Pergamon

Bioorganic & Medicinal Chemistry Letters, Vol. 5, No. 22, pp. 2717-2720, 1995

0960-894X/95 $9.50+0.00

0960-894X(95)00464-5

6,3'-DINITROFLAVONE, A NOVEL HIGH AFFINITY LIGAND FOR THE BENZODIAZEPINE RECEPTOR WITH POTENT ANXIOLYTIC PROPERTIES.

Mariel Marder[a], Haydee Viola[b], Cristina Wasowski[a], Claudia Wolfman[b], Peter G. Waterman[c], Jorge H. Medina[b], Alejandro C. Paladini[a,*].

[a] *Instituto de Química y Fisicoquímica Biológicas, Facultad de Farmacia y Bioquímica, Junín 956 (1113), Buenos Aires, Argentina.*

[b] *Instituto de Biología Celular, Facultad de Medicina, Paraguay 2155 (1121), Buenos Aires, Argentina.*

[c] *Phytochemistry Research Laboratories, Strathclyde Institute for Drug Research and Department of Pharmaceutical Sciences, University of Strathclyde, Glasgow G1 1XW, Scotland, U. K.*

Abstract: 6,3'-Dinitroflavone (**2**) and 6,4'-dinitroflavone (**3**), prepared by direct nitration of flavone (**1**), were found to be ligands for the benzodiazepine receptor (BDZ-R). Compound **2**, with a K_i= 12 nM, produced potent anxiolytic effects in mice at a dose of 1 μg/Kg.

Introduction

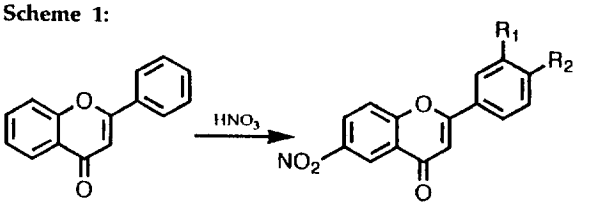
Some natural flavonoids were recently found to have anxiolytic properties,[1-3] presumably due to their affinity towards the central BDZ-R. Furthermore, these flavonoids, unlike benzodiazepines (BDZs), had neither sedative nor myorelaxant effects.[2,3] In an effort to improve this pharmacological activity of flavonoids we attempted the introduction of electronegative groups based on the favorable effect of these substitutions in the BDZs.[4] These experiments using halogen groups gave encouraging results,[5] but the most striking effects were obtained by nitration of the flavone nucleus, as described in this letter.

Chemistry

6,3'-Dinitroflavone (**2**) and 6,4'-dinitroflavone (**3**) were prepared as follows (Scheme 1). Anhydrous nitric acid (d=1.4, 750 μL) was added dropwise to flavone (**1**) (60 mg; 0.27 mmol). The vial containing **1** was kept in an ice bath during the addition. The resulting solution was allowed to stand for 30 min at room temperature. While stirring with a thin glass rod, water (10 mL) was added, and the vial placed in an ice bath to cool. The precipitated product was collected by vacuum filtration, washed with water and dried. Its toluene

solution was chromatographed in a silica gel column which was eluted in steps with increasing concentrations of acetone in toluene. Two major components could be isolated which were further purified by recrystallization from acetone-water rendering compounds **2** and **3**, whose structures were confirmed by spectroscopic data.[6,7]

Scheme 1:



1 flavone

2 $R_1 = NO_2$ $R_2 = H$
3 $R_1 = H$ $R_2 = NO_2$

Results and Discussion

Non-specific nitration of the flavone nucleus yielded the two nitrated flavones **2** and **3**. Compounds **2** and **3** inhibited ^{3}H-flunitrazepam (^{3}H-FNZ) binding[8] to extensively washed bovine cerebral cortical membranes[9] with a K_i of 12.0 ± 1.7 nM (n=7) and 17 ± 5 μM (n= 3), respectively.

Scatchard analysis of saturation curves for compound **2** revealed a competitive interaction showing a decline in the apparent affinity without changes in the maximal number of sites (Bmax) (Figure 1).

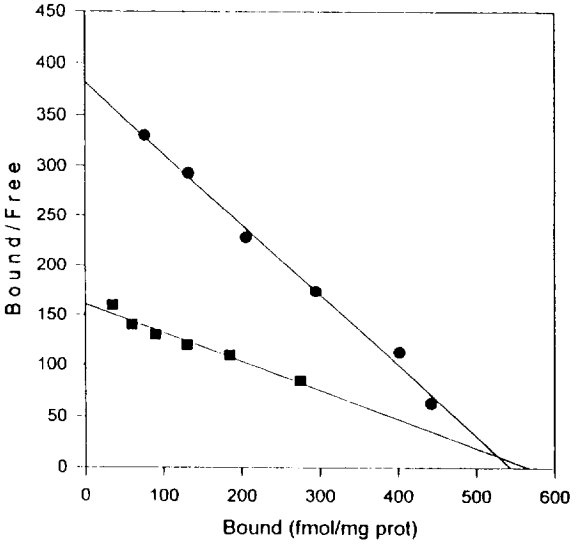


Figure 1: Scatchard plot of representative curves of ^{3}H FNZ binding to bovine synaptosomal membranes in the absence (●) or in the presence of compound **2** (■, 20 nM).

As compound **2** showed a very high affinity for the BDZ-R, it was further examined for pharmacological activity. Performance of mice on the elevated plus-maze,[10] a well validated test to measure anxiolytic actions in rodents,[11] following i.p. administration of vehicle or **2**, is shown in Figure 2.

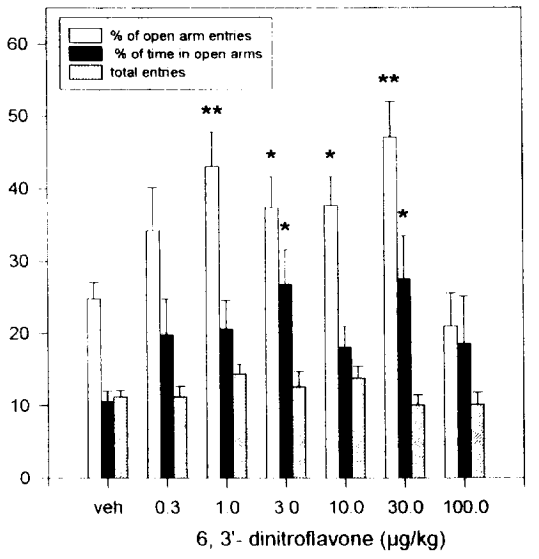


Figure 2: Performance of mice during a 5 min test on the elevated plus-maze test, 15 min after i.p. injection with vehicle (VEH) or **2** (0.3-100.0 µg/Kg). Results are expressed as mean ± SEM of the number of total arms entries (hatched bars), percentage of open arms entries (open bars) and percentage of time spent in the open arms (closed bars). *: $p<0.05$, **: $p<0.01$, Dunnet's multiple comparison test. The number of experimental mice per group ranged between 9-16.

Compound **2** at doses ranging from 1 to 30 µg/Kg increased the percentage of entries in the open arms, without affecting the total arms entries. For diazepam a minimum dose of 30 µg/Kg is necessary in order to produce similar anxiolytic effects (not shown). At a dose of 3 and 30 µg/Kg, **2** also enhanced the percentage of the time spent in the open arms (Figure 2).

In conclusion, we found that **2** is a very potent anxiolytic drug that interacts competitively and with high affinity with the BDZ-R. An on-going detailed pharmacological profile of this compound will tell us about its potential as a new tool in the therapeutics of anxiety.

References and Notes

1. Medina, J. H.; Paladini, A. C.; Wolfman, C.; Levi de Stein, M.; Calvo, D.; Diaz, L. E.; Peña, C. *Biochem. Pharmacol.* **1990**, *40*, 2227.

2. Wolfman, C.; Viola, H.; Paladini, A. C.; Dajas, F.; Medina, J. H. *Pharmacol. Biochem. Behav.* **1994**, *47*, 1.

3. Viola, H.; Wasowski, C.; Levi de Stein, M.; Wolfman, C.; Silveira, R.; Dajas, F.; Medina, J. H.; Paladini A. C. *Planta Medica* **1995**, *61*, 213.

4. Sternbach, L. H. *Prog. Drug Res.* **1978**, *22*, 229.

5. Marder, M.; Viola, H.; Wasowski, C.; Wolfman, C.; Waterman, P. G.; Medina, J. H.; Paladini, A. C. submitted to *J. Med. Chem.*

6. Compound **2**: yield 45%; yellow light crystals (from acetone-water); mp 246-248 °C; ^{1}H NMR (300 Mhz, DMSO-d_6): δ 8.93 (t, J= 2.1 Hz, H-2'), 8.72 (d, J= 2.6 Hz, H-5), 8.64 (dd, J= 9.3, 2.6 Hz, H-7), 8.63 (dt, J= 8.4, ca. 2.2 Hz, H-4'), 8.46 (ddd, J= 8.4, ca. 2.2 Hz, H-6'), 8.16 (d, J= 9.3Hz, H-8), 7.90 (t, J= 8.4 Hz, H-5'), 7.43 (s, H-3).
EIMS *m/z* 312 $[M]^+$, 284, 266, 238, 220, 165.

Compound **3**: yield 45%, yellow crystals (from acetone-water); mp 260-261 °C; ^{1}H NMR (300 Mhz, DMSO-d_6): δ 8.72 (br. s, H-5), 8.65 (br. d, J= 9.1 Hz, H-7), 8.41 (s, 4H, H-2', H-3', H-5', H-6'), 8.09 (d, J= 9.2 Hz, H-8), 7.42 (s, H-3).
EIMS *m/z* 312 $[M]^+$, 284, 266, 254.

7. Cushman, M.; Zhu, H.; Geahlen, R. L.; Kraker, A. J. *J. Med. Chem.* **1994**, *37*, 3353.

8. The binding of ^{3}H-FNZ (81.8 Ci/mmol; NEN) was carried out as described by Levy de Stein, M.; Medina, J. H.; De Robertis, E. *Mol. Brain Res.* **1985**, *5*, 9. In brief, for each assay, triplicate samples of the membranes, containing 0.2 to 0.4 mg protein were suspended in a final volume of 1 mL of 0.25 mM Tris-HCl buffer, pH 7.3. The incubation was carried out at 4 °C for 60 min with 0.6 nM ^{3}H-FNZ. To study the binding saturation, a range of 0.3 to 10 nM ^{3}H-FNZ was used. Non specific binding was determined in parallel incubations in the presence of 3 μM FNZ, and represented 5-15% of total. The assays were terminated by filtration under vacuum though Whatman GF/A glass-fiber filters, and three washes with 3 mL each of incubatiom medium. Filters were dried and counted after the addition of 5 mL of 2,5-diphenyloxazole/xylene as scintillation fluid.

9. Medina, J. H.; De Robertis, E. *J. Neurochem.* **1985**, *44*, 1340.

10. The animals used in the pharmacological test were male Swiss mice from our breeding stock, weighing 28-35 g. They were placed in groups of ten with free access to water and food, and maintained on 12h/12h day/night cycle. In all the tests the mice were i. p. injected with VEH or a solution of the drug, 15 min before the assay.
The elevated plus-maze set-up consisted of a maze of two open arms, 25 x 5 cm, crossed by two closed arms of the same dimensions, with free access to all arms from the crossing point. The closed arms had walls 35 cm high all around. The maze was suspended 50 cm from the room floor. Mice were placed on the central part of the cross facing an open arm. The number of entries and the time spent going into open and closed arms were counted during 5 min. A selective increase in the parameters corresponding to open arms reveals an anxiolytic effect. The total exploratory activity (number of entries in both arms) was also determined.

11. Pellow, S.; Chopin, P.; Fil, S.; Briley, M., *J. Neurosci. Meth.* **1986**, *14*, 149; Lister, R. G., *Psychopharmacology (Berl.)* **1987**,*92*, 180.

(*Received in USA* 8 *September* 1995; *accepted* 10 *October* 1995)