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Design and synthesis of new 2-substituted-5-(2-benzylthiophenyl)-1,3,4-oxadiazoles as benzodiazepine receptor agonists

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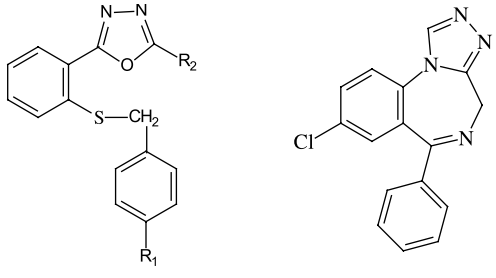
Abstract—A series of new 2-substituted-5-(2-benzylthiophenyl)-1,3,4-oxadiazoles was designed and synthesized as anticonvulsant agents. Conformational analysis and superimposition of energy minima conformers of the designed molecules on estazolam, a known benzodiazepine receptor agonist, revealed that the main proposed benzodiazepine pharmacophores were well matched. Electroshock and pentylenetetrazole-induced lethal convulsion tests showed that the introduction of an amino group in position 2 of 1,3,4-oxadiazole ring and a fluoro substituent at para position of benzylthio moiety had the best anticonvulsant activity. It seems this effect is mediated through benzodiazepine receptors mechanism.

Benzodiazepines (BZDs) are widely used in the treatment of central nervous system (CNS) disorders.[1] The pharmacological effects of BZDs result from their affinity for a specific binding domain on the $GABA_A$ receptors, known as the BZD receptor.[2,3] BZD agonists increase the frequency of the opening of the chlorine channel in response to GABA action, causing anxiolytic, sedative and muscle relaxant effects.[4] 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. Also, the presence of a second out-of-plane, aromatic ring could potentiate binding to the receptor.[5–9] On this basis, we reported 2-substituted-5-(2-benzyloxyphenyl) and (2-phenoxyphenyl)-1,3,4-oxadiazoles which showed considerable anticonvulsant activity.[10,11] In the present study, some new 2-substituted-5-(2-benzylthiophenyl)-1,3,4-oxadiazoles with a flexible second out-of-plane aromatic ring, benzylthio group, were designed (Fig. 1) which had all the suggested requirements for binding to the BZD receptors. In order to confirm that the designed compounds can mimic the proper conformation for binding to the BZD receptor, conformational analysis of designed molecules as well as a known benzodiazepine agonist, estazolam (Fig. 1) was performed followed by superimposition of energy minima conformers. As an in vivo model for evaluating BZD effects, pentylenetetrazole (PTZ)-induced lethal convulsion[12] and maximal electroshock[13] (MES) tests



1 Estazolam

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

Figure 1. The structure of designed compounds and estazolam.

Keywords: 1,3,4-Oxadiazole; Benzodiazepine receptor agonists; PTZ; MES.
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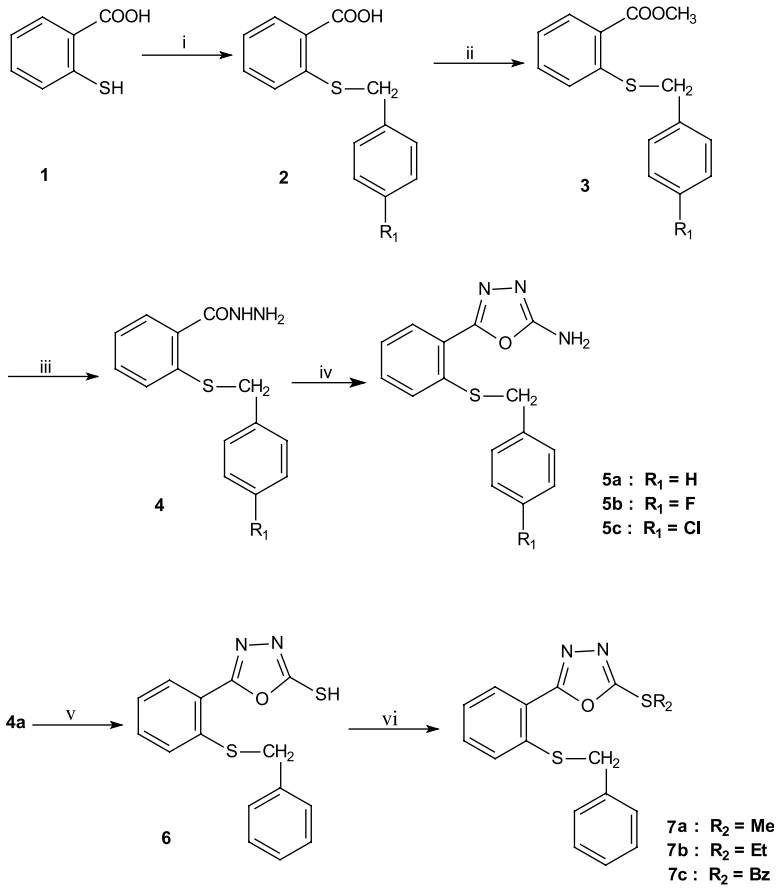
were performed on synthesized compounds. To clarify the mode of action of the synthesized compounds, the effect of flumazenil, a BZD receptor antagonist, on the anticonvulsant activity of the compounds was determined.

The designed compounds were synthesized according to Scheme 1. Reaction of thiosalicylic acid **1** with the appropriate benzyl chloride in alkaline hydromethanolic solution afforded corresponding 2-benzylthio benzoic acid **2**.[14] Esterification of **2** by methanol using acidic condition gave 2-benzylthio benzoic acid methyl ester **3**.[15] 2-Benzylthio benzoic acid hydrazides **4** were readily prepared by treatment of **3** with hydrazine hydrate in methanol.[16] The hydrazides were converted to 2-amino-5-(2-benzylthiophenyl)-1,3,4-oxadiazoles **5** using cyanogen bromide in methanol (56–68%).[17] 5-(2-Benzylthiophenyl)-2-mercapto-1,3,4-oxadiazole **6** was prepared by the reaction of hydrazide **4a** with carbon disulfide under basic condition.[18] Sonication of compound **6** in the presence of suitable alkyl halide in alkaline media afforded 2-alkylthio-5-(2-benzylthiophenyl)-1,3,4-oxadiazoles **7a**–**c** (71–88%).[19] The compounds were characterized by ^{1}H nuclear magnetic resonance, infrared, mass spectrometry and CHN analysis.

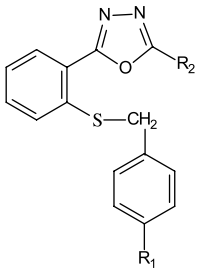
Conformational analysis of the synthesized compounds and estazolam were preliminarily performed by MMX force field method implemented in PCMODEL 6.0 software.[20] The conformers were optimized further by AM1 calculation using MOPAC 6.0 program.[21] Global energy minima conformers of the designed compounds were superimposed on corresponding conformer of estazolam molecule, which was considered as a reference BZD agonist.

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. Each one of the synthesized compounds, diazepam or vehicle was 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. As shown in Table 1, compound **5b** with amino group on position 2 of oxadiazole ring and fluoro substituent at para position of benzylthio group 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 that energy minima conformers of the compound **5b**, the



Scheme 1. Reagents and conditions: (i) BzCl, KOH 10%; MeOH, rt, 3 h; (ii) MeOH, H_2SO_4, reflux, 8 h; (iii) $NH_2NH_2{\cdot}H_2O$; EtOH, rt, 6 h; (iv) BrCN, $NaHCO_3$; MeOH, rt, 3 h; (v) CS_2, KOH, EtOH, reflux, 6 h; (vi) RI, NaOH 10%, EtOH, sonication, 20 min.

Table 1. Pharmacological evaluation of the synthesized compounds



Compd	R_1	R_2	ED_{50}[a] (mg/kg)	
			PTZ	MES
5a	H	NH_2	89.2 (60.1–135.9)[b]	78.9 (54.9–102.9)[b]
5b	F	NH_2	29.1 (16.8–42.1)[b]	43.3 (30.8–54.6)[b]
5c	Cl	NH_2	>100	>100
6	H	SH	>100	>100
7a	H	SCH_3	93.2 (65.1–188.4)[b]	>100
7b	H	SC_2H_5	>100	>100
7c	H	SBz	>100	>100
Diazepam			1.4 (1.1–2.0)[b]	1.8 (1.1–2.6)[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 ($P < 0.05$).

most potent synthesized analogue, and estazolam are superimposed. Obviously, the main BZD pharmacophores, aromatic rings and proton accepting groups, number 2 nitrogen 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 (**5c**) abolished the activity. This effect appears to be a steric hindrance effect since the unsubstituted analogue **5a** had a mild anticonvulsant activity. These results are in good agreement with the classical SAR data of BZDs[22] and our previous studies on 1,3,4-oxadiazole derivatives.[10,11] However, the benzylthiophenyl-1,3,4-oxadiazoles had less anticonvulsant activity compared with benzyloxy phenyl derivatives[11] in the both models. In the series of 2-alkylthio oxadiazoles, only compound **7a** had a weak anticonvulsant activity. Compounds **7b** and **c** did not have any significant anticonvulsant activity in both models. In conclusion, the results of this investigation indicate that some synthesized 2-amino-5-aryl-1,3,4-oxadiazoles with a simple non-rigid structure in which the flexible second out-of-plane aromatic ring, benzylthio group, has a suitable substituent can show benzodiazepine activity confirming the suggested SARs for benzodiazepine agonists.

Acknowledgements

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References and notes

1. Smith, G. B.; Olsen, R. W. *Trends Pharmacol. Sci.* **1995**, *16*, 162.
2. Kerr, I. B.; Ong, J. *Med. Res. Rev.* **1992**, *12*, 593.
3. Macdonal, R. I.; Olsen, R. W. *Annu. Rev. Neurosci.* **1994**, *17*, 569.

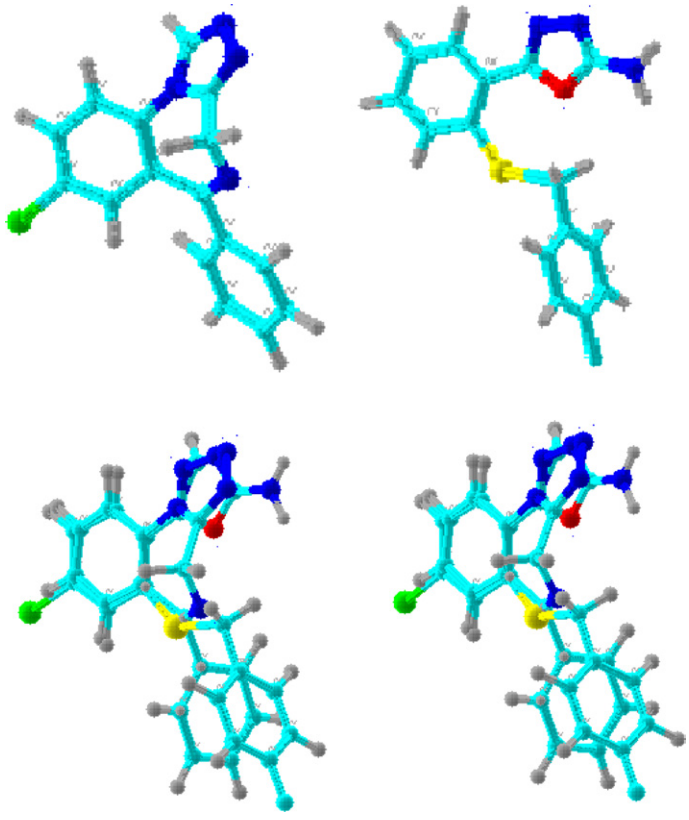

Figure 2. Stereoview of the superimposition of the energy minima conformers of estazolam (top left) and compound **5b** (top right).

4. Sieghart, W. *Pharmacol. Rev.* **1995**, *47*, 181.
5. Crippen, G. M. *Mol. Pharmacol.* **1982**, *22*, 11.
6. Codding, P. W.; Muir, A. K. *Mol. Pharmacol.* **1985**, *28*, 178.
7. Fryer, R. I.; Cook, C.; Gilman, N. W.; Wasler, A. *Life Sci.* **1986**, *39*, 1974.
8. He, X.; Zhang, J. M.; Cook, J. M. *Med. Chem. Res.* **2001**, *10*, 269.
9. Diaz-Arauzo, H.; Koehler, K. F.; Hagan, T. J.; Cook, J. M. *Life Sci.* **1991**, *49*, 207.
10. Almasirad, A.; Tabatabai, S. A.; Faizi, M.; Kebriaeezadeh, A.; Mehrabi, N.; Dalvandi, A.; Shafiee, A. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 6057.
11. Zarghi, A.; Tabatabai, S. A.; Faizi, M.; Ahadian, A.; Navabi, P.; Zanganeh, V.; Shafiee, A. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 1863.
12. Morpygo, C. *Arzneim.-Forsch.* **1971**, *11*, 1727.
13. Jachson, H. C.; Hasen, H. C.; Kristiansen, M.; Suzdaz, P. D.; Klitgaar, H.; Judge, M. E.; Swedberd, D. B. *Eur. J. Pharmacol.* **1996**, *306*, 21.
14. Bown, D. H.; Bradshaw, J. S. *J. Org. Chem.* **1980**, *45*, 2320.
15. Munch-Petersen, J. *Org. Synth.* **1973**, *V*, 762.
16. Firoozi, F.; Javidnia, K.; Kamal, M.; Fooladi, A.; Foroumadi, A.; Shafiee, A. *J. Heterocycl. Chem.* **1995**, *32*, 123.
17. Goswami, B. H.; Sarmah Kataky, J. C.; Baruah, J. N. *J. Heterocycl. Chem.* **1984**, *21*, 1225.
18. Almasirad, A.; Sheikha, M.; Hosseini, R.; Tabatabai, S. A.; Shafiee, A. *Arch. Pharm. Pharmacol. Med. Chem.* **2004**, *337*, 193.
19. Shafiee, A.; Naimi, E.; Mansoobi, P.; Foroumadi, A.; Shekari, M. *J. Heterocycl. Chem.* **1995**, *32*, 1235.
20. PCMODEL; Serena Software: PO Box 3070, Bloomington, IN 47402, USA.
21. QCPE; Department of Chemistry: Indiana University, Bloomington, IN 47405, USA.
22. Fryer, R. I. In *Comprehensive Medicinal Chemistry*; Hansch, C., Ed.; Pergamon: Oxford, 1990; Vol. 3, p 539.