 Hz), 7.86 (d, Phenyl H_6, J=7.8) ; Mass, *m/z* (%): 362.9 (M+, 20), 301.1 (100), 244.1 (50), 150.1 (60), 125 (90), 89.1 (30). Anal. Calcd. for $C_{17}H_{15}N_2OS_2Cl$: C, 56.26; H, 4.17; N, 7.72. Found: C, 56.35; H, 4.22; N, 7.66.

2-(Benzylthio)-5-{2-[(2-fluorobenzyl)thio]phenyl}-1,3,4-oxadiazole (5e)

Yield: 79%; mp: 109-110 °C; IR (KBr): ν (cm^{-1}) 1600-1480 (Aromatic); ^{1}HNMR ($CDCl_3$): δ (ppm) 4.27 (s, 2H, SCH_2), 4.59 (s, 2H, CH_2), 7.08-7.54 (m, 11H, ArH), 7.62 (dd, 1H, 2-fluorobenzyl H_3), 7.87 (d, Phenyl H_6, J=7.8 Hz); Mass, *m/z* (%): 408.5 (M+, 20), 317.1 (20), 285.4 (20), 150.1 (30), 125 (40), 91.1 (100). Anal. Calcd. for $C_{22}H_{17}N_2OS_2F$: C, 64.68; H, 4.19; N, 6.86. Found: C, 64.75; H, 4.24; N, 6.77.

2-(Benzylthio)-5-{2-[(2-chlorobenzyl)thio]phenyl}-1,3,4-oxadiazole (5f)

Yield: 75%; mp: 102-104 °C; IR (KBr): ν (cm^{-1}) 1580-1490 (Aromatic); ^{1}HNMR ($CDCl_3$): δ (ppm) 4.30 (s, 2H, SCH_2), 4.52 (s, 2H, CH_2), 7.13-7.48 (m, 11H, ArH), 7.52 (d, 1H, 2-chlorobenzyl H_3, J=7.9 Hz), 7.82 (d, Phenyl H_6, J=7.8) ; Mass, *m/z* (%): 425 (M+, 45), 301.1 (30), 244.1 (20), 150.1 (10), 125 (40), 91.1 (100). Anal. Calcd. for $C_{22}H_{17}N_2OS_2Cl$: C, 62.18; H, 4.03; N, 6.59. Found: C, 62.25; H, 4.10; N, 6.57.

General procedure for preparation of 5-{2-[(2-Halobenzyl)thio]phenyl}-N-phenyl-1,3,4-oxadiazol-2-amines 7

A mixture of **2** (8.5 mmol), phenyl isothiocyanate (8.5 mmol) and dry THF (50 ml) was stirred at room temperature for 10 hours. It was then concentrated and cooled. The obtained solid was filtered and dispersed in ethanol (50 ml) and to this suspension, aqueous sodium hydroxide (5 N, 8 ml) was added with stirring to obtain a clear solution. To this solution, iodine in potassium iodide solution (5%) was added gradually with stirring till the colour of iodine persisted at room temperature. The reaction mixture was refluxed for 2 hour on a steam bath. It was then cooled and poured onto crushed ice. The solid product that separated was filtered, dried and recrystallized from ethanol [20].

5-{2-[(2-Fluorobenzyl)thio]phenyl}-N-phenyl-1,3,4-oxadiazol-2-amine (7a)

Yield: 60%; mp: 176-178 °C; IR (KBr): ν (cm^{-1}) 1580-1490 (Aromatic); ^{1}HNMR ($CDCl_3$): δ (ppm) 4.30 (s, 2H, SCH_2), 7.07 (brs, 1H, NH), 7.13-7.48 (m, 11H, ArH), 7.61 (dd, 1H, 2-fluorobenzyl H_3), 7.82 (d, Phenyloxadiazole H_6, J=7.8 Hz); Mass, *m/z* (%): 377.4 (M+, 10), 301.4 (30), 268.1 (20), 150.1 (10), 125 (40), 91.1 (100). Anal. Calcd. for $C_{21}H_{16}N_3OSF$: C, 66.83; H, 4.27; N, 11.13. Found: C, 66.95; H, 4.34; N, 11.11.

5-{2-[(2-Chlorobenzyl)thio]phenyl}-N-phenyl-1,3,4-oxadiazol-2-amine (7b)

Yield: 70%; mp: 185-187 °C; IR (KBr): ν (cm^{-1}) 3200 (NH), 1560-1460 (Aromatic); ^{1}HNMR ($CDCl_3$): δ (ppm) 4.30 (s, 2H, SCH_2), 7.05 (brs, 1H, NH), 7.10-7.45 (m, 11H, ArH), 7.58 (d, 1H, 2-chlorobenzyl H_3, J= 7.8 Hz), 7.89 (d,

Phenyloxadiazole H_6, J=7.8 Hz); Mass, *m/z* (%): 393.8 (M+, 10), 317.7 (10), 246.7 (10), 230.8 (20) 150.1 (20), 89.1 (100). Anal. Calcd. for $C_{21}H_{16}N_3OSCl$: C, 64.03; H, 4.09; N, 10.67. Found: C, 64.10; H, 4.14; N, 10.62.

2. Pharmacology

Anticonvulsant evaluation of the synthesized compounds was performed following the standard procedure provided by the antiepileptic drug development program [23, 24]. It includes qualitative assays using MES and PTZ tests. The first assay is related to electrical induction of seizure and the second test generates convulsion by chemical induction. The compounds were administered to adult male albino mice (25–30 g) intraperitoneally at four or five doses (0.1,1, 10, and 100 or 150 mg/kg), and all the results are summarized in Table 2. Diazepam and all tested compounds were dissolved in DMSO. The synthesized compounds, diazepam or vehicle were administered 30 min before injection of PTZ 100 mg/kg or application of electroshock (60 Hz, 37.2 mA and 0.25 s). After 30 min, the dead mice were counted in PTZ test and occurrences of HLTE (hind limb tonic extension) were observed in MES Model. In general, the dose-response curves were estimated by testing three or four doses using at least 10 mice for each dose. To clarify the mode of action of the synthesized compounds, the effects of flumazenil (10 mg/kg), a BZD receptor antagonist, on the anticonvulsant activity of the compounds were determined. The Institutional Animal Ethics committee approved the protocol adopted for the experimentation of animals.

3. Statistical analysis

Statistical analysis of the anticonvulsant activity of the synthesized compounds on animals was evaluated using a one-way analysis of variance (ANOVA). In all cases, post-hoc comparisons of the means of individual groups were performed using Fisher's Exact Probability test. Differences with $P< 0.05$ between experimental groups at each point were considered statistically significant. All values were expressed as mean ± SD (standard deviations). For statistical analysis

we used SPSS Software 13.0 version. (SPSS Software 13.0 version, Inc. Chicago, Ilinois, USA).

Conclusions

The results of this investigation indicate that 1,3,4-oxadiazoles having an amino group at C-2 position with a benzylthio moiety possessing a suitable ortho electron withdrawing substituent can show moderate benzodiazepine activity confirming the suggested SARs for benzodiazepine agonists.

Acknowledgment

This work was partially supported by a grant from the research council of Shahid Beheshti University of Medical sciences.

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Received March 16th, 2008
Accepted (after revision) April 14th, 2008
Available online at www.scipharm.at April 20th, 2008