Design and Synthesis of New 2-Substituted-5-[2-(2-halobenzyloxy)phenyl]-1,3,4-oxadiazoles as Anticonvulsant Agents

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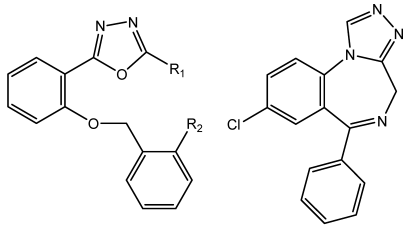
A new series of 2-substituted-5-[2-(2-halobenzyloxy)phenyl]-1,3,4-oxadiazoles was designed and synthesized as anticonvulsant agents. Electroshock and pentylenetetrazole-induced lethal convulsion tests showed that the introduction of an amino group at position 2 of 1,3,4-oxadiazole ring and a fluoro substituent at *ortho* position of benzyloxy moiety had the best anticonvulsant activity. Our results showed that this effect is mediated through benzodiazepine receptors mechanism.

Key words benzodiazepine receptor; 1,3,4-oxadiazole; anticonvulsant; structure–activity relationship (SAR)

The benzodiazepines (BZDs) are widely used in the treatment of central nervous system (CNS) disorders such as anxiety, insomnia and epilepsy.[1)] The pharmacological effects of the BZDs result from their affinity for a specific binding domain on the gamma amino butyric acid ($GABA_A$) receptors, known as the BZD receptor. The interaction of BZD agonists with $GABA_A$ receptor complex increases the GABA-induced chloride channel opening frequency, which results in membrane hyperpolarization, thus reducing neuronal excitability.[2,3)] 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 (5 Å). 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 BZDs 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-halobenzyloxy)phenyl]-1,3,4-oxadiazoles (Fig. 1) with a flexible second out-of-plane aromatic ring, benzyloxy group which has all the suggested requirements for binding to the BZD receptors.



R_1= NH_2, NHPh, NHPh, SH, SMe, SEt, SBz
R_2=F, Cl

Estazolam

Fig. 1. The Structure of Designed Compounds and Estazolam

Results and Discussion

Chemistry The target 1,3,4-oxadiazole derivatives were synthesized according to Chart 1. Accordingly, reaction of 2-halo-2-benzyloxy benzoic acid methyl ester **1** with hydrazine hydrate in *N*,*N*′-dimethyleformamide (DMF) at room temperature afforded corresponding 2-halo-2-benzyloxy benzoic acid hydrazide **2** high yields (87—90%).[16)] The hydrazide were converted to 2-amino-5-(2-halo-2-benzyloxyphenyl)-1,3,4-oxadiazoles **3** using cyanogen bromide in methanol (71—90%).[17)] 5-(2-Halo-2-benzyloxyphenyl)-2-mercapto-1,3,4-oxadiazole **4** was prepared by the reaction of hydrazide **2** with carbon disulfide under basic condition (60—70%).[18)] Sonication of compound **5** in the presence of suitable alkyl halide in alkaline media afforded 2-alkylthio-5-(2-halo-2-benzyloxyphenyl)-1,3,4-oxadiazole **5a**—**f** (58—90%).[19)] Treatment of hydrazide **2** with phenylisothiocyanate followed by reaction with KI/I_2 in alkaline hydro-ethanol gave 2-anilino-5-(2-halo-2-benzyloxyphenyl)-1,3,4-oxadiazole **7** (57—80%).[20)] The compounds were characterized by ^1H nuclear magnetic resonance, infrared, mass spectrometry and CHN analysis. Physicochemical data of these compounds are shown 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 MOPAC 6.0 program.[21)] Global energy minima conformers of the designed compounds were superimposed on corresponding conformer of estazolam molecule which was considered as a reference BZD agonist.

Anticonvulsant Activity The BZD activity of the synthesized compounds was determined through the evaluation of the ability of the compounds to protect mice against con-

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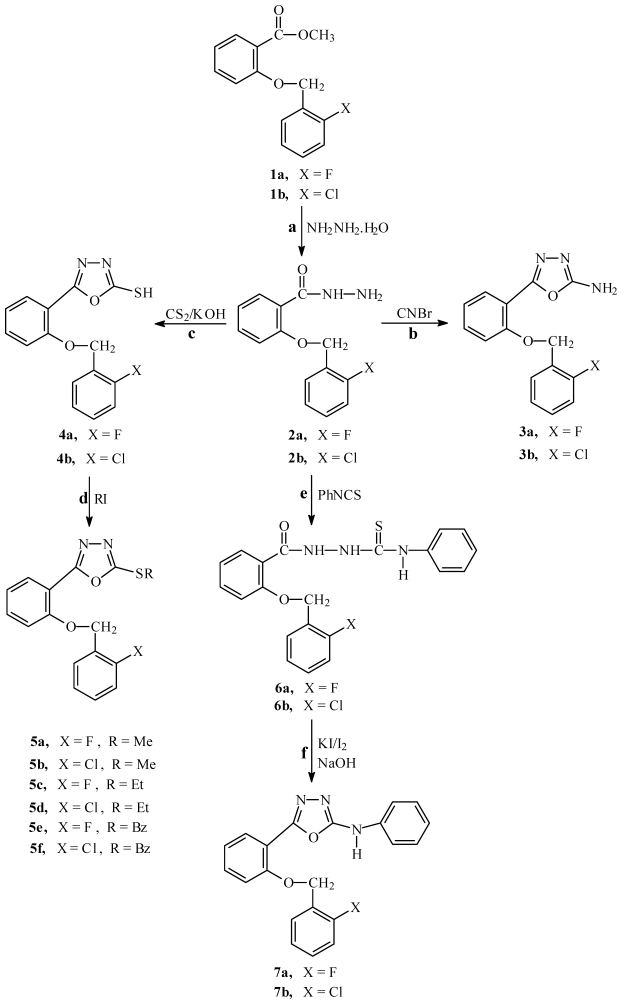
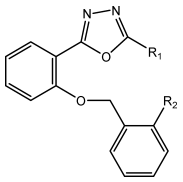


Reagents: (a) $NH_2NH_2 \cdot H_2O$, EtOH, rt, 10 h; (b) BrCN, $NaHCO_3$, MeOH, rt, 3 h; (c) CS_2, KOH, EtOH, reflux, 12 h; (d) RI, NaOH 10%, EtOH, sonication, 20—45 min; (e) PhNCS, EtOH, reflux, 3 h; (f) I_2/KI, NaOH, H_2O/EtOH, rt→reflux, 1 h.

Chart 1

vulsion induced by a lethal dose of pentylenetetrazole (PTZ) and electroshock as two routine models.[23,24] Diazepam was considered as a reference BZD agonist with anticonvulsant effect in both models. 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 maximal electroshock (MES) Model. As shown in Table 2, compound **3a** with amino group on position 2 of oxadiazole ring and fluoro substituent at *ortho* position of benzyloxy group has the best anticonvulsant activity in both PTZ and MES models. The ac-

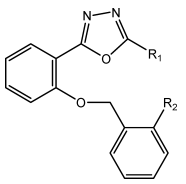
Table 2. Pharmacological Evaluation of the Synthesized Compounds



Compd.	R_1	R_2	ED_{50} (mg/kg)[a]	
			PTZ	MES
3a	NH_2	F	9.0 (7.3—10.8)[b]	16.2 (11.4—22.8)[b]
3b	NH_2	Cl	37.5 (22.5—61.5)[b]	49.5 (31.5—76.5)[b]
4a	SH	F	>100	>100
4b	SH	Cl	>100	>100
5a	SCH_3	F	59.6 (40.8—88.2)[b]	75.1 (52.7—110.5)[b]
5b	SCH_3	Cl	85.6 (59.1—136.4)[b]	79.5 (50.1—126.4)[b]
5c	SC_2H_5	F	71.5 (45.1—106.4)[b]	75.3 (52.6—110.7)[b]
5d	SC_2H_5	Cl	>100	81.4 (50.6—118.5)[b]
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 compoounds >300 mg/kg. *b*) ED_{50} significantly increased in the presence of flumazenil 10 mg/kg ($p<0.05$).

Table 1. Physical Data of the Synthesized Compounds



Compound	R_1	R_2	mp (°C)[a]	Yield (%)[b]	Molecular formula	Molecular weight
3a	NH_2	F	143	90	$C_{15}H_{12}N_3O_2F$	285.27
3b	NH_2	Cl	200	71	$C_{15}H_{12}N_3O_2Cl$	301.73
4a	SH	F	175—177	70	$C_{15}H_{11}N_2O_2SF$	302.38
4b	SH	Cl	188—190	60	$C_{15}H_{11}N_2O_2SCl$	318.53
5a	SCH_3	F	69—70	95	$C_{16}H_{13}N_2O_2SF$	316.41
5b	SCH_3	Cl	85—87	85	$C_{16}H_{13}N_2O_2SCl$	332.86
5c	SC_2H_5	F	64—66	80	$C_{17}H_{15}N_2O_2SF$	330.43
5d	SC_2H_5	Cl	88—90	82	$C_{17}H_{15}N_2O_2SCl$	346.89
5e	SBz	F	125—127	58	$C_{22}H_{17}N_2O_2SF$	392.50
5f	SBz	Cl	99—101	75	$C_{22}H_{17}N_2O_2SCl$	408.56
7a	NHPh	F	175	80	$C_{21}H_{16}N_3O_2F$	361.37
7b	NHPh	Cl	184	57	$C_{21}H_{16}N_3O_2Cl$	377.82

a) Recrystallized from EtOH. *b*) Yield after recrystallization.

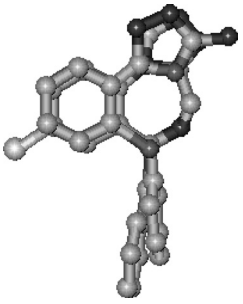


Fig. 2. Superimposition of the Energy Minima Conformers of Estazolam and Compound **3a**

tivity was antagonized with flumazenil, a BZD antagonist, which establishes the involvement of BZD receptors in this effect. In previous related work,[13] we also showed that an amino group on position 2 of oxadiazole or triazole ring and a fluorine substituent at *ortho* position of phenoxy group of 2-substituted-5-[2-(2-fluorophenoxy)phenyl]-1,3,4-oxadiazoles and 1,2,4-triazoles had the best anticonvulsant activity. 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. Replacement of 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.[13—15] In the series of 2-alkylthio oxadiazoles, compounds **5a** and **5b** possessing a small alkylthio group at C-2 of 1,3,4-oxadiazole ring had moderate anticonvulsant activity in both models. Increasing the size of alkyl group (**5c**—**f**) significantly decreases the anticonvulsant activity in both PTZ and MES models. Accordingly, compounds **5e** and **5f** with a bulky benzyloxy 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 on anticonvulsant activity in both PTZ and MES models. In addition, the size of electron withdrawing substituent at *ortho* position of benzyloxy moiety is also important for their anticonvulsant effects. In conclusion, the results of this investigation indicate that 1,3,4-oxadiazoles having amino group at C-2 position with a benzyloxy moiety possessing a suitable *ortho* electron withdrawing substituent can show high BZD activity confirming the suggested SARs for BZD agonists.

Experimental

Melting points (mp) were determined using a Thomas Hoover capillary apparatus (Philadelphia, U.S.A.). Infrared spectra acquired on a Perkin-Elmer 1420 ratio recording spectrometer. A Bruker FT-500 MHz instrument (Brucker Biosciences, U.S.A.) was used to acquire ^{1}H-NMR spectra; chloroform-*d*, dimethyle sulfoxide (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 °C. Elemental analyses were carried out with a Perkin Elmer Model 240-C apparatus (Perkin Elmer, Norwalk, CT, U.S.A.). 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 (U.S.A.) or Merck (Germany) and were used without further purification.

General Procedure for Preparation of 2-Halo-2-benzyloxy Benzoic Acid Hydrazide 2 To a solution of 2-halo-2-benzyloxy benzoic acid methyl ester **1** (10 mmol) in DMF (10 ml), hydrazine hydrate (50 mmol) was added and stirred at room temperature for 10 h. After this time, 100 ml water was added and the solid thus separated out was filtered, dried and recrystallized from ethanol.

2-Fluoro-2-benzyloxy Benzoic Acid Hydrazide **2a**: Yield 87%; mp 105 °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-benzyloxy 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 2-Amino-5-(2-halo-2-benzyloxyphenyl)-1,3,4-oxadiazoles 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 h. The resulting solution was neutralized with sodium bicarbonate solution. The solid thus separated out was filtered, washed with water, dried and recrystallized from ethanol.

2-Amino-5-(2-fluoro-2-benzyloxyphenyl)-1,3,4-oxadiazoles **3a**: Yield 90%; mp 143 °C; IR (KBr): ν (cm^{-1}) 3380, 3290 (NH_2), 16010—1490 (aromatic); ^{1}H-NMR (methanol-d_4): δ (ppm) 5.34 (s, 2H, CH_2), 7.10—7.38 (m, 5H, ArH), 7.62 (m, 1H, phenyl H_4), 7.69 (t, 1H, 2-fluorophenyl H_3), 7.81 (dd, 1H, phenyl H_6, $J_{5,6}$=7.8 Hz, $J_{4,6}$=1.6 Hz); Mass, *m/z* (%): 285.1 (M^+, 10), 243.1 (20), 121.1 (80), 150.1 (50), 109 (100), 83.1 (60). *Anal.* Calcd for $C_{15}H_{12}N_3O_2F$: C, 63.15; H, 4.24; N, 14.73. Found: C, 63.35; H, 4.42; N, 14.85.

2-Amino-5-(2-chloro-2-benzyloxyphenyl)-1,3,4-oxadiazoles **3b**: Yield 71%; mp 200 °C; IR (KBr): ν (cm^{-1}) 3300, 3150 (NH_2), 1600—1480 (aromatic); ^{1}H-NMR (methanol-d_4): δ (ppm) 5.33 (s, 2H, CH_2), 7.10—7.41 (m, 5H, ArH), 7.50 (m, 1H, phenyl H_4), 7.70 (d, 1H, 2-chlorobenzyl H_3, J=7.7 Hz), 7.78 (dd, 1H, phenyl H_6, $J_{5,6}$=7.7 Hz, $J_{4,6}$=1.6 Hz); Mass, *m/z* (%): 301.1 (M^+, 10), 228.1 (10), 207.1 (10), 125.0 (100), 109.1 (50), 89.1 (40). *Anal.* Calcd for $C_{15}H_{12}N_3O_2Cl$: C, 59.71; H, 4.01; N, 13.93. Found: C, 59.50; H, 3.92; N, 14.15.

General Procedure for Preparation of 5-(2-Halo-2-benzyloxyphenyl)-2-mercapto-1,3,4-oxadiazole 4 A mixture of **2** (3.6 mmol), KOH (10 mmol) and carbon disulphide (10 mmol) in ethanol (10 ml) was refluxed on a steam bath for 12 h. The solution was then concentrated, cooled and acidified with dilute HCl. The solid product that separated out was filtered, washed with cold ethanol, dried and recrystallized from ethanol.

5-(2-Fluoro-2-benzyloxyphenyl)-2-mercapto-1,3,4-oxadiazole **4a**: Yield 70%; mp 175-177 °C; IR (KBr): ν (cm^{-1}) 2550 (SH), 1600—1490 (aromatic); Mass, *m/z* (%): 302.4 (M^+, 20), 227.1 (10), 150.1 (40), 136 (70), 109 (100), 83.1 (45). *Anal.* Calcd for $C_{15}H_{11}N_2O_2SF$: C, 59.59; H, 3.67; N, 9.27. Found: C, 59.75; H, 3.85; N, 8.99.

5-(2-Chloro-2-benzyloxyphenyl)-2-mercapto-1,3,4-oxadiazole **4b**: Yield 60%; mp 188—190 °C; IR (KBr): ν (cm^{-1}) 2580 (SH), 1590—1480 (aromatic); ^{1}H-NMR (DMSO-d_6): δ (ppm) 5.35 (s, 2H, CH_2), 7.08—7.51 (m, 6H, ArH), 7.76 (d, 1H, 2-chlorobenzyl H_3, J=7.6 Hz), 7.82 (d, phenyl H_6, J=7.9 Hz); Mass, *m/z* (%): 318.0 (M^+, 50), 245.1 (20), 151.1 (20), 125 (100), 89.1 (40). *Anal.* Calcd for $C_{15}H_{11}N_2O_2SCl$: C, 56.52; H, 3.48; N, 8.79. Found: C, 56.26; H, 3.65; N, 8.54.

General Procedure for Preparation of 2-Alkylthio-5-(2-halo-2-benzyloxyphenyl)-1,3,4-oxadiazole 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 on a sonication bath for 30—60 min. The reaction mixture was poured in cold water (50 ml) and the solid thus separated out was filtered, washed with water, dried and recrystallized from ethanol.

2-Methylthio-5-(2-fluoro-2-benzyloxyphenyl)-1,3,4-oxadiazole **5a**: Yield 95%; mp 69—70 °C; IR (KBr): ν (cm^{-1}) 1590—1460 (aromatic); ^{1}H-NMR ($CDCl_3$): δ (ppm) 2.76 (s, 3H, CH_3), 5.34 (s, 2H, CH_2), 7.12—7.54 (m, 6H, ArH), 7.79 (t, 1H, 2-fluorobenzyl H_3), 8.01 (d, phenyl H_6, J=7.7 Hz); Mass, *m/z* (%): 316.2 (M^+, 20), 269.2 (50), 243.2 (65), 150.1 (70), 121.1 (80), 109.1 (100), 92.1 (60). *Anal.* Calcd for $C_{16}H_{13}N_2O_2SF$: C, 60.74; H, 4.14; N, 8.85. Found: C, 60.96; H, 4.32; N, 8.64.

2-Methylthio-5-(2-chloro-2-benzyloxyphenyl)-1,3,4-oxadiazole **5b**: Yield 85%; mp 85—87 °C; IR (KBr): ν (cm^{-1}) 1580—1480 (aromatic); ^{1}H-NMR ($CDCl_3$): δ (ppm) 2.75 (s, 3H, CH_3), 5.31 (s, 2H, CH_2), 7.08—7.54 (m, 6H, ArH), 7.80 (d, 1H, 2-chlorobenzyl H_3, J=7.6 Hz), 7.96 (d, phenyl H_6, J=7.6 Hz); Mass, *m/z* (%): 333.1 (M^+, 90), 297.1 (25), 259.1 (20), 166.1

(20), 125 (100), 89.1 (20). *Anal.* Calcd for $C_{16}H_{13}N_2O_2SCl$: C, 57.74; H, 3.94; N, 8.42. Found: C, 57.56; H, 3.80; N, 8.21.

2-Ethylthio-5-(2-fluoro-2-benzyloxyphenyl)-1,3,4-oxadiazole **5c**: Yield 80%; mp 64—66 °C; IR (KBr): ν (cm^{-1}) 1570—1480 (aromatic); ^{1}H-NMR ($CDCl_3$): δ (ppm) 1.51 (t, 3H, CH_3), 3.29 (q, 2H, CH_2), 5.33 (s, 2H, OCH_2), 7.12—7.53 (m, 6H, ArH), 7.80 (t, 1H, 2-fluorobenzyl H_3), 8.01 (d, phenyl H_6, J=7.7 Hz); Mass, *m/z* (%): 330.2 (M^+, 15), 269.2 (40), 243.2 (65), 150.1 (70), 121.1 (90), 109.1 (100), 92.1 (50). *Anal.* Calcd for $C_{17}H_{15}N_2O_2SF$: C, 61.80; H, 4.58; N, 8.48. Found: C, 61.65; H, 4.22; N, 8.25.

2-Ethylthio-5-(2-chloro-2-benzyloxyphenyl)-1,3,4-oxadiazole **5d**: Yield 82%; mp 88—90 °C; IR (KBr): ν (cm^{-1}) 1580—1470 (aromatic); ^{1}H-NMR ($CDCl_3$): δ (ppm) 1.50 (t, 3H, CH_3), 3.28 (q, 2H, CH_2), 5.30 (s, 2H, OCH_2), 7.08—7.54 (m, 6H, ArH), 7.85 (d, 1H, 2-chlorobenzyl H_3, J=7.9 Hz), 7.98 (d, phenyl H_6, J=8.0 Hz); Mass, *m/z* (%): 346.9 (M^+, 80), 311.1 (20), 285.1 (30), 150.1 (40), 125 (100), 89.1 (40). *Anal.* Calcd for $C_{17}H_{15}N_2O_2SCl$: C, 58.87; H, 4.36; N, 8.08. Found: C, 58.65; H, 4.12; N, 7.86.

2-Benzylthio-5-(2-fluoro-2-benzyloxyphenyl)-1,3,4-oxadiazole **5e**: Yield 58%; mp 125—127 °C; IR (KBr): ν (cm^{-1}) 1600—1480 (aromatic); ^{1}H-NMR ($CDCl_3$): δ (ppm) 4.50 (s, 2H, SCH_2), 5.34 (s, 2H, OCH_2), 7.14—7.53 (m, 11H, ArH), 7.77 (t, 1H, 2-fluorobenzyl H_3), 8.01 (d, phenyl H_6, J=7.7 Hz); Mass, *m/z* (%): 392.2 (M^+, 5), 269.2 (10), 243.2 (10), 150.1 (10), 121.1 (40), 109.1 (100), 91.2 (65). *Anal.* Calcd for $C_{22}H_{17}N_2O_2SF$: C, 67.33; H, 4.37; N, 7.14. Found: C, 67.55; H, 4.64; N, 7.01.

2-Benzylthio-5-(2-chloro-2-benzyloxyphenyl)-1,3,4-oxadiazole **5f**: Yield 75%; mp 99—101 °C; IR (KBr): ν (cm^{-1}) 1580—1490 (aromatic); ^{1}H-NMR ($CDCl_3$): δ (ppm) 4.49 (s, 2H, SCH_2), 5.31 (s, 2H, OCH_2), 7.08—7.54 (m, 11H, ArH), 7.72 (d, 1H, 2-chlorobenzyl H_3, J=7.6 Hz), 7.97 (d, phenyl H_6, J=7.8 Hz); Mass, *m/z* (%): 409.1 (M^+, 10), 373.1 (20), 285.1 (20), 166.1 (10), 125 (80), 91.1 (100). *Anal.* Calcd for $C_{22}H_{17}N_2O_2SCl$: C, 64.62; H, 4.19; N, 6.85. Found: C, 64.35; H, 4.30; N, 6.67.

General Procedure for Preparation of 2-Anilino-5-(2-halo-2-benzyloxyphenyl)-1,3,4-oxadiazole 7 A mixture of **2** (8.5 mmol), phenyl isothiocyanate (8.5 mmol) and dry tetrahydrofran (THF) (50 ml) was stirred at room temperature for 10 h. 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 h on steam bath. It was then cooled and poured over crushed ice. The solid product that separated out was filtered, dried and recrystallized from ethanol.

2-Anilino-5-(2-fluoro-2-benzyloxyphenyl)-1,3,4-oxadiazole **7a**: Yield 80%; mp 175 °C; IR (KBr): ν (cm^{-1}) 3250 (NH), 1580—1490 (aromatic); ^{1}H-NMR ($CDCl_3$): δ (ppm) 5.36 (s, 2H, OCH_2), 7.09—7.54 (m, 9H, ArH), 7.57 (d, 2H, 2-aminophenyl H_2, H_6, J=8.3 Hz), 7.60 (br s, 1H, NH), 7.74 (t, 1H, 2-fluorobenzyl H_3), 7.96 (dd, phenyloxadiazole H_6, $J_{5,6}$=7.7 Hz, $J_{4,6}$=1.6 Hz); Mass, *m/z* (%): 361.2 (M^+, 10), 243.2 (10), 124.1 (25), 109.1 (100), 93.1 (30). *Anal.* Calcd for $C_{21}H_{16}N_3O_2F$: C, 69.79; H, 4.46; N, 11.62. Found: C, 69.95; H, 4.64; N, 11.31.

2-Anilino-5-(2-chloro-2-benzyloxyphenyl)-1,3,4-oxadiazole **7b**: Yield 57%; mp 184 °C; IR (KBr): ν (cm^{-1}) 3300 (NH), 1560—1460 (aromatic); ^{1}H-NMR ($CDCl_3$): δ (ppm) 5.21 (s, 2H, OCH_2), 6.88 (t, 1H, aminophenyl H_4),6.99—7.35 (m, 8H, ArH), 7.50 (d, 2H, 2-aminophenyl H_2, H_6, J=8.3 Hz), 7.77 (m, 2H, 2-chlorobenzyl H_3 and phenyloxadiazole H_6), 9.59 (br s, 1H, NH); Mass, *m/z* (%): 377.8 (M^+, 10), 317.7 (10), 246.7 (10), 230.8 (20) 150.1 (20), 89.1 (100). *Anal.* Calcd for $C_{21}H_{16}N_3O_2Cl$: C, 66.76; H, 4.27; N, 11.12. Found: C, 66.97; H, 4.45; N, 10.92.

Pharmacological Evaluation 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 effect 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.

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. A significance level of $p<0.05$ considered significance in all cases. All values were expressed as mean±S.D. (standard deviations). For statistical analysis we used SPSS Software 13.0 version. (SPSS Software 13.0 version, Inc. Chicago, Ilinois, U.S.A.).

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

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